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Recent trends in conservation of the Himalayan Yew

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Abstract

The Himalayan yew (*Taxus wallichiana* Zucc., syn. *Taxus baccata* L. subsp. *wallichiana* (Zucc.) Pilger, Taxaceae) is a medium sized, evergreen, unisexual gymnosperm growing in the temperate and subalpine Himalayan regionsat altitudes between 1800 and 3300 m amsl. The plant has immense medicinal and ethnobotanical importance. Apart from being used in the traditional systems of medicine, the Himalayan yew is also a source of paclitaxel (Taxol®), a broad-spectrum antineoplastic agent, being widely used in the treatment of cancer. It is also effective against noncancerous conditions like polycystic kidney diseases. *T. wallichiana* is also an important source of 10-deacetyl baccatin III (10-DAB), that is a precursor of paclitaxel and docetaxel (Taxotere®), an analogue of paclitaxel. Thus, the plant is in high demand. But it is slow growing with poor regeneration, has a long seed dormancy period of 1.5-2 years and germinates slowly. It has a small population size, narrow range of distribution and is affected by climatic changes. In addition, to meet the growing demand of paclitaxel, plant parts have been indiscriminately collected from the wild. There has been a decline in its population over the last few decades and the plant has been enlisted as 'endangered'. Thus, theconservation of this precious, endangered species has become a matter of concern and a lot of effort has been put in the past few decades to safeguard its existence; ex *situ*, *in situ* and *in vitro* conservation strategies have been adopted.The present review summarizes thevarious conservation strategies adopted in the last decade for protecting the Himalayan yew from being extinct.

Key words: cancer, endangered, paclitaxel, Taxol, *Taxus wallichiana*

Introduction

The Himalayan yew [*Taxus wallichiana* Zucc., syn. *Taxus baccata* L. subsp. *wallichiana* (Zucc.) Pilger, Taxaceae] is a medium sized (10 to 28 m in height),long lived, evergreen, cross-pollinatedgymnosperm. In Asia, it is distributed from Afghanistan to Philippines through the Himalayas, with wide distribution in Pakistan, India, Nepal, Bhutan, China, Malaysia, Indonesia and Vietnam (Thomas, 2011; Juyal *et al.*, 2014; Pandey *et al.*, 2020).In the Indian subcontinent, it is distributed in the temperate and subalpine Himalayan regionsin Himachal Pradesh, Uttarakhand, Northern-Western States of Jammu and Kashmir, Sikkim, Assam, and Arunachal Pradeshat altitudes between 1800 and 3300 m amsl and at an altitude of 1500m amsl in the Meghalaya and Manipur hills (Karki and Gupta, 2000;Khan *et al.*, 2006;Xu *et al.*, 2009). The species, growing in cool, well drained,moist, forested habitats, has needle shaped, dark green, flat leaves with spiral arrangement on the stemsand

brilliant red berries (seed covered by arils) (Garyali *et al.*, 2014;Nazir *et al.*, 2018;Iqbal *et al.*, 2020a). Also known as 'Thuner/Thuno/Thoon' in the western Himalayas (Thakur *et al.*, 2023), this shade-tolerant species is commonly found growing in association with other coniferous and broad-leaved forest species such as *Abies pindrow*, *A. spectabilis*, *Cedrus deodara*,*Pinus wallichiana*, *Picea smithiana*, *Acer cesium*, *Rhododendron arboretum* and *Quercus semicarpifolia* forming a canopy (Rikhari *et al.*, 1998; Samant *et al.*, 2002; Joshi, 2002; Pant, 2005; Aslam *et al.*, 2009). Flowers bloom between March-April and September-November is the ripening period for the seeds (Iqbal *et al.*, 2020a).

*T. wallichiana*Zucc. has immense pharmaceuticaland ethnobotanical importance. In the traditional system of medicine, decoctions, herbal teas and juices from this plant are used for the treatment of high fever, painful inflammations, cold and cough, respiratory infections, tuberculosis and epilepsy (Ahmed *et al.*, 2004; Juyal *et al.*, 2014). Rheumatism is treated with the leaves and bark being used in steam baths and bark paste is traditionally used to treat fractures and headaches (Juyal *et al.*, 2014). In the Ayurvedic system of medicine, literature shows the use of young shoots of this plant in the form of a tincture for the treatment of headache, giddiness, feeble and falling pulse, diarrhoeaand biliousness(Khan *et al.*, 2006).'Zarnab', a drug prepared from the bark and leaves is prescribed for the treatment of asthma, bronchitis, epilepsy, snake bite and scorpion stings and is also used as a sedative and aphrodisiac in the Unani system of medicine (Purohit *et al.*, 2001).The leaves have been used in the treatment of hysteria, nervousness, as carminative and have antimalarial properties (Chauhan, 1999), while the arils have diuretic and laxative properties (Orwa *et al.*, 2009).

A highly valued medicinal tree, extracts from different plant parts of *T. wallichiana* Zucc. have shown to possess analgesic, antispasmodic, anti-inflammatory, antiallergic, anticonvulsant, antibacterial, antifungal, immunomodulatory as well as vasorelaxing effects (Chattopadhyay *et al.*, 2006; Nisar *et al.*, 2008a, b; Khan *et al.*, 2011; Qayum *et al.*,2012). The seeds, bark and needle leaves of the Himalayan yew also contain several unique bioactive taxoids in addition to paclitaxel and 10-deacetyl baccatin III (10-DAB), that is a precursor of paclitaxel. 10-DAB is also the precursor for the semi synthesis of docetaxel (Taxotere®) (Chattopadhyay *et al.*,1996), an analogue of paclitaxel (Furmanowa and Syklowska-Baranek, 2000). Paclitaxel (Taxol®: is the registered trade name of a formulated drug based on the chemical paclitaxel, first commercially developed by the pharmaceutical company Bristol-Myers Squibb Company-Princeton, NJ); a diterpene alkaloid, is a broad-spectrum antineoplastic agent, being widely used for the treatment of advanced, progressive and drug refractory ovarian cancer and breast cancer(McGuire *et al.*,1989; Einzig *et al.*, 1991;Markman, 1991; Holmes *et al.*, 1991). Since its approval for the treatment of ovarian cancer by the FDA (Food and Drug Administration, U.S.) in 1992, it has also been used for the treatment of lung, head and neck, renal, prostrate, colon, cervix, gastric and pancreatic cancers(Ettinger, 1992; Forastiere *et al.*,1993; Arbuck *et al.*,1993; Roth *et al.*,1993; Brown *et al.*,1993). Originally isolated from the Pacific yew, *T. brevifolia* Nutt., paclitaxel has a unique mode of action; it stabilizes

microtubules against depolymerisation. This unusual stability blocks the cell's ability to disassemble the mitotic spindle during cell division; cells are blocked in the G2/M phase of the cell cycle and this finally leads to cell death (Schiff *et al.,* 1978; Horowitz *et al.,* 1986). In addition to being an excellent antineoplastic agent, it is also effective against noncancerous conditions like polycystic kidney diseases (Woo *et al.,*1994) and has shown promising results in multiple sclerosis(Theodoridis *et al.*, 1999) and AIDS related Kaposi's sarcoma(Skeel, 1999). Taxol is expensive because of its high demand in chemotherapy and limited supply of the drug from its natural source.

Apart from its medicinal importance, the Himalayan yew is used for other purposes as well. Considered the hardest of all coniferous wood and resistant to rot and insect attack,the colourful wood of *T. wallichiana* Zucc.is in high demand because of its strength and durability and is used for making furniture, veneers, fancy articles, for construction of beehives, eaves, roofs, panelling, for gates, fences, ploughs, carts and also for cremation of dead bodies (Purohit *et al.,* 2001; Aslam *et al.,*2017; Iqbal *et al.,*2020a). The wood is also used in bow making, as incense in Tibet and Nepal and as a fuelwood (Dar and Dar, 2006; Kala, 2010). The gelatinous and sweet pulp of the fleshy aril, the only edible part of the tree, is used in making jams, ice creams, as well as added in cosmetics (Edward, 1998; Aslam *et al.,*2009; Pandey *et al.,*2020).

The Himalayan yew is a dioecious, slow growing tree with poor regeneration (Rikhari *et al.,* 1998; Aslam *et al.,* 2017). Seeds have a long dormancy period of 1.5-2 years and germinate slowly(Chee, 1994). Its existence is threatened because of its small population size, slow propagation, specific habitat, narrow range of distribution, habitat loss, change in climatic conditions, lack of awareness andillicit, destructive harvesting (to gather firewood for cooking and heating purposes) and grazing for leaves as forage by cattle, sheep, goat and other livestock (Samant, 1999; Pant and Samant, 2008; Iqbal *et al.,*2020a). The vegetative propagation of the plant, anatomy, physiology, behaviour are affected by harsh climatic conditions, extreme temperatures (Gegechkori, 2018). Yews are also severely affected by external factors such as forest fires and genetic isolation of wild populations (Camprodon *et al.,*2016). They do not grow in clear areas devoid of forests as young plants require dense shade and shelter (Iqbal *et al.,*2020a). In addition, to meet the growing demand of paclitaxel plant parts have been indiscriminately collected from the wild.The worldwide demand of the Taxol is 800 -1000 kg per annum (Nazir *et al.,* 2018). 50-150 mg pure Taxol is obtained from at least 1 kg of dried yew bark which is gathered by peeling the bark, damaging the plants immensely and nearly three trees, each about 60 years old, are required to get 1 g of the compound (Aslam *et al.,*2017). A reduction in the thickness of the bark to less than 0.43 cm affects both the survival and growth of this species (Purohit *et al.,* 2001). In addition, canopy destruction by lopping and overgrazing are also causing extensive damage to the plants, seriously affecting natural regeneration and survival of the species (Rikhari *et al.,*1998; Iqbal *et al.,*2020a). Even after imposing severe restrictions on removal of yew trees from forest areas by the State Forest Departments, illegal removal of barks, shoots and even trees continuepersistently.In northern India, there has been a 90 per cent decline in

*T. wallichiana*Zucc. population over the last few decades and the species has been listed as 'endangered' by the IUCN(WCMC, 2002; Thomas and Farjon, 2011; Aslam *et al.*,2017; IUCN, 2020; Gowthami *et al.*, 2021). It is also listed under CITES Appendix II (Thomas and Farjon, 2011; Gowthami *et al.*, 2021).

Thus,the conservation of this precious, endangered natural wealthand its habitat has become a matter of concernand a lot of effort has been put in the past few decades to safeguard the existence of this species; *ex situ, in situ*and *in vitro* conservation strategies have been adopted. In addition to propagation by conventional and *in vitro* techniques(Dubey, 1997; Nandi *et al.*,1996; Nandi *et al.*,1997;Mitter and Sharma, 1999;Mishra *et al.*, 2000;Saini, 2001;Khali and Sharma, 2003;Datta and Jha, 2004;Datta *et al.*, 2006;Datta and Jha, 2008;Aslam and Rather, 2008; Kaul, 2008),biotechnological approaches using cell and tissue cultures have proved to be attractive alternatives for the production of paclitaxel and 10-DAB from the Himalayan yew (Jha and Jha, 1995; Banerjee *et al.*,1996; Jha *et al.*,1998; Ghosh *et al.*,2002;Veeresham *et al.*, 2003;Das *et al.*, 2008). This review summarizes the different *ex situ, in situ*and *in vitro* techniques adopted in the last decade to conserve the Himalayan yew.

Recent *ex situ*and *in situ* approaches for conservation of the Himalayan yew

Regeneration of *Taxus* through seeds is difficult, often requiring complex treatments. It is a slow process with rapid loss of seed viability and low survival percentage (Rajewski *et al.*, 2000). Root induction is also difficult and a lengthy process in *T. wallichiana*Zucc. (Fordhamand Spraker, 1977). Clonal propagation through branch cuttings thusseems to be a practical approach for conservation, providing genetic uniformity with the original source plant and multiplication of a stock with superior characteristics in a short span of time (Aslam *et al.*, 2017). Elite plant genetic resources have been successfully conserved through adventitious root formation (ARF)(Aslam *et al.*, 2017).Previous reports indicate that *Taxus* species could be regenerated by adventitious rooting of fresh stem cuttings (Schnek, 1996; Khali and Sharma, 2003). In keeping with this view, Das and Jha, 2014, investigated the effect of shoot type (softwood, semi-hardwood and hardwood), wounding (light and severe), auxins(indole-3-butyric acid (IBA) and α-naphthalene acetic acid (NAA)) and their interactive effect on adventitious rooting from shoot cuttings of *T. wallichiana* Zucc. collected from East Khasi Hills, India. Auxins are typically used in nursery conditions to stimulate rooting (Kulenova, 2011). Higher survival rate was noted in case of less perishable semi-hardwood and hardwood cuttings (29.7% and 26.1% respectively) compared to soft wood cuttings (5.6%). Soft wood cuttings had high mortality rate because of their delicate nature, resulting in excessive water loss through transpiration and wilting. Semi-hardwood cuttings also exhibited better rooting response. The extent of wounding also had an impact on the timing of root development, with severely wounded (4-5 incisions, 2-3 cm on the basal shoot to a depth of 5-10 mm, reaching secondary xylem) shoot cuttings exhibiting faster and increased root initiation, although severely wounded softwood cuttings exhibited high mortality rate due to excessive tissue damage. Of the two auxins used, IBA induced better rooting response in comparison to NAA.

In another study, juvenile apical shoot cuttingsof *T. wallichiana* Zucc.collected from Pahalgam range of Lidder Forest Division (Jammu and Kashmir, India) were treated with different concentrations of three auxins(indole-3-acetic acid (IAA), IBA and NAA)to induce rooting (Aslam *et al.,* 2017). In conformity with the results of Das and Jha, 2014, IBA was the most effective auxin for the induction of higher number of primary roots with greater length from the juvenile branch cuttings.In a similar study, Nazir *et al.*, 2018, investigated the effect varying concentrations of these three auxins on adventitious root induction from shoot cuttings of mature Himalayan yew collected from Tangmarg region of Jammu and Kashmir in four different seasons (spring, summer, autumn and winter) under nursery conditions. Analogous to the previous results, 1000 ppm IBA showed best response in terms of rooting percentage, root length and root number in the spring season (March-May), followed by winter and autumn. Iqbal *et al.*, 2020b, also demonstrated that IBA (7000 ppm) application to stem cuttings resulted in induction of maximum number of roots with increased length, number of leaves, sprouts and higher survival percentage. These studies indicate that IBA is a suitable plant growth regulator (PGR) for the rooting of cuttings of the Himalayan yew and thus preferablefor its vegetative propagation and conservation.

Kishor *et al.*, 2015, performed an inexpensive, easy, *in situ* air layering experiment in a natural habitat of the Himalayan Yew in high altitude forests of Munsyari region of Kumaun Himalayas. The stimulatory effect of root formation of various auxins (IAA, IBA, NAA) was studied in air layered shoots by applying the auxins with soil paste and cotton plugs on girdled shoots of healthy plants. As demonstrated before, maximum rooting was induced by 1000 µM IBA. Well rooted clonal plants raised through the technique were successfully established both *in situ* and *ex situ*.

Research in plant-microbe interaction has proved that root microbiome activity helps in establishing a host plant species in an ecological niche (Pandey *et al.,* 2020). The root microbiome assists in the uptake of nutrients by the host plant and protects it against biotic and abiotic stresses (Bakker *et al.,* 2018). Reports indicate that the Himalayan yew, at various forest sites in the Indian Himalayan Region, is colonized by arbuscular mycorrhizae, with *Glomus* as the dominating genus (Chaurasia *et al.,* 2004) and a mutually beneficial symbiotic relation has been observed between the host, *T. wallichiana*Zucc. and these microbes (Pandey *et al.,* 2020). In an interesting study, Pandey *et al.,* 2020, tried to propagate *T. wallichiana*Zucc. at the nursery stage utilizing root-associated microbiome. Collected from Uttarakhand, India, root samples of the Himalayan yew with heavy microbial colonization were crushed and stem-cuttings were inoculated with the root-microbe mixture. A rhizosphere developed within a year and well-established *T. wallichiana* Zucc. plants were transferred to the field.

Endophytes recently isolated from the Himalayan yewas alternative sources of Taxol

Traditional methods of extraction of Taxol from the bark of yew trees are not only inefficacious but also cause destruction of the natural source with low yields of the drug in pure form (Garyali *et al.,* 2014). Also, semi synthesis

using Taxol precursors is insufficient to meet the global demand (Kumar *et al.*, 2019). Thus,several alternative strategies for production of Taxol have been developed. A newly developed technology, microbial fermentation, has emerged as an alternative, economically feasible approach with higher productivity of Taxol (Kumar *et al.*, 2019).Specifically, isolation and identification of Taxol producing endophytic fungi is a prospective approach for large scale production of Taxol.High growth rate coupled with short generation time make these fungi suitable as alternative Taxol producers. Although several endophytic microorganisms (species of *Alternaria, Aspergillus, Fusarium, Cladosporium, Monochaetia,Ozonium, Pestalotiopsis, Pithomyces, Taxomyces, Tubercularia* etc.) have been reported to produce Taxol (Stierle *et al.*, 1993; Gangadevi and Muthumary, 2008; Zhao *et al.*, 2008, 2009;Zhou *et al.*, 2010), strains isolated from yew species producing paclitaxel are few(Wang *et al.*, 2000;Yijian *et al.*, 2003).

Garyali *et al.*, 2014, investigated the endophytic fungal diversity of Taxol-producing endophytes from bark samples of the Himalayan yewcollected from different locations of northern Indian Himalayan region (Jammu and Kashmir, Himachal Pradesh and Uttarakhand). Of the 60 fungal endophytes isolated from the inner bark of the Himalayan yew, five species showed amplification of a 530-bp fragment of the *bapt* gene (C-13 phenylpropanoid side chain-CoA acyltransferase, involved in Taxol biosynthesis) and sequence analysis confirmed the Taxol biosynthetic capacity of all the fungal strains. The five fungal isolates were identified as belonging to the phylum Ascomycota based on morphological and unique phenotypic characters. Among the five Taxol producing strains, *Fusarium redolens* yielded the maximum amount of Taxol. The antimitogenic effect of the fungal Taxol was proved by the inhibition of tumour formation in potato discs. In a similar attempt, Zaiyou *et al.*, 2015, isolated 435 endophytic fungal strains from surface sterilized bark of *T. wallichiana* var. *mairei*, collected from Taihang Mountain in Henan Province of China, out of which only one strain belonging to the genus *Fusarium* produced paclitaxel. In a subsequent study, Zaiyou *et al.*, 2017, isolated 528 endophytic fungal strains from the same variety of *Taxus*and a unique strain efficiently producing paclitaxel was identified as *Phoma medicaginis*, a group producing ample secondary metabolites including antibiotics.

In an attempt to isolate a hyper Taxol producing endophyte having the potential of industrial application, Kumar *et al.*, 2019, isolated 34 endophytes from bark, stem and needles of *Taxus* collected from Shimla, Himachal Pradesh (India). Molecular screening confirmed that one of the fungal isolates was positive for *dbat* (10-deacetylbaccatin III-10-O-acetyl transferase, essential for Taxol biosynthesis)and *ITS* (Internal Transcribed Spacer, universal DNA barcode marker for fungal identification) genes, which was characterized and identified as *Aspergillus fumigatus.*Taxol was detected and quantified by HPLC and characterized by Thin Layer Chromatography (TLC), Ultraviolet (UV) spectroscopy, Mass spectrometry (MS), Fourier-Transform Infrared Spectroscopy (FTIR) and Nuclear Magnetic Resonance (NMR) spectroscopy. Yield of Taxol was 1.60 g/L after microbial fermentation, claimed by the authors to be the highest production from an

endophytic fungus, thus making *A. fumigatus* a potential strain for commercial production of Taxol.

***In vitro*approaches for conservation of the Himalayan yew**

Plant *in vitro* culture or plant tissue culture (PTC) is an efficient technique for rapid growth and proliferation of plant cells, tissues and organs under defined physical and chemical conditions, aseptically. The technique is extensively used for the conservation of elite germplasm, by taking advantage of totipotency of plant cells. In contrast to field grown plants, which have a limited lifespan and are susceptible to various diseases and stresses, plant *in vitro* cultures can be propagated disease-free indefinitely and rapidly, free from seasonal and geographic barriers and are easily available on demand (Doran, 2009).PTC has remained a method of choice for the conservation of various endangered, rare and threatened plant species which are slow growing, less abundant in nature and have recalcitrant seeds.The technique of PTC also allows *de novo* organ development *via* organogenesis or somatic embryogenesis from mature plant cells by manipulating the combination and concentration of PGRs in the culture medium (Halder *et al.*, 2021). Independent of plant developmental barriers, biotechnological approaches using plant cell and organ cultures also appear to be attractive alternatives for the production ofdiverse commercially as well as pharmaceutically important plant secondary metabolites, reducing the pressure on natural habitat.

As mentioned, successful efforts have been made before to conserve the Himalayan yew through various *in vitro* techniques (callogenesis, organogenesis, embryogenesis, establishment of cell suspension cultures etc.). Hussain *et al.*, 2013, used Murashige and Skoog's (1962) (MS) medium supplemented with various PGRs (2,4-dichlorophenoxy acetic acid (2,4-D), NAA, IBA, kinetin) alone or in combination with activated charcoal to induce callus from stem and leaf explants of *T. wallichiana* Zucc. collected from Hattar, Pakistan. The best response was obtained from stem explants on medium supplemented with 2 mg/L 2, 4-D along with 5 mg/L activated charcoal, although the induced callus failed to regenerate shoots even after supplementation with BAP, kinetin and IBA. When shoot tip meristems were cultured on MS medium supplemented with BAP and IBA, they elongated and roots were successfully induced from such elongated shoot tips on full or half strength MS medium supplemented with IBA. In Vietnam, Nhut *et al.*, 2014, studied the effect of light emitting diodes (LEDs) on callus induction, growth and paclitaxel content of needle and petiole-derived calli of *T. wallichiana* Zucc.For callus induction, the authors used B5 medium (Gamborg *et al.*, 1968)supplemented with various concentrations of 2,4-D, with or without kinetin, under LEDs in different red:blue ratios (100:0, 75:25, 50:50, 25:75, 0:100). Callus was induced from both the types of explants with the petioles showing better response and 100% blue LEDs proved to be the best for callus induction and growth. Analysis of paclitaxel and 10-DAB by high performance liquid chromatography (HPLC) from the calli showed that there were differences in contents between needle and petiole induced calli. Although calli induced from petioles showed better growth, in terms of productivity, the needle derived calli showed higher paclitaxel content and 10-

DAB was also detected only from the needle derived calli of the Himalayan yew.

In yet another study, callus was induced from leaf explants of *T. wallichiana* on MS medium supplemented with 2,4-D, kinetin and BAP (6-benzylaminopurine) (Gul *et al.*,2020). Shoots regenerated on medium supplemented with IAA in combination with BAP. However, no rooting was observed. Mujib *et al.*, 2020,collected newly grown twigs of *T. wallichiana* Zucc. from Jammu and Kashmir provinces of India and leaves, stems and miniature cone explants were tested for their ability to induce callus on MS medium supplemented with 2,4-D, NAA and BAP. Stem explants were more responsive in inducing callus on 2,4-D and NAA supplemented medium. Taxol yield, as quantified by high-performance thin layer chromatography (HPTLC), was also higher in callus tissues cultured on 2,4-D containing medium.The authors concluded that 2,4-D functioned as a signalling element and a stressor, as indicated by high levels of antioxidant enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX) and the level of proline, in calli grown on 2,4-D supplemented medium.

Gauchan *et al.*, 2021, inoculated needle leaves and young stem explants of the Himalayan yew on B5 medium supplemented with 2,4-D and NAA for callus induction. Media containing 2,4-D proved to be the best for callus induction compared to media containing NAA. The authors studied the effect of different parameters (*viz.*explant source, sucrose concentration, sugar source, phytohormone concentration, inoculum volume and macronutrient concentration) on cell suspension cultures established with fully developed friable, viable calli. In suspension, calli induced from needles exhibited better cell growth than the ones from stems. Although a higher concentration of sucrose gave better results,fructose gave the best result in terms of cell count when different carbon sources (fructose, sucrose, glucose, xylose and mannitol) were used. Also, 2mg/L 2,4-D and 5 mg/L NAA gave the best cell count and viability. An inoculum volume of 20ml produced the highest growth rate in suspension culture. Changes in the concentration of different macronutrients of the B5 medium showed that the amounts maintained in the basal medium are sufficient for cell division. Extra addition of macronutrients other than magnesium inhibited growth of cells. Taxol could be detected by HPLC analysis both from the calli as well as cell suspension cultures. The authors also studied genetic relationships among *T. wallichiana* population samples collected from 11 sites of Nepal using random amplified polymorphic DNA (RAPD) technique.

'Hairy root culture' is an interesting form of culture. In higher plants, the hairy root syndrome is characterized by the development of adventitious roots with profuse root hairs at or next to the site of infection by a soil borne bacterium known as *Agrobacterium rhizogenes*(Sevon and Oksman-Caldentey, 2002).Following infection of wound sites in plants by *A. rhizogenes*, certain genes (the transferred DNA or T-DNA genes) are transferred from the bacterium to the plant, which get stably integrated in the plant genome and are expressed, resulting in the development of the hairy root

phenotype. These roots are highly branched, plagiotropic, capable of rapid growth on phytohormone free culture medium, remain genetically and biochemically stable over extended periods (Sevon and Oksman-Caldentey, 2002). Often referred to as 'phytochemical factories', these roots can biosynthesize higher or analogous amounts of compounds which are naturally produced by the roots of the mother plant (Lorence *et al.,* 2004;Halder *et al.*, 2021).As an alternative for the production of Taxol, *A. rhizogenes* was used to induce hairy roots from *T.baccata* subsp. *wallichiana* explants using acetosyringone (a phenolic compound) and low frequency sonication (Sahai and Sinha, 2021). Biomass of the hairy roots was further increased in root induction media without acetosyringone. The cultures produced Taxol, thereby paving a way for large scale production of the compound, bypassing destruction of this immensely important plant species from the wild.

Conclusion

The Himalayan yew or *T. wallichiana* Zucc. is a plant of immense medicinal importance, particularly because of the presence of the antineoplastic agent,paclitaxel, and its precursor, 10-DAB. As mentioned, both natural as well as anthropogenic factors have led to a decline of the species in its natural habitat, resulting in its categorization as 'endangered' by the IUCN. So various *ex situ*, *in situ* and *in vitro* conservation measures have been adopted by scientists and researchers over the past few decades to protect this species from being extinct. The present literature survey summarized the conservation strategies formulated and implemented in the last decade to protect and make sustainable use of the Himalayan yew.

References

Ahmed, E., Arshad, M., Ahmad, M., Saeed, M. and Ishaque, M. 2004. Ethnopharmacological survey of some medicinally important plants of Galliyat Areas of NWFP, Pakistan. *Asian J7. Plant Sci.*3: 410-415.

Arbuck, G., Christian, M.C., Fisherman, J.S., Cazenave, L.A., Sarosy, G., Suffness, M., Adams, J., Canetta, R., Cole, K.E. and Friedman, M.A. 1993. Clinical development of Taxol. *J. Natl. Cancer Inst. Monograph.* 15: 11-24.

Aslam, M., Ali, M., Reshi, Z.A. and Siddiqi, T.O. 2009. Status of Himalayan yew (*Taxus baccata* Linn.) in a mixed coniferous forest of the Kashmir Himalaya. *Natl. J. Life Sci*. 6: 55-61.

Aslam, M., Raina, P.A., Rafiq, R.U., Siddiqi, T.O. and Reshi, Z.A. 2017. Adventitious root formation in branch cuttings of *Taxus wallichiana* Zucc. (Himalayan yew): A clonal approach to conserve the scarce resource. *Curr. Bot.* 8: 127-135.

Aslam, M. and Rather, M.S. 2008. Macro-propagation of *Taxus baccata* Linn: a novel method for conserving a critically endangered medicinal plant. *Indian For*. 134: 1058-1066.

Bakker, P.A.H.M., Pieterse, C.M.J., de Jonge, R. and Berendsen, R.L. 2018. The soil-borne legacy. *Cell*.172: 1178-1180.

Banerjee, S., Upadhyay, N., Kukreja, A.K., Ahuja, P.S., Kumar, S., Saha, G.C., Sharma, R.P. and Chattopadhyay, S.K. 1996. Taxanes from *in vitro* cultures of the Himalayan Yew *Taxus wallichiana*. *Planta Med.*62: 333-335.

Brown, T., Tangen, C., Flemming, T. and Macdonald, J. 1993. A phase II trial of taxol and granulocyte colony stimulating factor (G-CSF) in patients with adenocarcinoma of pancreas. *Proc. Am. Soc. Clin. Oncol.*12 (abstracts). Pp. 200.

Camprodon, J., Guixé, D., Casals, P., Caritat, A., Buqueras, X., García-Martí, X., Reverté, J., Rios, A.I., Beltrán, M., Llovet, J., Taüll, M., Vives, A., À guila, V. and Casas, C. 2016. Conservation of Mediterranean yew forests. Best practice handbook. Life TAXUS Project. Forest Science Centre of Catalonia.

Chattopadhyay, S.K., Pal, A., Maulik, P.R., Kaur, T., Garg, A. and Khanuja, S.P. 2006. Taxoid from the needles of the Himalayan yew *Taxus wallichiana* with cytotoxic and immunomodulatory activities. *Bioorg. Med. Chem. Lett.*16: 2446-2449.

Chattopadhyay, S.K., Saha, G.C., Sharma, R.P., Kumar, S. and Roy, R. 1996. A rearranged taxane from the Himalayan Yew *Taxus wallichiana*. *Phytochem.*42: 787-788.

Chauhan, N.S. 1999. *Medicinal and Aromatic Plants of Himachal Pradesh*. 1st ed. New Delhi: Indus Publishing Company New Delhi, India.

Chaurasia, B., Pandey, A. and Palni, L.M.S. 2004.*Occurrence of arbuscular mycorrhizae in the rhizosphere of Himalayan Yew* (*Taxus baccata* L. subsp. *wallichiana* (Zucc.) Pilger)—*a case study*. Podila, G.K. and Varma,A.K. (eds.),Basic research and applications of Mycorrhizae. IK International Pvt Ltd, New Delhi.Pp. 25-35.

Chee, P.P. 1994. In vitro culture of zygotic embryos of *Taxus* species. *Hort. Sci.* 29: 695-697.

Dar, A.R. and Dar, G.H. 2006. The wealth of Kashmir Himalaya gymnosperms. *Asian J. Plant Sci.* 5: 251-259.

Das, K., Dang, R., Ghanshala, N. and Rajasekharan, P.E. 2008. *In vitro* establishment and maintenance of callus of *Taxus wallichiana* Zucc. for the production of secondary metabolites. *Nat. Prod. Radiance*.7: 150-153.

Das, S. and Jha, L.K. 2014. Effect of wounding and plant growth regulators (IBA and NAA) on root proliferation of *Taxus wallichiana* shoot cuttings. *Res. J. Agri. Forest Sci.*2: 8-14.

Datta, M.M. and Jha, S. 2004. Embryo culture of *Taxus wallichiana* (Zucc.). *J. Plant Biotechnol.*6: 213-220.

Datta, M.M. and Jha, S. 2008. Plant regeneration through somatic embryogenesis in *Taxus wallichiana. J. Plant Biochem. Biotechnol*. 17: 37-44.

Datta, M., Majumder, A. and Jha, S. 2006. Organogenesis and plant regeneration in *Taxus wallichiana* (Zucc.). *Plant Cell Rep*. 25: 11-18.

Doran, P.M. 2009. Application of plant tissue cultures in phytoremediation research: Incentives and limitations. *Biotechnol. Bioeng*. 103: 60-76.

Dubey, K.P. 1997. Himalayan yew (*Taxus baccata*) conservation: A vegetative approach. *Indian For.*123: 1150-1154.

Edward, E.A. 1998. *Taxaceae: The Genera and cultivated species*. L.H. Bailey (ed.),The Botanical Review. Vol.64. New York: Hortorium Cornell University Ithaca.

Einzig, A.I., Wiernik, P.H. and Schwartz, E.L. 1991. Taxol: a new agent active in melanoma and ovarian cancer. *Cancer Treat. Res.*58: 89-100.

Ettinger, D.S. 1992. Taxol in the treatment of lung cancer., In: Abstracts of Second National Cancer Institute Workshop on Taxol and Taxus, Alexandria, Virginia, 23-24 September.

Forastiere, A.A., Neuberg, D., Taylor, S.G., DeConti, R. and Adams, G. 1993. Phase II evaluation of taxol in advanced head and neck cancer: an Eastern Cooperative Oncology Group Trial. *J. Natl. Cancer Inst. Monographs.* 15: 181-184.

Fordham, A.J. and Spraker, L.S. 1977. Propagation manual of selected gymnosperms. *Arnoldia*. 37: 1-88.

Furmanowa, M. and Syklowska-Baranek, K. 2000. Hairy root cultures of *Taxus* x *media* var. *Hicksii* Rehd. as a new source of paclitaxel and 10-deacetylbaccatin III. *Biotechnol. Lett.*22: 683-686.

Gamborg, O.L., Miller, R.A. and Ojima, K. 1968. Nutrient requirements of suspension cultures of soyabean root cells. *Exp. Cell Res.*50: 151-158.

Gangadevi, V. and Muthumary, J. 2008. Taxol, an anticancer drug produced by an endophytic fungus *Bartalinia robillardoides*, Tassi, isolated from a medicinal plant, *Aegle marmelos* Correa ex Roxb. *World J. Microbiol. Biotechnol.*24: 717-724.

Garyali, S., Kumar, A. and Reddy, M.S. 2014. Diversity and antimitotic activity of taxol-producing endophytic fungi isolated from Himalayan yew. *Ann. Microbiol*. 64: 1413-1422.

Gauchan, D.P., Bhuju, S., Lamichhane, J., Shakya, R. and García-Gil, M.S. 2021. Establishment of regenerative callus, cell suspension system, and molecular characterization of *Taxus wallichiana* Zucc. for the in vitro production of Taxol. *J. Appl. Pharm. Sci*. 11: 022-034.

Gegechkori, A. 2018. Patterns of distribution and survival of European yew (*Taxus b*accata L.) in an alpine tree line ecotone in the Greater Caucasus (Georgia). *Ann. Agrarian Sci.*16: 170-176.

Ghosh, B., Mukherjee, S., Jha, T.B. and Jha, S. 2002. *Taxol production in untransformed cell cultures of* Taxus wallichiana. Nandi, S.K., Palni, L.M.S. and Kumar,A. (eds.),Role of plant tissue culture in biodiversity conservation and economic development.Gyanodaya Prakashan, Nainital, India, Pp. 287-295.

Gowthami, R., Sharma, N., Pandey, R. and Agarwal, A. 2021. Status and consolidated list of threatened medicinal plants of India. *Genet. Resour. Crop Evol*. 68: 2235-2263.

Gul, N., Baig, S., Ahmed, R., Shahzadi, I., Zaman, I., Shah, M.M. and Baig, A. 2020.Conservation of an endangered medicinal tree species *Taxus wallichiana* through callus induction and shoot regeneration. *Plant Tissue Cult.& Biotech*. 30: 161-166.

Halder, M., Majumder, A., Ray, S. and Jha, S. 2021. *Medicinal plant research at crossroads: Biotechnological approaches for conservation, production and stability in tissue cultures and regenerated plants.* Ekiert, H.M.,Ramawat,K.G. and Arora,J. (eds.),Medicinal Plants. Sustainable Development and Biodiversity, vol 28. Springer, Cham. Pp. 459-544.

Holmes, F.A., Walters, R.S., Theriault, R.L., Forman, A.D., Newton, L.K., Raber, M.N., Buzdar, A.U., Frye, D.K. and Hortabagyi G.N. 1991. Phase II trial of taxol, an active drug in the treatment of metastatic breast cancer. *J. Natl. Cancer Inst.* 83: 1797-1805.

Horowitz, S.B., Lothsteia, L., Manfredi, J.J., Mellado, W., Parness, J., Roy, S.N., Schiff, P.B., Sorbara, L. and Zeheb, R. 1986. Taxol: Mechanism of action and resistance. *Ann. N. Y. Acad Sci*. 466: 733-743.

Hussain, A., Qarshi, I.A., Nazir, H., Ullah, I., Rashid, M. and Shinwari, Z.K. 2013. *In vitro* callogenesis and organogenesis in *Taxus wallichiana* Zucc. The Himalayan yew. *Pak. J. Bot*. 45: 1755-1759.

Iqbal, J., Meilan, R. and Khan, B. 2020a. Assessment of risk, extinction, and threats to Himalayan yew in Pakistan. *Saudi J. Biol. Sci*. 27: 762-767.

Iqbal, J., Khan, B., Khan, S., Ghaffar, N., Mian, I.A., Yousaf, N., Ahmad, I. and Manzoor, S. 2020b. Effect of hormones (IBA & IAA) on the propagation of Himalayan Yew in Pakistan: A conservation approach. *BMC Plant Biol.*doi.org/10.21203/rs.3.rs-27841/v1.

IUCN. 2020. The IUCN red list of threatened species. Version 2020–1. https://www.iucnredlist.org.

Jha, S. and Jha, T.B. 1995.A fast growing cell line of *Taxus baccata* L. (Himalayan yew) as a potential source of taxol precursor. *Curr. Sci.*69: 971-972.

Jha, S., Sanyal, D., Ghosh, B. and Jha, T.B. 1998. Improved taxol yield in cell suspension culture of *Taxus wallichiana* (Himalayan yew). *Planta Med.*64: 270-272.

Joshi, H.C. 2002. Assessment of habitat diversity, forest vegetation and human dependence in the buffer zone of Nanda Devi Biosphere Reserve of west Himalaya. *Int. J. Sustain. Develop., World Ecol.*11: 326-336.

Juyal, D., Thawani, V., Thaledi, S. and Joshi, M. 2014. Ethnomedical properties of *Taxus wallichiana* Zucc. (Himalayan Yew). *J. Tradit. Complement. Med*. 4: 159-161.

Kala, C.P. 2010. *Medicinal plants of Uttarakhand; Diversity, Livelihood and conservation.* Biotech Books, New Delhi.

Karki, M.M.S. and Gupta V.K. 2000. Natural product innovations: Taxol. *J. Intellect. Prop. Rights.*5: 206-210.

Kaul, K. 2008. Variation in rooting behaviour of stem cuttings in relation to their origin in *Taxus wallichiana* Zucc. *New For*. 36: 217-224.

Khali, R.P. and Sharma, A.K. 2003. Effect of phytohormones on propagation of Himalayan Yew (*Taxus baccata* L.) through stem cuttings. *Indian For.*129: 289-294.

Khan, I., Nisar, M., Shah, M.R., Shah, H., Gilani, S.N., Gul, F., *et al*. 2011. Anti-inflammatory activities of Taxusabietane A isolated from *Taxus wallichiana* Zucc. *Fitoterapia.*82: 1003-1007.

Khan, M., Verma, S.C., Srivastava, S.K., Shawl, A.S., Syamsundar, K.V., Khanuja, S.P.S. and Kumar, T. 2006. Essential oil composition of *Taxus wallichiana* Zucc. from the Northern Himalayan region of India. *Flavour Frag. J*. 21: 772-775.

Kishor, K., Upreti, B.M., Pangtey, Y.P.S., Tewari, A. and 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.* 4:1064-1067.

Kulevnova, S. 2011. Important medicinal and aromatic plants in South-East Europe in relation with their medicinal and other industrial utilization: Republic of Mecidonia Institute of organic chemistry with centre of phytochemistry, BAS, Sofia, Bulgaria.

Kumar, P., Singh, B., Thakur, V., Thakur, A., Thakur, N., Pandey, D. and Chand, D. 2019. Hyper-production of taxol from *Aspergillus fumigatus*, an endophytic fungus isolated from *Taxus* sp. of the Northern Himalayan region. *Biotechnol. Rep*. 24. doi.org/10.1016/j.btre.2019.e00395.

Lorence, A., Medina-Bolivar, F., Nessler, C. L., 2004. Camptothecin and 10- hydroxycamptothecin from *Camptotheca acuminata* hairy roots. *Plant Cell Rep*. 22: 437-441.

Markman, M. 1991. Taxol: an important new drug in the management of epithelial ovarian cancer. *Yale J. Biol. Med*. 64 : 583-590.

McGuire, W.P., Rowinsky, E.K., Rosenshein, N.B., Grumbine, F.C., Ettinger, D.S., Armstrong, D.K. and Donehower, R.C. 1989. Taxol: A unique antineoplastic agent with significant activity in advanced ovarian epithelial neoplasms. *Ann. Intern. Med*. 11: 273-279.

Mishra, A., Singh, A., Kukreja, A.K. and Patra, N.K. 2000. Adventitious root induction in stem cuttings of *Taxus baccata* Linn. for ex-situ cultivation. *J. Non Wood Forestry Prod.* 12: 63-66.

Mitter, H. and Sharma, A. 1999. Propagation of *T. baccata* Linn. by stem cuttings. *Indian For*. 125: 159-162.

Mujib, A., Tonk, D., Gulzar B., Maqsood, M. and Ali, M. 2020. Quantification of taxol by high performance thin layer chromatography in *Taxus wallichiana* callus cultivated *in vitro. Biotechnologia.* 101: 337-347.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *PlantPhysiol.*15: 473-497.

Nandi, S.K., Palni, L.M.S. and Rikhari, H.C. 1996. Chemical induction of adventitious root formation in *Taxus baccata* cuttings. *Plant Growth Regul*. 19: 11-22.

Nandi, S.K., Rikhari, H.C., Nadeem, M. and Palni, L.M.S. 1997. Clonal propagation of *Taxus baccata* Linn. Himalayan asset under threat. *Physiol. Mol. Biol. Plants.* 3: 15-24.

Nazir, N., Kamili, A.N., Shah, D. and Zargar, M.Y. 2018. Adventitious rooting in shoot cuttings of *Taxus wallichiana* Zucc., an endangered

medicinally important conifer of Kashmir Himalaya. *Forest Res.*7.doi: 10.4172/2168-9776.1000221.

Nhut, D.T., Nguyen, P.L.H., Don, N.T., Hien, N.T.T., Huy, N.P., Nam, N.B., Vinh, B.T. and Luan, T.C. 2014. Induction, growth and paclitaxel content of needle and petiole-derived calli in Himalayan yew (*Taxus wallichiana* Zucc.) under light-emitting diodes. *Acta Biol. Crac. Ser. Bot.*56: 107-114.

Nisar, M., Khan, I., Simjee, S.U., Gilani, A.H., Obaidullah and Perveen, H. 2008a. Anticonvulsant, analgesic and antipyretic activities of *Taxus wallichiana* Zucc. *J. Ethnopharmacol.*116:490-494.

Nisar, M., Khan, I., Ahmad, B., Ali, I., Ahmad, W. and Choudhary, M.I. 2008b. Antifungal and antibacterial activities of *Taxus wallichiana* Zucc. *J. Enzyme Inhib. Med. Chem*. 23: 256-260.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. and Simons, A. 2009. Agroforestry Database: A Tree Reference and Selection Guide. Available from: http://www.worldagroforestry.org/af/ treedb.

Pandey, A., Adhikari, P., Dhyani, A. and Palni, L.M.S. 2020. Use of root microbiome in conservation of Himalayan Yew (*Taxus wallichiana* Zucc.): An ecofriendly approach. *Natl. Acad. Sci. Lett*. 44: 249-251.

Pant, S. 2005. *Assessment of plant diversity and ethnobotany of Mornaula reserve forest in Kumaon, West Himalaya*. Doctoral dissertation, Ph. D. Thesis. Kumaon University, Nainital.

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. *J Mountain Sci.*5: 257-264.

Purohit, A., Maikhuri, R.K., Rao, K.S. and Nautiyal, S. 2001. Impact of bark removal on survival of *Taxus baccata* L. (Himalayan yew) in Nanda Devi Biosphere Reserve, Garwhal Himalaya, India. *Curr. Sci.*81: 586-90.

Qayum, M., Nisar, M., Shah, M.R., Adhikari, A., Kaleem, W.A., Khan, I., *et al.* 2012. Analgesic and antiinflammatory activities of taxoids from *Taxus wallichiana* Zucc. *Phytother. Res.*26: 552-556.

Rajewski, M., Lange, S. and Hattemer, H.H. 2000. Problems of reproduction in the genetic conservation of rare tree species: The examples of common yew (*T. baccata*). *Forest Snow Landsc. Res.*75: 251-266.

Rikhari, H.C., Palni, L.M.S., Sharma, S. and Nandi, S.K. 1998. Himalayan yew: stand structure, canopy damage, regeneration and conservation strategy. *Environ. Conserv*. 25: 334-341.

Roth, B.J., Yep, B.Y., Wilding, G., Kasimes, B., McLeod, D. and Loehrer, P.J. 1993. Taxol in advanced hormone refractory carcinoma of the prostrate: a phase II trial of the Eastern Cooperative Oncology Group. *Cancer*. 72: 2457-2460.

Sahai, P. and Sinha, V.B. 2022. Development of hairy root culture in *Taxus baccata* subsp. *wallichiana* as an alternative for increased Taxol production. *Mater: Today: Proc.* 49: 3443-3448.

Samant, S.S. 1999. *Diversity, nativity, and endemism of vascular plants in a part of Nanda Devi Biosphere Reserve in west Himalaya I*. Himalayan Biosphere Reserve, Biannual Bull. 1 (1 & 2): 1-28.

Samant, S.S., Joshi, H.C., Arya, S.C. and Pant, S. 2002. *Studies on the structure, composition, and changes of the vegetation in Nanda Devi Biosphere Reserve of West Himalaya.* Biosphere Reserves in India and their Management. New Delhi, Kerala Forest Research Institute and Ministry of Environment and Forests. Pp. 133-139.

Saini, R.P. 2001. Vegetative propagation in Silviculture (Hills) Division, Darjeeling (West Bengal). *Indian For*. 127: 389- 408.

Schiff, P.B., Fant, J., Auster, L.A. and Horowitz, S.B. 1978. Effects of taxol on cell growth and *in vitro* microtubule assembly. *J. Supramol. Struct.* 8: 328.

Schneck, V. 1996. Studies on influence of clone on rooting ability and rooting quality in the propagation of cuttings from 40 to 350-year-old *Taxus baccata* L. ortets. *Silvae Genet*. 45: 246-249.

Sevon, N. and Oksman-Caldentey, K.M. 2002. *Agrobacterium rhizogenes*-mediated transformation: root cultures as a source of alkaloids. *Planta Med.*68: 859-868.

Skeel, R.T. 1999. *Handbook of Chemotherapy* (5th ed.) Lippincott, Williams and Wilkins, Baltimore.

Stierle, A., Strobel, G.A. and Stierle, D. 1993. Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew. *Science*.260: 214-216.

Thakur, K., Chaudhary, J. and Thakur, R. 2023. *Taxus wallichiana:* endangered medicinal plant of Seraj valley in Himachal Pradesh. *Just Agriculture*: 43-44

Theodoridis, G., Laskaris, G. and Verpoorte, R. 1999. HPLC analysis of taxoids in plants and plant cell tissue culture. *Am. Biotechnol. Lab*. 17: 40-44.

Thomas, P. 2011. A review of the distribution and conservation status of *Taxus* in the Himalaya, China and Southeast Asia. *Span. J. Rural Dev.*2: 35-42.

Thomas, P. and Farjon, A. 2011. *Taxus wallichiana. The IUCN Red List of Threatened Species 2011*: e.T46171879A9730085. http://dx.doi.org/10.2305/IUCN.UK.2011-2.RLTS.T46171879A9730085.en

Veeresham, C., Mamatha, R., Prasad Babu, Ch., Srisilam, K. and Kokate, C.K. 2003. Production of Taxol and its analogues from cell cultures of *Taxus wallichiana*. *Pharm. Biol*. 41: 426-430.

Wang, J., Li, G., Lu, H., Zheng, Z., Huang, Y. and Su, W. 2000. Taxol from *Tubercularia* sp. strain TF5, an endophytic fungus of *Taxus mairei*. *FEMS Microbiol. Lett*. 193: 249-253.

WCMC. 2002. World Conservation Monitoring Centre. www.wcmc.org.uk/trees/trade/tax_wal.htm

Woo, D.D.l., Miao, S.Y.P., Pelayo, J.C. and Woolf, A.S. 1994. Taxol inhibits progression of congenital polycystic kidney disease. *Nature.* 368: 750-753.

Xu, J., Grumbine, R.E., Shrestha, A., Eriksson, M., Yang, X., Wang, Y.U.N. and Wilkes, A. 2009. The melting Himalayas: cascading effects of

climate change on water, biodiversity, and livelihoods. *Conserv. Biol.*23: 520-530.

Yijian, C., Zhuo, Z., Yan, W., *et al.* 2003. Screening endophytic fungus to produce taxol from *Taxus yunnanensis*. *Biotechnol*. 13:10-11.

Zaiyou, J., Hongsheng, W., Ning, W., Li, M. and Guifang, X. 2015. Isolation and identification of an endophytic fungus producing paclitaxel from *Taxus wallichiana* var. *mairei*. *Nutr. Hosp.* 32: 2932-2937.

Zaiyou, J.M.D., Meng Li, B.S. and Hu Xiqiao, M.S. 2017. An endophytic fungus efficiently producing paclitaxel isolated from *Taxus wallichiana* var. *mairei*. *Medicine.* 96: 27-30.

Zhao, K., Ping, W., Li, Q., Hao, S., Zhao, L., Gao, T. and Zhou, D. 2009. *Aspergillus niger* var. *taxi*, a new species variant of taxol-producing fungus isolated from *Taxus cuspidata* in China. *J. Appl. Microbiol*. 107: 1202-1207.

Zhao, K., Zhao, L.F., Jin, Y., Wei, H.X., Ping, W.X. and Zhou, D.P. 2008. Isolation of a taxol-producing endophytic fungus and inhibiting effect of the fungus metabolites on HeLa cell. *Mycosystema*5: 210- 217.

Zhou, X., Zhu, H., Liu, L., Lin, J. and Tang, K. 2010. Recent advances and future prospects of taxol-producing endophytic fungi. *Appl. Microbiol. Biotechnol*. 86: 1707-1717.