E-Training Manual

Conservation and Cultivation practices of Endangered Himalayan Yew (*Taxus contorta* Griff.) in Himachal Pradesh

Developed under

National Mission on Himalayan Studies (NMHS) Funded Project

"Returning Taxus to the Forests and the People: a study in Shimla and Kullu Districts of the Indian Himalayan Region"

Year-2021

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About the NMHS-Taxus Project

Project title "Returning *Taxus* to the Forests and the People: a study in Shimla and Kullu Districts of the Indian Himalayan Region"

Funded by National Mission on Himalayan Studies (NMHS), Govt. of India

The west Himalayan yew (*Taxus contorta* Griff.) previously known as *Taxus wallichiana or baccata* has suffered a severe range wide population decline of upto 90% in the Indian Himalayan Region (IHR), mainly because of overexploitation for its medicinal properties, especially for the commercial extraction of the anti-cancer drug Taxol (Thomas & Farjon, 2011; Aslam, 2016). The last population assessment of *Taxus* in Himachal Pradesh was conducted in 2008 in Khokhan Wildlife Sanctuary (Pant & Samant, 2008) and the current extent and possible locations of this species is not known. Hence there is a need to assess and map the current distribution using species niche modeling technique. Pant & Samant (2008) reported a very poor situation of regeneration of this species and have predicted the extirpation of this species from the sanctuary. Hence, there is an urgent requirement of understanding the causes of decline in regeneration and to try to decipher the factors that have resulted in the successful regeneration, in the few habitats where *Taxus* has been able to establish itself. Hence this project would focus on trying to establish those conditions, be it nurse shrubs, shrubs or trees to which the birds responsible for the dispersal of *Taxus* seeds are attracted, or inoculating the soil with mycorrhizae that help in the early establishment of the seedlings, litter thickness that provides the appropriate temperature, soil fauna that aid in creation of the ideal soil environment and help to prevent seed predation, and the soil compaction, pH level, temperature and litter C:N ratio that ultimately help to establish *Taxus* seedlings in the habitat for in situ conservation. At the same time it is important to reduce the dependency of the indigenous people on the forest individuals of *Taxus* by helping them in establishing *Taxus* plantlets in their gardens and community lands. This is where we need to develop and standardize mass multiplication protocols and plantlet establishment involving the forest department and the local people for successful planting for ex situ conservation of this endangered medicinal tree. Such community monitored plantations and trees planted in forests would generate livelihood and income for the local people, creating an incentive for conserving the species for extraction of plant parts for medicinal uses, and this would develop into a self-sustaining conservation strategy. Without undertaking these steps it would be impossible to save this species from extinction from wild in the IHR and an important source of anti-cancer medicines would be lost forever.

Considering the above, G. B. Pant National Institute of Himalayan Environment Mohal, Kullu - 175 126, Himachal Pradesh, India in collaboration with Shoolini University, Bajhol, PO Sultanpur, Solan - 173229, India is implementing a project entitled "Returning Taxus to the Forests and the People: a study in Shimla and Kullu Districts of the Indian Himalayan Region" funded by National Mission on Himalayan Studies (NMHS) of G.B. Pant "National Institute of Himalayan Environment"(NIHE), Kosi-Katarmal, Almora, Uttarakhand.

Project Objectives, Deliverables and Monitoring Indicators

The Project Objectives, Quantifiable Deliverables and Monitoring Indicators are as follows:

Project Objectives	Quantifiable Deliverables	Monitoring Indicators
• Assessment and mapping of populations of *Taxus* in Shimla and Kullu district. • Investigation of the physicochemical and biotic factors associated with regeneration of *Taxus*. • Development and standardization of protocols for mass multiplication of *Taxus* and comparison of its phytochemistry relative to natural individuals. • Establishment of plantlets of *Taxus* involving the forest department and the local communities.	• Develop the Distribution map for *Taxus contorta* • Generation of knowledge about different ecological factors affecting natural regeneration of *Taxus*. • Development and standardization of mass propagation techniques for mass multiplication of *Taxus contorta* (production of >100000 propagules) • New conservation Model for plantation drives through strengthening local nurseries and forest department, and community monitored plantations • A manual and guidelines for conservation and sustainable use of target species • Creating awareness among farmers in 10 villages	• Number of distribution map developed (Nos.) • Mass propagation of selected species (Nos.) • Conservation Model developed (Nos.) • Number of nurseries strengthened (No/ Area) • The number of Beneficiaries (Nos.) • No. of Reports/Research articles/Policy documents/Manual prepared and published (Nos.)

The role and responsibilities of project partners

The role and responsibilities of project partners are as follows

S.No.	Name of organizations	Roles & Responsibilities
1.	Shoolini University, Bajhol, PO Sultanpur, Solan - 173229, India	• Collection of invertebrates and soil fauna associated with Taxus. • Identification of fauna and analysis of soil nutrients. • Studying the role of seed dispersers and seed predators. • Studying the extent of herbivory and the associated animals. • Statistical analysis of data. • Guiding a project fellow for collection, identification, soil analysis, statistical analysis and • local community participation and outreach activities.
2.	G. B. Pant National Institute of	• Mapping the distribution of Taxus in Kullu district. • Identification of plants associated with Taxus.

	Himalayan Environment and Sustainable Development Mohal, Kullu - 175 126, Himachal Pradesh, India	• Development and standardization of mass multiplication methods for cultivating Taxus. • Establishing plantlets of seedlings involving forest department and local people. • Guiding a project fellow for mass multiplication of seedlings and • local community participation and outreach activities.
3.	Shoolini university of Biotechnology and Management Sciences, Solan, Himachal Pradesh	• Isolation and identification of mycorrhizae associated with Taxus. • Phytochemistry of mass multiplied saplings and its comparison to naturally propagated individuals. • Outreach activities.

Context of the project:

Biodiversity Conservation and Management*:* The west Himalayan yew (*Taxus contorta* Griff.) has been warranted with the endangered status in the IUCN RedList (Thomas & Farjon, 2011). The main reasons for the decline of population of *Taxus* are overexploitation of the tree because of its commercially important medicinal use and the lack of regeneration. To reduce the overexploitation of the species, research would be conducted to successfully establish plantlets of *Taxus* in the village lands for its ex situ conservation. For in situ conservation, the factors responsible for successful regeneration of *Taxus* in the wild would be investigated, such that establishing those conditions should aid in successful germination and growth of the seedlings in the forest. The current distribution of the species would be mapped using niche distribution modeling, which would help in recognizing potential areas where this species is growing or could grow, so that those areas could be given protection.

Skill Development and Capacity Building: There would be involvement of the local communities in establishment of the plantlets in the community lands, with proper training so that employment opportunities if created for planting *Taxus* in forest lands, would help in generating some income to the local people. This would also empower the local people for identification, monitoring and protection of the species, creating conditions conducive to the regeneration of the species, and for generating nurseries for growing the species and establishing plantations for sustainable exploitation. The people will also be trained as to how to harvest the bark so as to not kill the tree.

Livelihood options and Employment Generation: Once the local people get trained in establishing the plantlets of *Taxus*, they can plant it in their own lands and after some years, they would be able to generate income by extracting plant parts is a sustainable way for utilizing the medicinal properties of this tree, thereby generating income continually in the future and also saving the species from extinction.

General Introduction about *Taxus contorta*

Scientific name - *Taxus contorta*

Family - Taxaceae

Local name - Rakhal, The Himalayan Yew, Thuner

Useful parts - Leaves and bark

Medicinal uses - It is a prime source of taxol, a potent anticancer drug; used for the treatment of high fever and painful inflammatory conditions; consumed as decoctions, herbal tea for treating cold, cough, respiratory infections, indigestion.

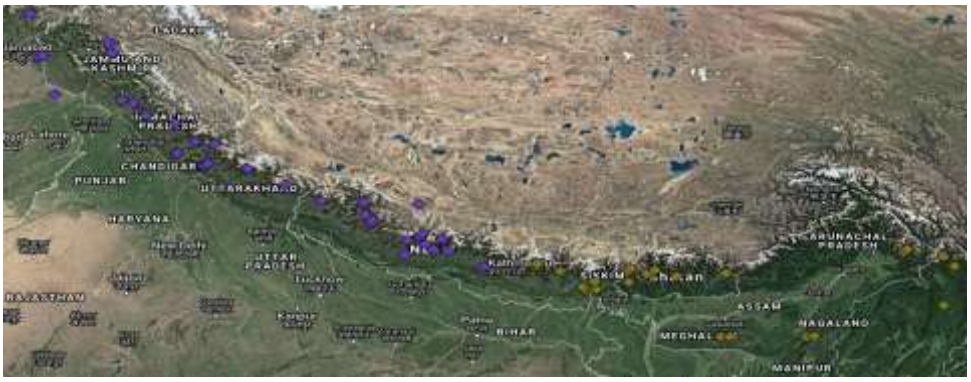
General details

- It is a medium-sized, temperate, endangered, native high value medicinal plant of the Indian Himalayan region.
- It attains a height of 9-20 meters with a massive trunk.
- The leaves are flat, dark green, 1–4 centimeters (0.39–1.57 in) long and 2–3 millimeters (0.079–0.118 in) broad, twisted at the base and arranged spirally on the stem.
- The stem is profusely branched and remains covered with a thin brown-colored bark.
- Plants are mainly dioecious with globose male flowers and female flowers appearing as small stalked conical buds in the leaf axils.
- Seeds are brown and nut-like and are covered with a red fleshy aril, ripening in the first year.
- It is closely associated with *Abies pindrow*, *Quercus semecarpifolia*, *Q.floribunda*, *Q.leucotrichophora*, *Betula utilis*, *Acercaesium*, *Pinus wallichiana*, *Rhododendron arboreum* and *Betula alnoides*

Conservation approach for Taxus contorta in Himachal Pradesh

Taxus contorta Griff. (Western Himalayan Yew, locally known as Rakhal/Thuner), belonging to family Taxaceae, is an endangered native high value medicinal plant species of the North Western Himalaya (as per IUCN red list). Total ten species under the genus *Taxus* were recorded globally. Out of which *T. contorta* is native to Indian Himalayan Region (IHR) covering the temperate and sub-alpine zones. In India, it covers the area of J&K, Himachal Pradesh, Uttarakhand, Meghalaya, Nagaland and Manipur.

The native population in the IHR has been using its bark for making herbal tea concoctions, for treating cold, cough, fever and headache. It is well known for it's anti-cancerous product Taxol, which is obtained from the leaves and bark of tree. High anthropogenic pressure; particularly over exploitation from its natural habitats, biotic pressure on yew from lopping, peeling bark and grazing, several medicinal properties of the bark and leaves of this species has led to a drastic decrease in its population. Due to over-harvesting of its bark and leaves, most wild populations are threatened with extinction and are endangered in the Himalayas. Himalayan yew is a very slow growing plant, with poor natural regeneration potential primarily due to low seed production and germination. It was observed that the population of *Taxus* has been decreasing drastically in the last few years in the Western Himalayan Region. The forest has been cut down by the local communities for domestic uses like, for timber wood, fuel wood. Local people also use its branches for support and for decorative purposes.



Distribution of Taxus in Indian Himalayan Region

Climate change is projected to alter species' natural distribution and drive biodiversity loss in forest ecosystems. Habitat destruction, changing climate, pollution, invasion of alien species and pathogens, overexploitation and increasing human population are the most important factors responsible for ecosystem degradation worldwide that alters the structural and functional integrity of the ecosystems. Such alterations have brought approximately one fifth of the plant species to the brink of extinction. To mitigate the effects of climate change on forest ecosystems, effective target conservation strategies such as modeling species distributions to identify areas where sensitive species exist or are likely to exist will be needed. To effectively model species distributions, detailed and reliable information about the spatial distribution of species is required.

The present study is focused on (i) to investigate population size and altitudinal distribution of *T. contorta* in Kullu district of Western Himalaya, (ii) to test which bioclimatic parameters influence the species presence (iii) to test species distribution models and compare the species' extent of occurrence under current climatic situation and under climate change scenarios and (iv) to make a conservation strategy for its protection.

To remodel the forest of *Taxus contorta*, it is important to understand distribution of the species, its current status, and to assess the vegetation related to it. The species do not regenerate well from seed and that is another risk factor. The objective of this research is to investigate the population ecology of the species as a foundation for its conservation and to remodel the forest communities of Kullu valley in order to understand the distribution of the species, and its current status. The species re-introduction is a successful ecological technique for recovering and maintaining the position of degraded species populations, degraded habitats and ecosystems. For the successful re-introduction and rehabilitation of species in an ecosystem, a detailed knowledge on the potential habitats and distribution of species is essential. Species Distribution Models (SDMs) are commonly used to predict the geographic range of a species, given presence-only occurrence data and environmental variables assumed to influence its distribution.

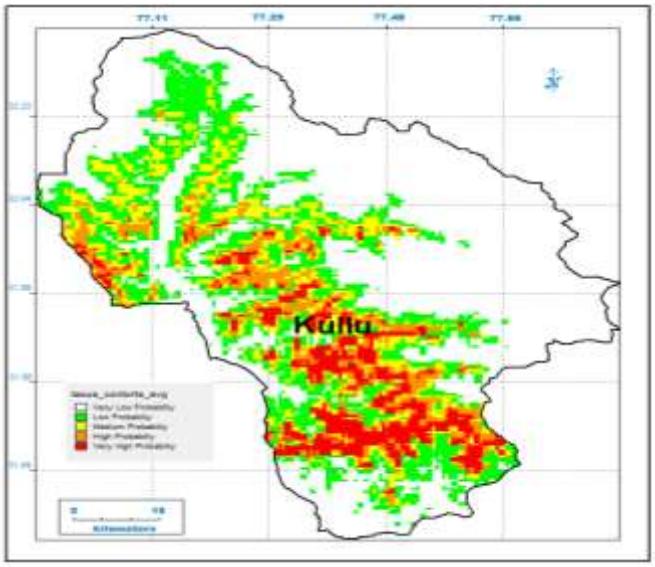
Of many species distribution model algorithm methods, Maxent (Maximum Entropy, Phillips, 2004) has proved powerful when modeling rare species with narrow ranges and scarce presence-only occurrence data. Twenty eight (28) secondary and 3 primary occurrence points (Ground control points) were collected through field survey and literature review. Remotely sensed data on elevation and bioclimatic variables in highest resolution (*i.e.*, ~1km) were downloaded in BIL format, converted to ACS II raster grids and rescaled to ~250 m resolution in Arc GIS 10.5 software. The 31 occurrence points and environmental layers – current global climate (19 bioclimatic variables), altitude, slope and aspect were used for the MaxEnt modeling. The Maxent model shows the probability of occurrence of *Taxus contorta* in Kullu district of Himachal Pradesh. The model AUC was 0.860, which is greater than the minimum acceptable value of 0.75 and the standard deviation was 0.138. BIO13 showed the greatest relative contribution to the model. BIO7 showed the highest Permutation importance. On the basis of habitat suitability for *Taxus contorta*, the areas with very high, high, medium, low and no probability were identified; the area with very high probability is 419 Km^2 (7.60%) and area with high probability is 429 Km^2 (7.79%). The information from collection sites of the herbarium specimens and some of our own collections were used to validate model predictions. Predictions on potential sites of *Taxus contorta* could help in the conservation of this species. The model with eco-physiologically important bioclimatic variables seems to perform best, covering most potential sites of *Taxus contorta*. This study also revealed that the most suitable

altitudinal range was from 2600-3000 m a.s.l., while temperature and precipitation played important role in the species. Results from our models can be utilized for developing conservation strategies for the species in the Himalayan region. Mass multiplication of *Taxus contorta* cutting was also carried out in the institute nursery. These mass multiplied saplings have been transplanted in most suitable area/high probability areas marked by the Maxent model with the support of local communities and state forest department.

A conservation strategy of *Taxus contorta* has been formulated under the project. It will provide a template for conservation in other locations where the species is at risk. The study thus suggests that there is an immediate need to protect *Taxus* forests from harvesting (lopping, peeling bark, etc.), grazing and other destructive activities. Using efficient biotechnological tools (like tissue culture), artificial regeneration of this species needs to be promoted in nurseries to produce healthy planting material on a large scale. Besides in-situ conservation and management, large-scale afforestation with the participation of local communities and

forest department would also be needed for conservation of endangered and valuable *Taxus contorta* species in the Himalayan region.

Unsustinable harvesting threats to Taxus contorta in Kullu, Himachal Pradesh



Habitat suitability for *Taxus Contorta* in Kullu district of Himachal Pradesh

Mass multiplication Package of Practice of Taxus contorta

1. **Name of medicinal plant**

a) **Scientific name:** *Taxus contorta* Zucc.

b) **Pharmacopoeial name:** Sthauneya [1]

c) **Local name (specify language):** English: Himalayan Yew; Hindi : Thuner, Talispatra Bhed; Bengali: Birmi, Bhirmie, Talish Patra, Bhada Getela; Gujarati: Gethela Barmi; Oriya: Talisabhed, Chalisa Patra; Tamil: Talisapatri-Bhedam; Telegu: Taleesa Patri Bhedamu and in Sanskrit: Sukapuspa, Vikarna [1].

d) **Varieties/Germplasm authentication**

e) **Characteristics/Diagram and Herbaria:**

Taxus contorta is a native evergreen non-resinous gymnosperm tree up to 20−28 m, often with multiple trunks and spreading, rounded or pyramidal canopy. It is capable of producing leafy branches from old branches and trunks, and sometimes from stools. Root system is shallow with extensive horizontal roots, often above ground on calcareous substrates. Branches long, not whorled. Twigs green and irregularly alternate; buds very small, bud scales dark-brown, rounded, imbricate and closely appressed. Leaves spirally attached but on lateral shoots twisted more or less into two ranks, can live for up to 8 years; 1–3(−4.5) cm long and 2–3 mm wide. It is normally dioecious, rarely monoecious; reproductive structures green, borne in leaf axils near the end of the previous summer's growth. Seed ovoid, smooth and shiny, brown-yellow, 6–7 × 5 mm at maturity, with a tough seed coat, partly surrounded by a fleshy red aril typically 9 × 7 mm which falls with the seed at maturity, the 'fruit' ripening in the first year. [2]

2. **Plant to be employed as the medicinal plant material (Description of the part of the plant used for medicinal purposes):**

Bark and Leaves

Himalayan yew has been used traditionally for the treatment of high fever and painful inflammatory conditions. It is consumed as decoctions, herbal tea, and juice for treating cold, cough, respiratory infections, indigestion, and epilepsy. As poultice, it is used locally on the infected wounds and burns. [3] Its **bark and leaves** are used in steam baths to treat rheumatism, and the paste made from its bark is used to treat fractures and headaches. Extracts from the tree are also used in medicinal hair oils. The **bark and leaves** of ***T. wallichiana*** are used in Unani medicine as a source of the drug Zarnab, which is prescribed

as a sedative, aphrodisiac, and as a treatment for bronchitis, asthma, epilepsy, snake bite, and scorpion stings.[6] **Young shoots** of the plant are used in Ayurveda to prepare a tincture for the treatment of headache, giddiness, feeble and falling pulse, coldness of extremities, diarrhea, and severe biliousness.[5] **Leaf paste** is used for the treatment of asthma and bronchial disorders. Tea, made out of the **stem bark** of Himalayan yew, has been popular in Himalayan tribal communities for curing cold, cough and hypertension.

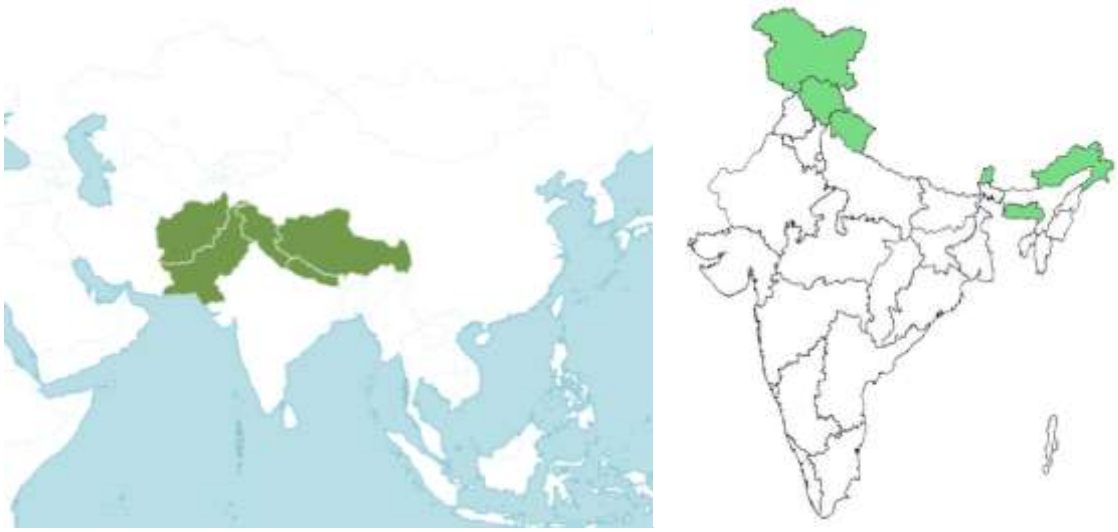
3. **Characteristics of the medicinal plant (Describe the ago-morphological characteristics including the key character following standard descriptors and descriptor state)**

T. wallichiana commonly known as 'Yew' is an evergreen tree attaining a height of 9-20 meters with a massive trunk. The stem is profusely branched and remains covered with a thin brown-coloured bark.

- **Leaves** - The leaves are linear, small, only 2-3 cm. long and 2–3 mm wide spirally arranged. Each leaf possesses a single strong vein and recurved margins, tapering to a petiole-like base. The upper surface is dark green while the lower surface is pale or rusty red in color. The stalk broadens into a flat persistent base which shows a slight twist.
- **Seed -** Taxus seeds are covered by a three-layered seed coat. The outermost layer is thin, brown and detaches soon. The middle layer is hard and stony while the innermost layer is fleshy. The mature seeds are covered by a red-coloured aril. The aril serves to attract birds and help in dissemination.
- **Stem** – It is profusely branched and remains covered with a thin brown-collared bark.
- **Flowering** - Plants are mainly dioecious with globose male flowers and female flowers appearing as small stalked conical buds in the leaf axils. The female flower resembles an axillary vegetative bud, but is usually decurved or pendent and is easily recognized on close inspection by the micropyle opening in the exposed ovule. The male flower or pollen cone has several sterile scales at the base, with a stalked globose head of 6 to 14 scales, each with 5 to 9 microsporangia or pollen sacs. [8]
- **Fruit** – It is a soft, bright red berry-like structure which is known as aril and they are long and wide open at the end. Subsequent to pollination, the aril matures within 6 to 9 months. Having a jelly-like consistency, the aril is not poisonous and is sweet to taste.

4. **Distribution of the species including map in Himalaya…; in India..; in world…;**

It is distributed from Pakistan to southwest China, Nepal and Bhutan, mainly at elevations of 1800–3300 m a.s.l. In Indian Himalaya, it is found in temperate and sub-alpine zone; Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Meghalaya, Nagaland and Manipur.



(A) **(B)**

Figure (A) Species distribution in the world

(http://www.plantsoftheworldonline.org/taxon/urn:lsid:ipni.org:names:263797-1)

Figure (B) Species distribution in India

(http://envis.frlht.org/maps/Taxus-18b10da2df66b026beeb71cfa4b4023c-wallichiana)

5. **Characteristics of accessions for cultivation**

i. **Key identification marks:** Similar to its agro-morphological characteristics.

ii. **Major chemical compound responsible for drug value along with chemical profile:** The diterpenoid alkaloid, Taxol (paclitaxel), was first isolated from the bark of *Taxus brevifolia*. Subsequently, taxol and taxoid derivatives were reported from foliage and bark of several other species of Taxus including *Taxus contorta*. The species is well recognized as source of anti-cancerous drug taxol for treating various forms of cancers. The needles, stem and bark have been reported to contain taxol, baccatin 111, and 10-deacetylbaccatin 111, a precursor for taxol synthesis. [7]

iii. **Preferred growing conditions**

a) **Soil conditions** (pH, Water retention capacity, nutrient status as per soil test report etc.)

In order to keep the soil moist and to suppress weed growth spread a 3-inch-thick layer of mulch in a 20-inch radius around the base of the yew tree. Use pine bark or other mildly acidic, lightweight mulch. It can tolerate frost, drought and strong winds, and usually grows on limestone-derived moist soil

b) Soil type: loamy soils of a slightly acidic or neutral pH having adequate moisture.

c) Shade requirement, if any: It is suitable for shady places as well as sunny spots. However, optimal is a half-shady location. If it is too dark, the growth rate decreases. In sunny locations, especially with alternating rain/snow and strong sun radiation, it can result in the drying up.

6. Cultivation methods [12]

i. Land preparation: The land is ploughed twice or thrice to make the soil more permeable. The weeds and stones are separated from the soil and let the land under the exposure of sun for a week.

ii. Propagation methods: The plant can be propagated through seeds and vegetative means (Air layering).

a) Seed: The seeds are collected from a medium-aged, healthy, and disease free tree.

- Time of year to collect seed: Autumn; Time of year to sow seed: Summer; Seeds should be covered with 1/2 inch of soil and proper cultural controls such as mulching and shading should be maintained to ensure proper moisture and temperature.
- It is best sown as soon as it is ripe when it should germinate 18 months later. Stored seed may take 2 years or more to germinate. For ideal germination conditions, seeds should be given 5-7 months of warm stratification at 65º F, followed by 2-4 months of cold stratification at 34º-40º F.

b) Cutting

- **Stem cutting**

➢ **Selection of Stem cuttings**

- 15 – 20 cm long and 4 – 5 cm in diameter
- Juvenile stem cuttings with leaves from lower part of the canopy of mature Taxus trees.
- At least 3 – 4 nodes.

Too many leaves in the cutting give negative effect because of higher transpiration rate and water deficiency that cause leaf shedding. At the same time, cuttings without leaves root poorly.

- **Preparation of Stem cuttings**
  - The base of each cutting is given a slanting cut and dipped in 0.1% aqueous solution of Bavistin for 5-10 min to protect cuttings from any chances of fungal infection, subsequently washed with distilled water.
  - 18 hours prior to planting, the cuttings are dipped in a hormonal solution of 500 ppm IBA.
- **Nursery Technique**
  - These cuttings are planted in the locally prepared beds in a slanting 45° position.
  - The cuttings should be protected from direct sunlight and high humidity (>70 percent) should be maintained by a thatch covering.
  - They require a lot of water. The soil should never dry out and should be kept appropriately wet. Make sure that no water logging occurs which a yew does not tolerate.
  - Spreading a layer of gravel or bark mulch on the surface of the soil or substrate is helpful especially for warm days. This prevents the evaporation of moisture and keeps the moisture longer inside the soil.
  - The cuttings are planted in the prepared beds during July-August.
  - 70-80% cuttings produced roots in the bed during September–October and they can then be transplanted in the field in early June.
- **Air layering:** The mature healthy plants are selected for air layering. The air layering experiment is carried out usually during rainy season. The layered shoots are treated with various concentrations test chemicals (500, 1000, 2000 and 5000μM) of indol-3-butyric acid (IBA), Indole-3-acetic acid (IAA) and α-naphthalene acetic acid (NAA) and systematic fungicide Bavistin. The shoots of 5-8 mm diameter (250-320 cm, CBH trees) are used for each treatment and untreated is used for control. The careful removal of bark (1.5-2cm width) with sharp knife at the node of shoot is followed by application of various test chemicals mixed with soil paste or directly by using test solution dipped cotton plug around the layer. The layer is then covered by moss pad and wrapped with perforated polythene sheet, tied with thread at both the end and observations are taken at 20-25days intervals. Afterwards well rooted shoots are cut 5 cm below the girdle with help of sharp secateurs. Out of these 50% rooted cuttings are directly planted in their natural habitat and 50% are transported in laboratory in wet cotton sheets. These rooted cuttings are transferred in pots containing mixture of soil and farmyard manure and kept inside mist chamber. [13]

- **Tissue culture:** The shoot cuttings of *T. wallichiana* are taken and washed using detergent solution (Tween 20, two drops) under running tap water for 15-20 minutes. Afterwards, they are sterilized with a solution of 0.1% Bavistin for 10-20 minutes and Mercuric chloride 0.05 % for 30sec-2 minutes and rinsed with distilled water thoroughly under laminar air flow cabinet. They are then cultured in Murashige and Skoog (MS) medium containing various concentrations and combinations of BA – Benzyl adenine, NA - 1-Naphthaleneacetic acid, and GA3 - Gibberellic acid and then placed in the culture room (25-27° C temperature, 16hr light 8hr dark conditions).

c) Sowing methods

- **Seed:** Time of year to sow seed: Summer; Seeds should be covered with 1/2 inch of soil and proper cultural controls such as mulching and shading should be maintained to ensure proper moisture and temperature.
- **Transplanting:** The seedlings are very slow-growing and will probably require at least 2 years of pot cultivation before being large enough to plant out.

d) Manures & fertilizers including microbial fertilizers

1.5 inches of soil, 1.5 inches of sand, and 1.5 inches of organic fertilizer.

- **Irrigations:** They require a lot of water. The soil should never dry out and should be kept appropriately wet. Make sure that no water logging occurs which a yew does not tolerate.

e) Pest managements including diseases

i) Pest / disease type:

Disease	Symptoms	Pathogen/Cause
Bleeding canker	Sap oozes from the lower trunk near the soil line. Needles yellow and fall as branches die back.	*Phytophthora*
Dieback	Needles on branches yellow as the branch dies.	Excessive soil moisture.
Edema	Bumps of scab-like tissue form on the underside of needles. [15]	Excessive soil moisture.

ii) Control measures used:

Remove the infected plant and do not replace it with a Taxus or other *Phytophthora*-susceptible plant. Do not plant *Taxus* in poorly drained locations, especially in areas of heavy clay. [11]

Viii. Harvesting stage, time & procedures:

- **Stage of Maturity:** Only harvest from trees with a trunk diameter of more than 20cm
- **Time of Harvesting:** Conventionally, harvesting time of Himalayan Yew can be variable depending on the component, the plant collected for. Collection of leaves for essential oil can be done early in the morning before the day temperature starts increasing due to strong essential oil activity in morning.
- **Method of harvesting**: Only harvest from trees with a trunk diameter of more than 20cm. Ensure that the branch which the twig was taken from has been pruned. Never completely remove a branch of its twigs. These methods ensure that the branch can quickly recover, and not kill the tree. Snip a small twig from the tree approximately 10-15cms.

(a) Postharvest handling & processing:

(i) Cleaning & washing: The harvested material should be washed with clean water... Do not use contaminated water for washing. Do not wash seeds and delicate parts of the medicinal plants. All excess water should be drained from the herb before drying.

(ii) Drying: Small branches with leaves and the collected leaves are shade dried during sunlight on a tarpaulin / cement floor for 3 - 4 days and protect them from fog and frost.

(iii) Storage: Leaves can be stored when wrapped in appropriate packing in air tight bags. For long term storage leaves should be dried well and drying should avoid any breakage to avoid loss of essential oil and taxanes.

(iv) Packaging: leaves are then tied up in bundles and put in gunny bags to avoid fungal attack.

(b) Expected yield with desired quality: depends on the age of the plant, a 10-year-old plant gives a yield of 200kg/ 0.61 acres, for paclitaxel extraction a yield of 0.1% - 0.45% per kg.

7. Quality evaluation of the medicinal plant material

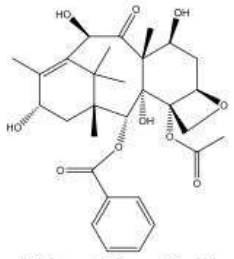
i) National quality standard of the medicinal plant material API (defined as the quality and quantity standard) [1]

- **Foreign matter -** Not more than 2 Per cent, Appendix 2.2.2
- **Total ash -** Not more than 6 Per cent, Appendix 2.2.3
- **Acid-insoluble ash -** Not more than 1.5 Per cent, Appendix 2.2.4
- **Alcohol-soluble extractive -** Not less than 10 Per cent, Appendix 2.2.6
- **Water-soluble extractive -** Not less than 16 Per cent, Appendix 2.2.7

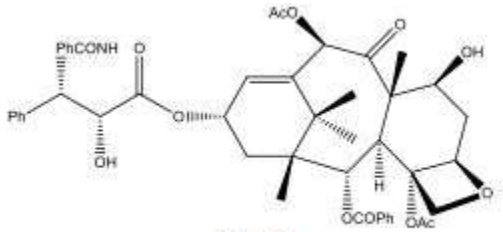
ii) Name of major chemical/chemicals constituents and its percentage

Taxol (a diterpenoid alkaloid) is the major taxoid which is mainly obtained from bark and varies in concentration from 0.007 to 0.01%, in different Taxus species. Other potent taxoids found in Taxus *contorta* include baccatin-III (0.084%), cephalo mannine (0.031%), and 10-deacetyl baccatin-III. Another important class of compounds (lignans) is found in heartwood of plant. These include isoliovil, conidendrin, α-conidendrin, hydroxymatairesinol, texiresinol, β-conidendrin, (–)-secoisolariciresinol, isotexiresinol. These lignans have anticancer as well as antiulcer potentials. Major bioflavonoids found in T. wallichiana include sciadopitysin and amentoflavone. Phytosterols (daucosterol, 4-desmethylsterol type, and βsitosterol), and phytoecdysteroids (ponasterone and ecdysone), have also been reported from T. wallichiana bark. [9]

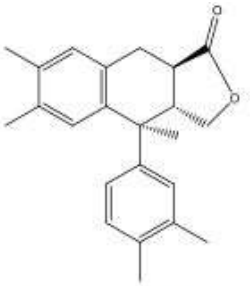
iii) Chemical structure of selected major constituents [9]



10-deacetyl baccatin-III

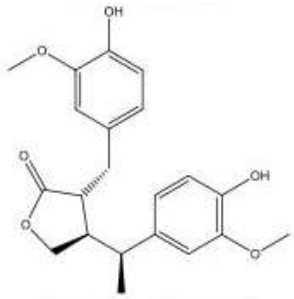


Taxol

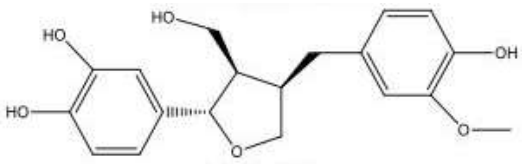


α –conidendrin

(A) (B) (C)



Hydroxymatairesinol



Texiresinol

(D) (E)

Figure: Major chemical constituents of *Taxus contorta*

A) 10-deacetyl baccatin-III B) Taxol C) α-conidendrin D) Hydroxymatairesinol E) Texiresinol

8. Cultivation calendar

A tabulated schedule of cultivation practices whichever to be followed indicating the type of care and management work/actions and their timing during the entire growing period.

Cultivation practices	Month	Course of work [12]
Seed collection	October- November	Collect seeds from the natural forest of Taxus. Clean seeds with drinking water by removing the outer fleshy part and dry them in shade. Store dry seeds in a cold but not damp place. While storing protect them from fungus, insects and mice.
Nursery bed preparation	March	Create a bed, containing 3 levels. 1.5 inches of soil, 1.5 inches of sand, and 1.5 inches of organic fertilizer
Seed sowing	March-April	Seeds should be covered with 1/2 inch of soil and proper cultural controls such as mulching and shading should be maintained to ensure proper moisture and temperature.
Seed germination	First week of June	After 20-25 days of sowing, the seeds start germinating; keep them in the nursery bed for one month and then transfer to polybags where they remain for 8-12 months
Transplanting	July-August	Seedlings will take one and half year before being ready to plant in the field.

Harvesting	August to October	Only harvest from trees with a trunk diameter of more than 20cm. Ensure that the branch which the twig was taken from has been pruned. Never completely remove a branch of its twigs. These methods ensure that the branch can quickly recover, and not kill the tree. Snip a small twig from the tree approximately 10-15cms. You can also dry the twigs - they fetch a higher price. Dry the twigs in open sunlight and away from livestock or wildlife. This can be done by fencing or elevating the twigs.

Mass multiplication method of *Taxus contorta*

Stem cuttings treated with IBA 500 ppm

9. Background data and other information, if any

Photographs of plant and plant material;

1. (B) (C) (D)

Figure: A) Leaves, B) Aril, C) Male Flower, D) Dried leaves

10. Extract attributes, if applicable

A. Plant part: Leaves

B. Extraction method: Four types of solvent extraction methods (ultrasound and microwave assisted extraction, pressurized liquid extraction, and extraction in the Soxhlet apparatus) for paclitaxel, cephalomannine, and 10-deacetylbaccatin, taxoids can be employed for extraction process. [14]

11. Quality parameter for certifications

Quality parameter addressing below is based on Ayurvedic Pharmacopoeia of India part 1 volume IX. It suggests the parameter should be used in Compliance with the prescribed limits which are shown in the appendix section of the book. [10]

A. Pesticide residue (if pesticides used, any)

B. Heavy metal:

S. No.	Heavy metal	Permissible limits
1.	Lead	10 ppm
2.	Arsenic	3 ppm
3.	Cadmium	0.3 ppm
4.	Mercury	1 ppm

C. Microbial load:

Microbial Contamination Limits:

Sl. No.	Parameters	Permissible limits for herbal extracts and Powders	Permissible limits for plant materials which will be treated before use
1	Staphylococcus aureus/g	Absent	-
2	Salmonella sp./g	Absent	-

3	Pseudomonas aeruginosa/g	Absent	-
4	Escherichia coli	Absent	10
5	Total microbial plate count (TPC)	10^5/g	10^7/kg
6	Total Yeast & Mould	10^5/g	10^7/kg

D. Aflatoxin:

Permissible Limit of Aflatoxins:

Aflatoxin	Permissible Limit
B1	< 2 ppb
B1+B2+G1+G2	< 5 ppb

Pictorial representation of various activities of NMHS- Taxus project

On field demonstration *Taxus contorta* mass multiplication at Aloo ground nursery of Forest Department, Manali

Mass multiplication of *Taxus contorta* at GBNIHE, HRC

Awareness meeting cum Plantation of Taxus contorta at various locations of Kullu district

Bibliography/References

1. The Ayurvedic Pharmacopoeia of India, Part I, Volume – III, Ministry of Health and Family Welfare, Department of Ayush, New Delhi, pp 204-205.
2. Thomas, P. A., & Polwart, A. (2003). Taxus baccata L. *Journal of Ecology*, *91*(3), 489-524.
3. Nisar, M., Khan, I., Ahmad, B., Ali, I., Ahmad, W., & Choudhary, M. I. (2008). Antifungal and antibacterial activities of Taxus wallichiana Zucc. *Journal of enzyme inhibition and medicinal chemistry*, *23*(2), 256-260.
4. Gonzalez, J. (1980). Medicinal plants in Colombia. *Journal of ethnopharmacology*, *2*(1), 43-47.
5. Khan M, Verma SC, Srivastava SK, Shawl AS, Syamsundar KV, Khanuja SP, et al. Essential oil composition of *Taxus wallichiana Zucc*. from the Northern Himalayan region of India. *Flavour Frag J.* 2006;21:772–5.
6. Purohit A, Maikhuri RK, Rao KS, Nautiyal S. Impact of bark removal on survival of *Taxus baccata* L.(Himalayan yew) in Nanda Devi Biosphere Reserve, Garwhal Himalaya, India. *Curr Sci.* 2001;81:586–90.
7. Adhikari, P., & Pandey, A. (2017). Taxus wallichiana Zucc.(Himalayan Yew) in antimicrobial perspective. *Advances in Biotechnology and Microbiology*, *5*(4), 1-5.
8. Chadwick, L. C., & Keen, R. A. (1976). study of the genus taxus.
9. H. Sharma, M. Garg. (2015). A review of traditional use, phytoconstituents and biological activities of Himalayan yew, Taxus wallichiana. Journal of integrative medicine. 13(2): 80-90
10. https://main.ayush.gov.in/sites/default/files/Ayurvedic%20Pharmacopoeia%20of%20India%20part%201%20volume%20IX.pdf
11. https://extension.psu.edu/taxus-diseases
12. https://2019.igem.org/wiki/images/2/23/T--UNSW_Australia--IHP-Himalayan-Yew-Tree-Guideline.pdf
13. Kishor, K., Upreti, B. M., Pangtey, Y. P. S., Tewari, A., & Tewari, L. M. (2015). Propagation and conservation of Himalayan Yew (Taxus baccata L.) through air layering: a simple method of clonal propagation. *Ann. Plant Sci*, *4*(04), 1064-1067.
14. Wianowska, D., Hajnos, M. Ł., Dawidowicz, A. L., Oniszczuk, A., Waksmundzka-Hajnos, M., & Głowniak, K. (2009). Extraction methods of 10-deacetylbaccatin III, paclitaxel, and cephalomannine from Taxus baccata L. twigs: A comparison. *Journal of Liquid Chromatography & Related Technologies®*, *32*(4), 589-601.
15. https://extension.psu.edu/taxus-diseases

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The focus of the HRC is entire Himachal Pradesh state covering parts of north western Himalayan bio-geographic province. The region is recognized for its ecological and economic values manifested by ecosystem integrity, adaptability and ecosystem services. Its protective and productive functions for both upland and lowland dwellers are well known. The major thrust areas of activities include: (i) vulnerability assessment of biodiversity of the ecosystems in Trans and North Western Himalaya under biological, anthropogenic and climate scenarios and developing strategies for conservation management, (ii) assessment, monitoring and management of agricultural crops/farming systems for sustainability under chemical contamination and climate change scenarios along an altitudinal gradient in North Western Himalaya, (iii) assessment, characterization and valuation of ecosystem services for sustainable development of the native communities, (iv) development of strategies for monitoring and management of water resources under climate change scenario, (v) assessment and sustainable management of eco-tourism through entrepreneurship development, (vi) assessment, monitoring and analysis of the anthropogenic and natural environmental impacts for developing management strategies, and (vii) development and strengthening of institutional mechanism for information sharing and capacity building of the stakeholders for environmental management.

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