

# Comprehensive review on origin, distribution, micropropagation and agronomical practices of *Gymnema sylvestre* R. Br

## Abstract

*Gymnema sylvestre* (Retz.) R.Br. ex Sm. is a botanical that belongs to the Apocynaceae family and occurs throughout Asia, Africa, and Australia. This herb is used in several traditional medicine practices for a wide range of ailments, including in Ayurveda, for its potential to reduce blood glucose levels. It is used as a pharmacological ingredient, mainly for the presence of bioactive phytochemicals such as gurmarin, gymnemic acid, and gymnemasaponins, which have been shown to lower glucose levels. *Gymnema sylvestre* is also recognized as an important nutritional supplement for its antioxidant, antibacterial, anti-inflammatory, antiviral, gastro- and hepatoprotective, anti-cancer, and lipid-lowering properties. The main objective of this study is to have a comprehensive review of *Gymnema sylvestre* on origin and distribution, germplasm availability, a package of practices and post-harvest practices to increase the area under cultivation of this herb. Despite having a well-developed package of practices, this herb is rarely cultivated and nearly 80% of the required quantities are being collected from wild sources, which will lead to extinction. This study will provide an unabridged repository of references regarding the species for its effective and safe utilization as a "potential medicinal herb" for creating awareness on the use of plant-based medicine.

Keyword: Botany, Origin, Cultivation, Organic, Phytohormone and Propagation

## 1 Introduction

“More than 80% of the world’s population uses natural medicines and depends on medicinal plants for health care. In recent years, the growing demand for herbal products has led to a quantum jump in volume of plant materials traded within and across the countries. At present, 90% collection of herbal raw drugs used in the manufacture of Ayurveda, Siddha, Unani, and Homeopathy systems of medicine is largely from the wild out of which 70% collection involves destructive harvesting. Due to this spurt, medicinal plants are being overexploited and many of them are pushed to the brink of extinction” (Gowthami *et al.* 2021)[16]. Many medicinal plants are highly sensitive to the level of harvest and fragility of the ecosystem; one of them is Madhunashini (*Gymnema sylvestre* R. Br.) commonly known as Australian cow plant, small Indian Ipecaunha or periploca of the woods in English and ‘Gudmar’ in Hindi. It has been mentioned in literature like Ayurveda and Sushruta Sahitya to cure diabetes (glycosuria) and other urinary disorders. It neutralises the excess sugar in the body that is present in diabetes mellitus. When its fresh leaves are chewed in the mouth, it neutralises the taste of sweetness for some time. For these potential antidiabetic medicinal properties, it is popularly known as "Gudmar" or "Madhunashini" *G. sylvestre* is one of the most popular medicinal plant on the global market, requiring a cost-effective and simple method of cultivation to meet its growing demand. The availability of the species in natural forests is decreasing very fast due to overharvesting and unsustainable harvesting. The present demand is mostly met by wild collection. Therefore, the only way to meet the

increasing demand and reduce the pressure of harvesting in the wild is through large-scale cultivation.

“The World Health Organization (WHO) considers that the quality of raw materials and finished products, depend on many factors including cultivation techniques, collection methods, harvesting methods, post-harvest, processing, transport, and storage practices. WHO prescribed general guidelines for good cultivation and collection practices (GACP) for supply of quality medicinal herbs” (WHO, 2003) [55].

Table :1. Vernacular names of *Gymnema sylvestre* (Farooqui and Sreeramu, 2004) [14].

English	Ram’s horn, Small Indian Ipecacunaha, Australian cowplant and Periploca of the wood
Sanskrit	Meshshringi, Madhunashini
Hindi	Gudmar, Merasing, Gurmar
Tamil	Adigam, Cherukurinja
Telugu	Podapathri
Kannada	Sannagresehambu, Kadhasige
Malayalam	Chakkarakolli, Madhunashin
Gujarathi	Dhulet, Mardashingi
Marathi	Kavali, Kalikardori, vakundi
Bengali	Mera – singi

### 1.1 Origin and Distribution

In addition to India, it is widely distributed in many countries such as Malaysia, Sri Lanka, Australia, Indonesia, Japan, Vietnam, tropical Africa, and the southwestern regions of China (Di Fabio *et al.*, 2013) [13]. This plant is found in tropical and sub-tropical regions (Pandey 2012) [37]. In India, it is found in the forests of the Western Ghats, Konkan, Madhya Pradesh, Chhattisgarh, Bihar, Tamil Nadu, and Karnataka at an altitude of 100–1000 m (Farooqui and Sreeramu, 2004)[14]. Two allied species, *G. hirsutum*, found in Bundelkh, Bihar, and the Western Ghats, and *G. montanum*, growing wild in the Eastern Ghats and Konkan, are also used for the same purpose and are also called "Gurmar" (Thakur *et al.*, 1989)[51].

### 1.2 Soil and Climate

“The crop is found growing on a variety of soil in different localities. Red sandy loam or medium-deep black soil is reported to be ideal for this crop. The plant is sensitive to water logging and hence its cultivation on such soils should be avoided”. (Farooqui and Sreeramu, 2004) [14]. The soil has a pH of 7.6 was found ideal and no chemical fertilizers and pesticides were used in raising and managing the plantation (Pandey., 2012)[37]. Madhunashini prefers tropical and sub-tropical types of climates. It is found growing even in dry areas with a temperature range of 15-40 °C, up to an altitude of 1000 m. The areas with high or medium well-distributed rainfall are suitable for their cultivation.

### 1.3 Description of Plant Botany

*Gymnema sylvestre* (Retz.) R. Br. is a perennial, woody, medicinal vine with chromosome number  $2n = 22$  and is a member of the Apocynaceae family. It has several branches, grows slowly, and requires support to grow with pubescent young parts. The leaves are opposite, simple, pubescent on both sides, elliptic or ovate, and base-rounded or cordate. Flowers are small, yellow, and grouped in umbellate cymes, which are axillary clusters of solitary, tiny, bell-shaped flowers. Five sepals and a long corolla make up the umbel of flowers. One single, lengthy peduncle connects the stigma (corolla) to the inflorescence. The stigma is heavily lobed, curved, and frequently covered in buds. The style is often longer than the stamen. Flowering occurs from October to January, while fruits mature from March to May. Fruits are slender, and follicles are up to 7.5cm long. Seeds are about 1.3 cm long, narrowly ovoid-oblong, flat, and have a thin, broad, brown, and glabrous marginal wing. (Warrier, 1995)[54].

Table: 2. Taxonomy of *Gymnema sylvestre* (Kirtikar and Basu, 1987) [23].

Kingdom	Plantae
Sub kingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Gentianales
Family	Apocynaceae
Sub-family	Asclepiadaceae
Genus	<i>Gymnema</i>
Species	<i>Gymnema sylvestre</i> (Retz.) R. Br.ex Sm

## 1.4 Phytochemistry of *Gymnema sylvestre*

“*G. sylvestre* leaves contain triterpene saponins belonging to oleanane and dammarene classes. Oleanane saponins are gymnemic acids and gymnemasaponins, while dammarene saponins are gymnemasides. Besides this, other part plant constituents are flavones, anthraquinones, hentriacontane, pentatriacontane,  $\alpha$  and  $\beta$ -chlorophylls, phytin, resins, d-quercitol, tartaric acid, formic acid, butyric acid, lupeol,  $\beta$ -amyrin related glycosides and stigmasterol” (Laha, and Paul., 2019) [26]. “The plant extract also tests positive for alkaloids. Leaves of this species yield acidic glycosides and anthroquinones and their derivatives” (Dateo and Long, 1973) [11]. The drug had composed different medication in its formulation like Ayaskrti, Varunadi Kasya, Varunadighrtam, Mahakalyanakaghrtam, etc. Triterpenes and saponins found in the plant's leaves are thought to be responsible for its anti-diabetic properties. Gymnemic acids A, B, C, and D, which contain Gymnema genin and gymnestrogenins, have been designated these names (Goyal *et al.* 2022) [17] and (Tiwari *et al.*, 2014)[52]. Additionally, nonacosane and hentriacontane, which were extracted from leaves using hexane, are found in leaves. A trace component known as gymnamine, an alkaloid, has been isolated and identified (Farooqui and Sreeramu, 2004) [14].

## 1.5 Nutraceutical application

“This climber is extensively used in almost all the Indian systems of medicine (Rao and Ramakrishna., 2020) [40] to cure pitta, kapha, diabetic, ulcers, cough, dyspnea, and eye pain. Inflammations, hepatosplenomegaly, dyspepsia, constipation, jaundice, haemorrhoids, stranguria, renal and vesical calculi, helminthiasis, cardiopathy, cough, asthma, bronchitis, intermittent fever, amenorrhea, cataracts, and leucoderma might all benefit from the plant”. “Chewing on the fresh leaves has the unique effect of permanently paralysing the sense of taste for both sweet and bitter foods” (Warrier, 1995) [54]. “The medication is referred to as a destroyer of glycosuria (madhumeha) and other urinary diseases. Root has a long history of being used as a snakebite treatment. Castor oil and crushed leaves are applied externally to swollen glands and the growth of internal viscera like the liver and spleen” (Nadkarni, 1954) [33]. “The Nutraceutical is used to improve heart health and treat jaundice, piles, urinary calculi, problematic urination, and irregular fevers” (Sharma, 1983) [43].

## 2. Biodiversity of *Gymnema sylvestre*

### 2.1 Exploration and collection of germplasm

“Survey and exploration were carried out from Belgaum, Gadag, Uttara Kannada, Udupi and Shimoga districts of Karnataka in the Western Ghats for the collection of *Gymnema sylvestre*. Wild habitats of the targeted species, which included plain forest areas, tribal hilly areas, etc were thoroughly explored and a total of 30 accessions of *G. sylvestre* was collected. During the exploration, two types of variants of the species were identified i.e., narrow leaf type and broad leaf type. In a second exploration trip, Jabalpur, Damoh, and Sagar districts of Madhya

Pradesh were surveyed with the help of traditional healers and forest range officers, and old persons of the local society. In wild areas of Damoh and Sagar, the variability was observed in the case of fruit traits”. [2] “Two types of fruits were observed i.e., fruit of short length and fruits of longer length. A total of 14 accessions of *G. sylvestre* were collected from the area. It was noticed that the tribal people at Dhamoni use *Gymnema* leaves for eye problems, in addition to the popular use for the treatment of diabetes. A third exploration of *Gymnema* was taken up at Tumkur districts of Karnataka. The Siddarabetta of Tumkur is a hot spot for various medicinal and aromatic plants. In this area, a total of 15 accessions of *Gymnema* was collected with the help of local people. Another exploration conducted at Ranjendrangar, Hyderabad in the Telangana state and five accessions were collected from the wild source. Thus, altogether a total of 65 accessions were collected during the current year from diverse area of Madhya Pradesh, Karnataka, Telangana, and parts of western ghats. The collected genetic resources of *Gymnema sylvestre* are now maintained in the field gene bank under the nursery for the future multiplication, characterization, and evaluation for growth, yield, and quality traits to select elite accessions for specific traits of interest” (ICAR – DMAPR, 2018-19)[2].

## 2.2 Collection, characterization, evaluation, and maintenance of germplasm

“TNAU, Coimbatore: Sixty-six accessions of *Gymnema sylvestre* are maintained in the field at the Department of Medicinal and Aromatic Crops, Coimbatore and the morphological, yield, and quality characters were recorded. Among the 66 accessions, variations were observed for leaf shape, leaf base, leaf tip, leaf colour and leaf pubescence. Based on leaf shape, the accessions were grouped into ten different sets viz., elliptic (15 accessions), ovate (25 accessions), lanceolate (15 accessions), oblanceolate (1 accession), elliptic-ovate (1 accession), elliptic-lanceolate (1 accession), ovate-elliptic (1 accession), ovate-lanceolate (1 accession), oblong-ovate (1 accession) and ovate-oblong (1 accession). The leaf shape was oblong in three accessions; elliptic in 16; cordate in 2; ovate in 45 accessions. Based on leaf tip nature, the accessions were grouped into three viz., acute (40 accessions), acuminate (13 accessions) and attenuate (3 accessions). Accessions were grouped into four based on leaf base viz., round (51 accessions), cordate (7 accessions), obtuse (7 accessions) cuneate (3 accessions). Leaf pubescence was present in 56 accessions and absent in 10 accessions. Mid-rib pubescence was present in 49 accessions and absent in seven accessions. Variations were also observed for leaf length, breadth, petiole length and intermodal length” (ICAR – DMAPR, 2018-19) [2].

“The pooled mean for two years of data revealed that leaf length varied from 2.40 to 4.58 cm, and the accession Kolli Hills local-1 recorded the highest length (4.98 cm); leaf breadth varied from 1.57 to 2.90 cm, and the accession Anaikatti local-2 recorded the highest leaf breadth (2.90 cm); petiole length varied from 0.47 to 1.37 cm, and the accession Kolli Hills (5) recorded the highest petiole length (1.37 cm). The yield character, viz., dry leaf weight of leaves, ranged from 0.08 to 0.75 kg plant<sup>-1</sup>. Gs14 (Yercaud Local 5) recorded the highest leaf dry weight. The gymnemagenin content of the accessions ranged from 0.48 to 1.54%, and the accession Gs34 (Sirumalai local 4) recorded the highest gymnemagenin content (1.54%)” [2].

“14 genotypes were found to have high yields and high gymnemagenin contents out of the 66 genotypes. The 14 genotypes' leaf dry weights ranged from 0.49 kg per plant to 0.75 kg per plant. Gymnemagenin concentrations ranged from 0.72 to 1.54%. They were classified as genotypes with high biomass and low gymnemagenin content, genotypes with high gymnemagenin and low biomass, and genotypes with low biomass and high gymnemagenin content based on the average leaf dry weight of 0.62 kg plant<sup>-1</sup> and the gymnemagenin content (1.13%). Pooled data for two years revealed that the accession Gs14 (Yercaud local 5) recorded the highest leaf dry weight (0.75 kg plant<sup>-1</sup>) and the accession Gs34 (Sirumalai local 4) recorded the highest gymnemagenin content (1.54%)” (ICAR-DMAPR, 2018–19) [2].

Madhunashini **creepers** can be classified into two types based on the leaf size

**1. Small-leaved type:** Leaves are oval, measuring 1.0-3.5 cm in length and 1.5-2.5 cm in width, and very soft, found in dry regions.

**2. Broad** and pubescent type: The leaves are also oval, measuring 3-6 cm in length and 3.5-5.0 cm in width. Leaves are dark green compared **to small-leaved** types and are pubescent.

### **3 Propagation**

Madhunashini can be multiplied either by seeds or vegetative propagation methods, which are used for commercial cultivation.

#### **3.1 Seed Treatment and Germination Percentage**

“The study conducted stated that the germination was initially poor, but it was significantly affected by pre-treatments which varied from 28.50 - 42.50 percent. The highest germination was obtained when seeds were soaked in cold water for 24 hours” (Pandey., 2012) [37]. “Propagation of *G. sylvestre* through seed is not easy due to **the** difficulty in seed availability and **the** prevailing dormancy problem” (Arunakumara and Subasinghe, 2004)[4]. “Also reported that **the** dynamics of seed germination of *G. sylvestre* and **the** availability of high moisture content increases germination response” (Harikumar *et al.*, 2000)[19].

##### **3.1.1 Seeds**

The shrub produces fruit from February to April. The seeds of freshly picked fruits are collected and soaked in water overnight, the seeds are planted the following day in a seed pan that contains soil mixed with sand to support germination. Daily watering of the seed trays results in the seeds germinating after about 15 days. 40 to 50 days after sowing trays are transferred to polythene bags holding a mixture of soil, sand, and FYM mixed in equal proportions. Whereas seeds are sown in mixed soil using vermicompost instead of farmyard manure, the maximum germination percentage is up to 70%-85%. The plants are cared and constantly watered until they are transplanted (Harikumar *et al.*, 2000) [19]. (Farooqui and Sreeramu, 2004) [14].

### 3.1.2 Vegetative propagation

Semi-hardwood cuttings of terminal shoots 15 to 20 cm in length are used for vegetative propagation. *G. sylvestre* cuttings are planted in polybags filled with soil, sand, and FYM in a 1:2:1 ratio. Under North Indian conditions, February to March is the best time to take cuttings in the nursery. The cuttings adapt to humid conditions in shade houses or mist chambers for good root development and within a month, root formation begins. Before planting in polybag or nursery to promote rooting. The cutting is dipped in a 100-ppm solution of indole butyric acid (IBA) for six minutes (Farooqui and Sreeramu, 2004)[14]. The cuttings are also administered 1% Bavistin treatment prior to planting in the polythene bags to prevent root infectious diseases. Planted cuttings are regularly irrigated. After 90 days of planting, roots begin to emerge and about 6 months old, rooted cuttings are ready for transplanting in the main field. The study revealed higher rooting success (52%) in hardwood cuttings followed by semi-hardwood and softwood cuttings (26% and 15% respectively) without any hormonal treatment (Pandey., 2012) [37]. The propagation of *G. sylvestre* through rooted cuttings is preferred due to short supply and dormancy problems in seeds (Harikumar *et al.*, 2000) [19]. Hardwood cuttings had a higher potential for root production and the success rate is high in rooting of *G. sylvestre* when stem cuttings are placed with at least one node inside the planting medium which also produced the true to types of seedling (Singh *et al.*, 2008)[45] and Karoshi and Hedge, 2001)[21].

## 3.2 Micropropagation

“Micropropagation is a well-established process for mass-scale production of plants with clonal stability. In vitro multiplication of *Gymnema sylvestre* was first attempted” by Bahadur *et al.*, (2007) [7]. “Maximum number of shoots was achieved on MS medium containing BAP (5 mg l<sup>-1</sup>) and NAA (0.2 mg l<sup>-1</sup>). Multiplied shoots were brought to rooting on ½ strength MS medium without adding any plant growth regulator” Murashige and Skoog, 1962)[62]. “investigated the effect of various factors which remarkably affected in vitro regeneration of *Gymnema sylvestre* that were; seedling age, the nature of the explant, basal

medium, plant hormones, antioxidants (activated charcoal, ascorbic acid, citric acid, and poly vinyl pyrrolidone) and undefined supplements (coconut milk, yeast extract, casein hydrolysate and malt extract). A maximum of 57.2 shoots were induced from 30 day old seedling axillary node explants incubated on MS medium supplemented with  $0.1 \text{ mg l}^{-1}$  NAA,  $1.0 \text{ mg l}^{-1}$  BA,  $0.5 \text{ mg l}^{-1}$  Kn,  $100 \text{ mg l}^{-1}$  citric acid and  $100 \text{ mg l}^{-1}$  malt extract. Best root regeneration was observed on shoots derived from axillary nodal explant (50%) on  $\frac{1}{2}$  MS medium supplemented with  $3.0 \text{ mg l}^{-1}$  IBA. The rooted shoots were subjected to hardening in soil and successfully acclimatized to natural conditions” (Komalavalli and Rao, 2000)[24]. (Karthic and Seshadri, 2009) [22] developed “a cost-effective mass multiplication of *G.sylvestre* in hydroponic system. Effect of media and moisture on rooting of *Gymnema sylvestre* stem cuttings was also studied and concluded that the requirement of MS mediums for shoot bud activation and propagation confirms the requirement of rich salts for the regeneration of *Gymnema sylvestre*”. “Influence of various growth hormones like; 2, 4-D, IAA, BAP, and Kinetin on the breaking of axillary bud dormancy was studied and synergistic action of vitamin B<sub>2</sub> in relation to these plant growth regulators was also worked out. To minimize phenolic release by explants different antioxidants; activated charcoal, ascorbic acid and citric acid were also added in culture medium. Citric acid at a concentration of  $100 \text{ mg l}^{-1}$  prevented blackening of medium and enhanced the number of healthy micro propagated shoots in *Gymnema sylvestre*. Both qualitative and quantitative improvement on rooting was obtained on  $\frac{1}{2}$  strength MS medium, where 53 % of shoots were induced to root within 45 days. MS medium supplemented with  $1.0 \text{ mg l}^{-1}$  BA+ $0.5 \text{ mg l}^{-1}$  IAA+ $100 \text{ mg l}^{-1}$  vitamin B<sub>2</sub>+ $100 \text{ mg l}^{-1}$  citric acid was best for shoot proliferation and  $\frac{1}{2}$  strength MS medium with  $3.0 \text{ mg l}^{-1}$  IBA was best for root induction” (Arunakumara *et al.*, 2013) and (Subathra and Srinivasan, 2008). “Study have reported that highly reproducible plant regeneration protocol from young shoots of mature plant. Highest multiple shoots (80%) were observed on MS medium supplemented with  $5.0 \text{ mg l}^{-1}$  BAP with Mean shoot length of  $2.57 \pm 1.91 \text{ cm}$ ” (Solanki and Gupta (2013a) [48].

### 3.3 Somatic Embryogenesis

“Somatic embryos are induced in somatic tissue of plants by giving proper stimulus of a plant growth regulator in vitro. Applications of this technique include large-scale production of clones, virus elimination, providing source tissue for gene transfer, and production of synthetic seeds. Somatic embryos can be obtained on explant either directly or by the intervention of callus phase. It depends on the presence of competent cells in the explant. A proper stimulus by a specific growth regulator induces cells of explant to form an embryo. Whole plant regeneration by somatic embryo formation has been achieved by callus obtained from hypocotyl, cotyledon and leaf explants excised from in vitro raised seedlings of *Gymnema sylvestre*” (Gupta and Solanki, 2015) [18]. “MS medium containing (0.5–5.0  $\mu\text{M}$ ) 2,4-D + (0.5–2.0  $\mu\text{M}$ ) BA and 2.0 percent (w/v) sucrose, induced embryogenic callus within 6–8 weeks after initiation of culture. On this medium globular and heart shape stage embryo were obtained, which further developed into a torpedo and cotyledonary stage in a medium supplemented with MS salts, B<sub>5</sub> vitamins,  $0.5 \mu\text{M}$  BA and 2.0% sucrose. Subculturing on the same medium resulted in embryo germination and formation of plantlets, which were

successfully adapted to greenhouse conditions” (Ashok *et al.*, 2002) [6] and (Gopi and Vatsala, 2006)[15]. “An established procedure for the suspension culture of *Gymnema sylvestri* to produce somatic embryos. Callus cultures were induced on MS medium with growth regulators 0.5 mg l<sup>-1</sup> 2,4 -D (or) 1.0 mg l<sup>-1</sup> NAA and 10 percent coconut water. They were transferred into an MS liquid medium containing 1.0 mg l<sup>-1</sup> NAA, 1.0 mg l<sup>-1</sup> BA, 3.0 percent sucrose (w/v), 10 percent coconut water, citric acid 1.0 mg/l and glutamine 10 mg/l for induction of somatic embryos from callus. Various stages of somatic embryo development like; globular, heart, torpedo and cotyledonary were identified in suspension cultures within 8 weeks. The maturation of embryos was found to be considerably influenced by plant growth regulators and length of light and dark cycles. Plantlets were germinated from 5-7 % of embryos induced on semisolid MS salts with B<sub>5</sub> vitamins, 3.0 percent sucrose and 0.8 per cent agar (w/v). After transferring in field plantlets have shown similar traits as that of source plant. The various factors affecting callus production in *Gymnema sylvestri* have been investigated in detail, including the type and age of explant, media, carbon source and antioxidants. Leaves and stem cuttings from young plant were tested for their regeneration potential by inoculating on various concentrations of different combinations of auxins and cytokinins added in MS and B<sub>5</sub> medium. Callus induction was shown by (100 %) explants on all the levels of 2,4-D (0.5-5.0 mg/l) within 3-4 weeks. Callus obtained on 2,4-D was pale yellow and friable but on NAA compact callus was formed. On combinations of BAP and 2,4-D only leaf explants responded to form compact yellow, green callus within 25-30 days. Among the different media tried callus was obtained only on MS medium”. (Ahmed *et al.*, 2009) [1] and (Solanki and Gupta 2013b)[47]. “Among different types of antioxidants (adenine sulfate, ascorbic acid, and citric acid) and carbon source (glucose, maltose, and sucrose) tested, citric acid at 30 mg<sup>-1</sup> concentration and sucrose at 3% concentration produced the highest amount of light green, compact callus from leaves in 9-10 weeks after inoculation” (Solanki and Gupta 2013c) [46].

### 3.4 Mass multiplication of *Gymnema sylvestri* in hydroponic system

“Due to indiscriminate collection and over exploitation, natural stands of *G. sylvestri* are fast disappearing and threatened. Hence to avoid its disappearance, it requires cultivation in farmlands and this approach offers excellent scope for cultivation in the subtropical regions in southern India. Conventional propagation of this plant is hampered due to poor seed viability, low rate of germination, poor rooting ability of vegetative cuttings and low multiplication rate even in tissue culture” (Lee *et al.*, 2008)[27]. “It was observed that rooting of *G. sylvestri* using IBA in tissue culture technique was also very low” (Bahadur *et al.*, 2007)[7], (Komalavalli and Rao, 2000)[24]. “Hydroponics offers opportunities to provide optimal conditions for plant growth and enables the growers to manage the supply of essential nutrients to crops more efficiently and accurately than traditional field systems. Hydroponic system has been developed for growing several plants” (Jones, 2014)[20]. “Therefore, the hydroponic system was looked at as a potential alternative for rooting and mass multiplication of *G. sylvestri*. MCRCGY1, an accession collected from Muniyankudisai Village, Tamilnadu was micro propagated using MS basal medium supplemented with Benzyl aminopurine (BAP - 3.5 mg/L), Kinetin (KN 1.0 mg/L), Naphthaleneacetic acid (NAA

0.2 mg/L) and the grown plantlets were established at Sri AMM Arunachalam Technology Resource Centre, Vadakadambadi, Tamilnadu” (Karthic and Seshadri., 2009) [22] and (Cyriac *et al.* 2020)[10].

Preliminary studies showed that the explants with an actively growing side branches responding better than those actively growing single shoots and leaf less stem cuttings. According to research, IBA concentration and planting material size both have a significant impact on the development of roots in *G. sylvestre*. IBA supplementation of 1/10 MS basal salts medium resulted in greater root growth than the control. Within a week of incubation, root initiation was shown, and from the second week on, it became more apparent. The 30 cm long explants showed the highest root induction (93% rooting,  $17.8 \pm 1.1$  root numbers, and  $15.4 \pm 0.8$  cm root length) on 1/10 MS salts supplemented with 0.5 mg/L IBA, followed by 1.0 and 2.5 mg/L IBA and control. IBA and 1/10 MS media have been shown to reduce leaf fall and encourage shoot growth (Maene and Debergh, 1985)[30]. Plantlets were hardened in poly houses using red soil, river sand, and farmyard manure (1:1:1), with a 96% success rate for transplantation. This technique allows producing more plantlets in just 21 days. The use of plastic tanks with nutrient solutions for mass multiplication of *G. sylvestre* stem cuttings using a hydroponic system is the first study on the soilless culture, and it is a highly appealing approach (Karthic and Seshadri, 2009) [22].

### 3.5 In Vitro Response of *Gymnema sylvestre*

*Gymnema Sylvestre* is a plant that propagates itself in nature by producing seeds, but these seeds have very short viability times and lack endosperm, which results in very low germination rates under natural environmental conditions. This plant has undergone traditional methods of propagation. The factors that can enhance seed germination, vegetative propagation through stem cutting, and rooting of stem cuttings have been optimized to grow plants in the field and in vitro conditions (Singh *et al.*, 2008[45] and (Arunakumara *et al.*, 2013[5]). “Conventional propagation techniques do have drawbacks, however, such as being dependent on the environment, season, etc. For the extraction of gymnemic acid at commercial scales, it is now essential to multiply this valuable medicinal vine using tissue culture techniques. The review focuses on the ability of *Gymnema* to regenerate in vitro as well as the in vitro production of gymnemic acid, the chemical's principal anti-diabetic component. Gymnemic acid is found in plants only in trace amounts. A commercially viable production system is a major necessity to exploit its antidiabetic potential as a drug. In vitro regeneration of callus and maintenance of cell suspension culture allows the opportunity to not only produce gymnemic acid in large quantities but also maximize its production at the commercial level by manipulating cultural conditions” (Lee *et al.*, 2006)[28] (Subatra and Srinivasan., 2008)[49] and [Syedy and Nama, 2018)[50].

## 4 Argo- technique of cultivation

### 4.1 Land Preparation and Planting

The best time for planting is from June to August. Waterlogged conditions will adversely affect the establishment of plants and therefore, transplanting in such areas should be avoided. 50 cm<sup>3</sup> sized pits are dug after the soil has been ploughed and levelled, spacing of 2.5 m between rows and 1.8 m between plants is followed. The pits are opened 15 days before planting, filled with topsoil and green leaves, and 10 kg of well-rotten manure is put to each pit. If there is no rain, irrigate the pits for one week before planting the rooted cuttings and giving them irrigation. An optimum spacing of 1 m × 1.5 m is recommended for a crop stand of about 6700 plants per hectare. If the plant survival rate is 80%, then a total of 8400 plants will be required for one-hectare area. The rooted cuttings/seedlings may be planted by the crowbar method (Pandey., 2012) [37] and (Farooqui and Sreeramu, 2004)[14].

#### 4.2 Effect of planting time and spacing on growth and yield traits

“BAU, Ranchi, conducted a research experiment with three transplanting times (mid-July, mid-August, and mid-September) and three spacings (40×30 cm, 50×40 cm, and 50×50 cm). The maximum fresh biomass yield (0.98 kg plant<sup>-1</sup>) was recorded in a crop planted in mid-July at a spacing of 50 cm×50 cm and the minimum (0.66 kg plant<sup>-1</sup>) was recorded in a mid-August planting at a spacing of 50 cm× 50 cm with the grand mean of 0.80 kg plant<sup>-1</sup>. The maximum dry leaf yield (0.16 kg plant<sup>-1</sup>) was recorded in mid-July planting at 30 cm × 40 cm spacing, and the minimum (0.10 kg) was in mid-September planting at a spacing of 50 cm × 50 cm. Maximum dry biomass yield (0.49 kg plant<sup>-1</sup>) was recorded in mid-July planting with 50 cm × 50 cm spacing, and the minimum (0.33 kg plant<sup>-1</sup>) was in mid-August planting at 50 cm × 50 cm spacing” (ICAR – DMAPR, 2018-19)[2].

#### 4.3 Effect of Phytohormone

A fresh 12 to 15 cm long cutting with 2-4 nodes having a diameter of 11 to 15 mm was prepared. IBA rooting hormones were applied to the lower ends of cuttings for 30 minutes. The upper end of the cuttings was sealed with paraffin wax. The treated cuttings were planted with at least one node below the sand surface in trays filled with cleaned riverbed sand. The trays were kept moist by providing regular watering. After 3 months, the plantlets raised in trays were transferred to polybags 16 cm x 9 cm in size, filled with soil, sand, and FYM in a 1:3:1 ratio. The study found that cuttings dipped in 1000 ppm IBA solution for 30 minutes had showed the highest sprouting (77.87%), but the lowest rooting (52.50%) and survival (40.67%) (Pandey., 2012)[37]. Similarly, the study suggested that 2500 ppm IBA treatment to improve the rooting ability of apical shoot cuttings in *G. sylvestre* (Karoshi and Hedge, 2001) [21].

“The effect of plant growth regulators on rooting and sprouting in stem cuttings was studied at BCKV, Kalyani. The experiment was conducted in November 2018 with two growth hormones each at three levels (1000 ppm, 5000 ppm and 100 ppm) and pure honey, and compared with a control. After treatment, the cuttings were planted in sand in flat trays.

Optimal watering was done. Cuttings treated with higher doses of PGR (1000 ppm and 5000 ppm) sprouted less, even less than those in the control. A higher dose had a detrimental effect on the sprouting of the stem cutting. Stem cuttings treated with a lower dose of PGR gave good sprouting results (58–66%). Stem cuttings treated with pure honey also gave a moderately better result (46% sprouting)” (ICAR – DMAPR, 2018-19) [2].

#### 4.4 Effect of seasons and PGR on rooting

“The experiment was conducted with the objective to identify suitable season and plant growth regulator (PGR) doses for successful rooting in cuttings of madhunashini in JNKVV, Jabalpur. The cuttings were planted in three different seasons viz., July, August, and September by treating with IBA 250, 500, 750 ppm and without IBA to study the rooting success and survivability percent. The sprouting percentage of cuttings was observed maximum (59.07%) in July planting, but maximum survivability (28-46%) was found in August month. The cuttings treated with 750 ppm IBA sprouted early (9.61 days) followed by 500 ppm IBA solution (11.20 days). The cuttings treated with 750 ppm IBA exhibited maximum sprouting (67.08%), sprouting length (2.91 cm) followed by 500 ppm treatment. Maximum survivability (33.03%) was recorded with 750 ppm IBA-treated cutting. Considering the interaction effect, the maximum survivability (34.58%) was recorded in August month planting in 750 ppm IBA treated cuttings closely followed by 550 ppm IBA treatment” (ICAR – DMAPR, 2018-19)[2].

The study found that hardwood cuttings 15 cm long with 3-4 buds planted in July and August had the highest survivability. Among plant growth regulator (PGR) treatments, dipping cuttings in a 500 ppm IBA solution for 30 minutes was suitable for maximum survivability. The interaction between season and PGR revealed that 500 ppm IBA-treated cuttings planted in August showed the highest survivability by Vijay (2016) [53].

#### 4.5 Manures and Fertilizers

In the first year, in addition to the natural manure, the climber will grow and develop more quickly if 10 g of urea and 20 g of super phosphate are provided to each plant once before planting and then at intervals of one month. Fertilizers are applied in two split dosages beginning in the second year. During the first week of June, the crop is harvested, and the soil around the roots is loosened and treated with 40, 20, and 15 g of N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O per plant, or 90, 45, and 35 kg of NPK per hectare. To increase biomass production, a further equal dose may be added each year continued up to ten years. Application of FYM appeared to be the most promising, while vermicompost was found to be similarly efficient (Pandey., 2012)[37]. According to a previous study, the application of organic manures affected the biomass yield (Prakash and Adholeya,2004)[38].

“Treatment combination of FYM (25 t/ha) + recommended dose of fertilizer (90:45:35 kg/ha of NPK/ha) combined with foliar spraying of panchagavya and Manchurian mushroom extract each at 3% and humic acid at 0.3%, recorded highest plant height (227.53, 286.47, 300.1 and 334.54 cm), number of leaves (62.0, 70.0, 82.0 and 95.0) number of branches (36.048.055.058.0), leaf area (12.60, 15.52, 17.50 and 18.92 cm<sup>2</sup>), fresh biomass (2.55, 3.22,

3.88 and 4.10 kg/plant) and dry biomass (0.638, 0.782, 0.890 and 0.913 kg/plant) at 180, 240, 300 and 360 days after transplanting, respectively. Regarding the quality parameters, the treatment combination of FYM (25 t/ha) + Vermicompost (5 t/ha) + Neem Cake (250 kg) combined with foliar spraying of panchagavya and Manchurian mushroom extracts each at 3% and humic acid at 0.3% registered the highest crude gymnemic acid content of 485.74 mg per 100 g<sup>-1</sup> dry weight” (Padmapriya *et al.*, 2010)[14].

“The experiment was conducted to standardize a suitable source and dose of nutrients for maximizing dry leaf yield in JNKVV, Jabalpur. The number of leaves per plant and fresh and dry herbage yields varied significantly with different organic treatments. However, maximum fresh leaf weight (95.20 g plant<sup>-1</sup>) was recorded in the treatment receiving RDF (NPK @ 50:25:50 kg ha<sup>-1</sup>), which was on par with the treatment receiving FYM at 10 t ha<sup>-1</sup>. The maximum dry weight (48.7 g plant<sup>-1</sup>) was recorded in the treatment receiving FYM at 10 t ha<sup>-1</sup>, which was on par with the treatment receiving FYM at 15 t ha<sup>-1</sup>. The economic analysis of various treatments revealed that treatment of FYM at 10 t ha<sup>-1</sup> was most profitable with a B/C ratio of 2.87, followed by RDF with a B: C ratio of 2.11” (ICAR – DMAPR, 2018-19)[2].

**Application** of 1.2:0.8:0.4 kg NPK for 12, 18, and 24 MAT results showed the maximum plant height (188.96 cm, 293.29 cm, and 326.65 cm) and average number of branches per plant (7.85, 19.36 cm, and 22.46 cm) respectively. The highest dry leaf yield of 124.84 kg per acre was achieved by the application of 1.2:0.8:0.4 kg NPK in 12 MAT, while for 18 and 24 MAT, the highest yields of 348.84 and 381.44 kg were achieved by the application of 0.10:0.10:0.08 kg (Azospirillum, VAM, and PSB), respectively. Results concluded that the combined application of bio-fertilizers (Azospirillum, VAM, and PSB) significantly improve the growth and yield of *Gymnema sylvestre* (Bisht *et al.* 2019) [9].

The application of Vermicompost (1.0 kg) + Azospirillum (Root isolate (10 gm.) after four months of planting recorded highest plant height (264.36), Number of laterals/plant (52.19), Number of leaves/lateral (110.13), Leaf length (7.88 cm), Leaf breadth (5.87 cm), Leaf area (17.55 cm<sup>2</sup>), Single leaf weight (0.44 gm.), 100 leaves weight (44.18 gm.), Fresh leaves yield/plant (3.86 kg.) and Dry leaves/plant (2.91 kg.) which was followed by application of decomposed coir pith (5.0 kg) + Azospirillum (Root isolate 10 gm.) (Madhavan and Sha, 2019)[29].

#### 4.6 Training

*Gymnema* is a climber, hence it needs to be trained on a 2 m stone pillar with a 'Y'-shaped iron structure bent at 60 degrees to the sides. The lower side of the iron structure needs to be connected, and 3 iron wires should also be connected to each of the bending ends, for a total of 7 wires. The two main stems are trained on the lower wires so that they develop in opposing directions; additionally, the produced laterals are trained on different top wires. The climbers must take care to avoid falling to the ground. These plants can also be trained to grow along a wire fence, which will serve two purposes (Farooqui and Sreeramu, 2004) [14] and (Pandey, 2012)[37].

## 4.7 Irrigation

After harvest and manure application, irrigation is necessary. One irrigation is required immediately after transplanting for initial seedling establishment and growth. Thereafter, once every 5–6 days of irrigation is sufficient. The frequency of irrigation needs to be raised during the summer based on the weather and soil conditions. The plants, however, no irrigation was provided during the rainy season (Pandey, 2012) [37] and (Ashok *et al.* 2002)[6].

## 4.8 Drip irrigation

Irrigation through drip and drop-drop system is considered very successful. In a drip irrigation system, water is delivered through small diameter plastic pipes at a very low rate (2-20 litres/hour) to the plant roots through emitters or drippers outlets. The system consists of main pipeline, sub-level line and lateral pipelines. Water is delivered to the crop using a network of lines that have emission points along their length. Each dropper / emitter supplies water, along with nutrients and other growth substances, to the root zone of plants in a controlled manner. In this system, water is applied only to the root zone of the plants so that only that part of the soil gets wet which is the part of the roots. Unlike surface and sprinkler irrigation, which require wetting of the entire soil profile. In drip irrigation, water is applied more frequently (usually every 2-3 days) than in other methods, which maintains very favorable high moisture levels in the soil and improves plant growth and development (Kumar and Jnanasha, 2017) [25]. Similar results were reported in ginger and basil. (Santosh and Maitra, 2022) [42] and (Palada *et al.* 2000) [36].

## 4.9 Weed management.

After transplanting in *Gynmema sylvestre*, the transplanted area should remain weed free during initial growth. Since the growth of its plants is very slow, during the initial development, the gram crop can be grown as an intercrop. It is a creeper crop and alternatively, this crop can be raised under tree species. These tree species will also aid in its growth by acting as staking for its branches. Apart from this, bamboo poles can also be used for staking (Farooqui and Sreeramu, 2004) [14] and (Pandey, 2012) [37].

## 4.10 Pests & Diseases management

The risk of diseases is very low in Madhunashini farming, but due to the outbreak of insects, the crop suffers a lot. Among the pests, mainly aphids (*Aphis nerii*), and mealybugs (*Phenacoccus solenopsis*) in southern India and papaya mealybugs (*Paracoccus marginatus*) in western India are very destructive. Other pests include leaf weber, leaf minor, and cutworm.

### 4.10.1 Aphids

The aphid is a small, orange-coloured sap-sucking insect and comes in the Aphididae family. The bodies of these insects are soft and oval, the heads are small; the trunk is jointed, the tentacles are of seven segments; and there are four transparent wings (when wings are

present). Generally, aphids are the most destructive enemies of agricultural crops grown in temperate regions and cause great economic losses. Aphids are slow moving suck the sap of the plant so quickly and in such large quantities that most of the sap comes out of the body unchanged. This undigested food is in the form of a delicious, sugary liquid called honeydew. Due to its presence, many times there is an outbreak of shooty mould on the plants, which is harmful for vegetative growth (Shivakumara *et al.* 2022) [44] and (Prakash,2012)[39].

#### 4.10.2 Mealybug (*Paracoccus marginatus*)

They damage tender parts of *Gymnema sylvestre* by sucking the sap, which results in stunted plant growth. It damages various parts of the host plant, including the leaves, stem, and apical buds. It sucks the sap from the bark, leaves, tender twigs, and fruits of plants by inserting the stylet into the epidermis and injecting a toxic substance. This results in chlorosis, plant stunting, leaf deformity, and the loss of young leaves and fruits. The sap, which forms a honey-like substance, eventually becomes part of the plant. The formation of a thick white wax coat and death by heavy infection make the fruits inedible. Generally, 100% infestation of plants due to mealybugs has been observed. Its attack causes a lot of damage to the plants, along with a reduction in the quality and quantity of the leaves (Ravikumar *et al.* 2008) [41].

#### 4.10.3 Leaf Webber (*Spodoptera litura*)

*Gymnema sylvestre* leaves are also attacked by leaf webber (*Spodoptera litura*). The female adult lays about 850–1000 eggs together in a group, and these are covered with light, yellow-coloured hairs. It completes its life span in about 30 to 35 days. The larval stage of this insect infects the leaves of *Gymnema* and causes more damage. Newborn larvae are initially light green with a dark black colour, which should cover the colour of the larvae. *Gymnema* leaves are most severely affected by the insect's larvae. Newborn larvae are initially light green with a dark black colour, which later changes. Dark brown turns black with lines. In the beginning, it bites the leaves, and in cases of severe infection, only the skeletons of the leaves and shoots are left, which makes the crop 'almost unmarketable' and causes a lot of economic losses leaves (Ravikumar *et al.* 2008) [41].

#### 4.10.4 Management of all sucking pests

Keeping in view the importance and use of the crop, chemical pesticides should not be used in the madhunashini crop. Various types of suggestions have been recommended for its control. According to the recommendation of the Tamil Nadu Agricultural University, neem should be applied to the soil at a rate of 250 kg / hectare under the eco-friendly IPM module. Simultaneously, spray the first time with Neem, Seed Kernel Extract (NSKE) 5% at 2.5 ml.

/Litre After this, a second spray of *Beauveria bassiana* (5 g/litre) and a third spray of *Bacillus thuringiensis* (5 g/litre) should be done. Release of 10000 grub/hectare of *chrysopralla* should be done, and cow pea should be grown as a border crop to encourage the activities of natural enemies. spray Fish oil resin soap @ 125g / 5L of water release Australian ladybird beetle, *Cryptolaemus montrouzieri* @ 10 beetles/tree, Band the trees with 20 cm wide degradable polythene sheets (150 gauge) for mealybugs. Organic practises include control measures using neem-based formulations; fish oil resin soap can be used to manage all sucking pests. Botanicals such as garlic, Vitex negundo, Lantana camera, *Clerodendron inerme*, and *Calotropis gigantean* extracts are frequently combined and sprayed on a regular basis to control pests (Mohan *et al.* 2010) [31], (Antwi and Reddy, 2015) [3] and (Ravikumar *et al.* 2008)[41].

Powdery mildew and leaf spots are common diseases of this crop, and they can be controlled by spraying organic-based sources *trichoderma* and *pseudomona* for leaf spot at intervals of 10 to 15 days based on the incidence level and crop rotation use of resistant varieties for powdery mildew (Mohan *et al.* 2010) [31].

#### 4.11 Harvesting and Yield

After one year of planting, the commercially important part of the harvest the leaves begin. When plants begin to bloom, the crop is ready for harvesting at the end of June or the first week of July. The plucking of leaves can be done manually or using a sickle or knife. The collected leaves are spread thinly on the cleared ground and allowed to dry under shade for about 7-8 days. Care should be taken to ensure that the leaves are not dried in the sun. It is preferable to stagger the harvesting to achieve optimum drying. (Padmapriya *et al.* 2016)[35]. The crop is only plucked once a year. At 3–4-year-old vine may yield 5–6 kg of dried leaves on average per plant, which equals to 9–10 tonnes of dried leaves per hectare. Under proper management, the crop can be grown for 10–15 years (Binnisha *et al.* 2021) [8].

#### 4.12 Packaging

To prevent spoilage, be sure to dry the leaves thoroughly. It is then packed in polythene bags when the moisture content is less than 8-9%. In excess of this moisture, mold *mould* can develop on the packed leaves. The risk of an outbreak remains. Create the creative quality of the leaves. To keep it, it should not be dried in direct sunlight (Dhanani *et al.* 2015) [12].

### Conclusion

*Gymnema sylvestre* is an important species used in traditional medicine, as well as a nutritional supplement, for a wide range of reported health benefits. With *the* increasing demand for this ingredient in Southeast Asian pharmaceutical formulations, the plant is threatened. Bringing *Gymnema sylvestre* into commercial cultivation with improved

agronomical practices will support the conservation of wild populations and help meet market demands.

### COMPETING INTERESTS:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

### NOTE:

The study highlights the efficacy of "Ayurved" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

### References:

1. Ahmed AB, Rao AS, Rao MV. Somatic embryogenesis and plant regeneration from cell suspension culture of *Gymnema sylvestre* (Retz) R. Br. Ex Roemer & Schultes. Curr. Appl. Sci. Technol. 2009;9(1):18-26.
2. Annual Report of ICAR - Directorate of Medicinal and Aromatic Plants Research, Boriavi, Anand, Gujarat, India. 2018-19.
3. Antwi FB, Reddy GV. Toxicological effects of pyrethroids on non-target aquatic insects. Environ Toxicol. Pharmacol. 2015;40(3):915-923.
4. Arunakumara KK, Subasinghe S. Seed germination dynamics of *Gymnema sylvestre* as influenced by sowing media and storage period Trop. Agri. Res. 2004;16: 339-441.
5. Arunakumara KK, Walpola BC, Yoon MH. Mass multiplication of an important medicinal plant *Gymnema sylvestre* R. Br.: A review. Int. J. Med. Plants. 2013; 105:250-259.
6. Ashok Kumar HG, Murthy HN, Paek KY. Somatic embryogenesis and plant regeneration in *Gymnema sylvestre*. Plant Cell, Tissue Organ Cult. 2002; 71:85-88.
7. Bahadur B, Reddy KJ, Rao ML. Medicinal plants: an overview. Advances in medicinal plants. Universities Press, Hyderabad. 2007.
8. Binnisha NV, Nalina L, Mahendiran R, Rajamani K, Uma D. Effect of different drying methods on quality parameters of *Gymnema* (*Gymnema sylvestre* R. Br). J. Pharm Innov. 2021;10(10):41-46.
9. Bisht VK, Chandorkar MS, Uniyal RC, Pathak JM, Dhutraj SB. Effect of Different Manure on the Plant Growth and Yield of *Gymnema sylvestre* R. Br. J. Appl. Sci. 2019;19(3):223-228.

10. Cyriac A, Thomas T, Thomas TD. Mass multiplication of *Gymnema sylvestre* (Retz.) R. Br. ex Schult. through in vitro shoot organogenesis from callus. Plant Tissue Cult. Biotechnol. 2020;30(1):27-32.
11. Dateo Jr GP, Long Jr L. Gymnemic acid, the antisaccharine principle of *Gymnema sylvestre*. isolation and heterogeneity of gymnemic acid A1. J. Agric. Food Chem. 1973;21(5):899-903.
12. Dhanani T, Singh R, Waman A, Patel P, Manivel P, Kumar S. Assessment of diversity amongst natural populations of *Gymnema sylvestre* from India and development of a validated HPLC protocol for identification and quantification of gymnemagenin. Ind. Crops Prod. 2015; 23;77:901-909.
13. Di Fabio G, Romanucci V, Zarrelli M, Giordano M, Zarrelli A. C-4 gem-dimethylated oleanes of *Gymnema sylvestre* and their pharmacological activities. Molecules. 2013 ;18(12):14892-14919.
14. Farooqui AA, Sreeramu BS. Cultivation of medicinal and aromatic crops. Universities Press; 2004.
15. Gopi C, Vatsala TM. In vitro studies on effects of plant growth regulators on callus and suspension culture biomass yield from *Gymnema sylvestre* R. Br. Afr. J. Biotechnol. 2006;5(12). 1215-1219.
16. Gowthami R, Sharma N, Pandey R, Agrawal A. Status and consolidated list of threatened medicinal plants of India. Genet. Resour. Crop Evol. 2021 ;68(6):2235-2263.
17. Goyal MR, Birwal P, Mishra SK, editors. Phytochemicals and Medicinal Plants in Food Design: Strategies and Technologies for Improved Healthcare. CRC Press; 2022 May 18.
18. Gupta D, Solanki A. In vitro response of *Gymnema sylvestre*: A review. Indian J. Plant Sci. 2015;4(1):52-59.
19. Harikumar C, Malarkodi K, Srimathi P. Density grading on seed quality and seed recovery in *Gymnema Sylvestre*. Madras Agric. J. 2000;87(1/3):166-167.
20. Jones Jr JB. Complete guide for growing plants hydroponically. CRC Press; 2014 Feb 13.
21. Karoshi VR, Hegde GV. Vegetative propagation of *Gymnema sylvestre*, an important medicinal plant-a research report. Indian Forester. 2001;127(9):1067-8.
22. Karthic R, Seshadri S. Cost effective mass multiplication of *Gymnema sylvestre* in hydroponic system. Nat. Preced. 2009 ;16:1-3.

23. Kirtikar KR, Basu LM. Indian Medicinal Plants. Allahabad. Jayyd Press, New Delhi, India. 1987; 2:146.
24. Komalavalli N, Rao MV. In vitro micropropagation of *Gymnema sylvestre*—A multipurpose medicinal plant. Plant Cell, Tissue Organ Cult. 2000; 61:97-105.
25. Kumar A, Jnanesha AC. Agriculture Practices for Diabetes Medicinal Plants *Gymnema Sylvestre* R. Br.(Gurmar). 2017;16 (06):13-18.
26. Laha S, Paul S. *Gymnema sylvestre* (Gurmar): A potent herb with anti-diabetic and antioxidant potential. Pharmacogn. J. 2019;11(2): 201-206.
27. Lee EJ, Hahn EJ, Paek KY. Effects of chemical and physical environments on cell culture of *Gymnema sylvestre*. International Workshop on Medicinal and Aromatic Plants. 2008 Jan 15 pp. 273-278.
28. Lee EJ, Mobin M, Hahn EJ, Paek KY. Effects of sucrose, inoculum density, auxins, and aeration volume on ceil growth of *Gymnema sylvestre*. J. Plant Biol. 2006; 49:427-431.
29. Madhavan S, Sha K. Effect of organics along with azosprillum (Root and Soil Isolate) on growth and leaf yield in *Gymnema* (*Gymnema sylvestre* R. Br). Int. J. Pharmacogn. Phytochem. 2019;8(2S):582-585.
30. Maene L, Debergh P. Liquid medium additions to established tissue cultures to improve elongation and rooting in vivo. Plant cell, tissue and organ cul. 1985;5:23-33.
31. Mohan S, Devasenapathy D, Venilla C, Gill MS. Pest and disease management in organic ecosystem. IPM Booklet, Tamil Nadu University, India. 2010.
32. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 1962; 15:473-497.
33. Nadkarni AK. Indian Materia Medica :Popular book depot. Mumbai. 1954; edition-1956, page-461.
34. Padmapriya S, Kumanan K, Rajamani K. Studies on effect of organic amendments and bio-stimulants on morphology, yield and quality of *Gymnema sylvestre* R. Br. Afr. J. Agric. Res. 2010;5(13):1655-1661.
35. Padmapriya S, Rajamani K. Standardization of post-harvest technology for *Gymnema sylvestre* and *Plectranthus forskohlii* (Wild) Briq (Syn: *Coleus forskohlii*). J. Med. Aromatic Plants Sci. 2016;4(4):11-17.
36. Palada MC, Crossman SM, Kowalski JA, Collingwood CD. Evaluation of organic and synthetic mulches for basil production under drip irrigation. J. Herbs Spices Med. Plants.2000;6(4):39-48.

37. Pandey AK. Cultivation technique of an important medicinal plant *Gymnema sylvestre* R Br (Gurmar). Academic J.Plant Sci. 2012;5(3):84-89.
38. Prakash A, Adholeya A. Effect of different organic manures/composts on the herbage and essential oil yield of *Cymbopogon winterianus* and their influence on the native AM population in a marginal alfisol. Bioresour. Technol.2004 1;92(3):311-319.
39. Prakash OM. IPM schedule for aonla pests. Ext. Bull. no. 5, National Horticulture Mission, Ministry of Agriculture and Cooperation, Government of India, Delhi, India, 2012.
40. Rao S, Ramakrishna A. Indian Medicinal Plants: Uses and Propagation Aspects. CRC Press; 2020 Oct 26.
41. Ravikumar A, Rajendran R, Chinniah C, Irulandi S, Pandi R. Evaluation of certain organic nutrient sources against mealy bug, *Coccidohystrix insolitus* (Green.) and the spotted leaf beetle, *Epilachna vigintioctopunctata* Fab. on Ashwagandha, *Withania somnifera* Dunal. J. Biopestic.2008;1(1):28-31.
42. Santosh TD, Maitra S. Effect of drip irrigation and plastic mulch on yield and quality of ginger (*Zingiber officinale*). Res. Crop. 2022;23(1):211-219.
43. Sharma PV. Dravyaguna vijñana, Reprint. Varanasi: Chowkhamba Krishnadas Academy. 1983:10-15.
44. Shivakumara KT, Keerthi MC, Polaiah AC, Thondaiman V, Manivel P, Roy S. Seasonal abundance of Oleander aphid, *Aphis nerii* Boyer de Fonscolombe and its predator on *Gymnema sylvestre* (R. Br) in relation to weather parameters from India. Int. J. Trop. Insect Sci. 2022;42(2):1925-1932.
45. Singh BG, Anandalakshmi R, Warriar RR, Sivakumar V and Kumar AM. Root ability of *Gymnema sylvestre* stems cuttings as influenced by the presence of nodes. J. Non-Timber Forest Products. 2008; 12(11): 36-37.
46. Solanki A, Gupta D. Effect of Antioxidants and Carbon source on Callus Culture of *Gymnema sylvestre* R. Br. Res. J. chem. Environ. Sci. 2013c;1(5):121-125.
47. Solanki A, Gupta D. In vitro dedifferentiation from different explants of *Gymnema sylvestre*. Biologix. 2013b;2(1):116-121.
48. Solanki A, Gupta D. In vitro shoot multiplication of *Gymnema sylvestre* R. Br. Adv. Plant Sci. 2013a;26(2):321-323.
49. Subatra DC and Srinivasan MV. In vitro propagation of *Gymnema sylvestre*. Asian J. Plant Sci. 2008;7: 660-665.

50. Syedy MO, Nama KS. In vitro propagation of shoots and callus induction of *Gymnema sylvestre* R. Br. "an important anti-diabetic plant". Int. J. Curr. Pharm. Res. 2018;10(3):60-64.
51. Thakur RS, Puri HS, Hussain A. Major medicinal plants of India, Central Institute of medicinal and aromatic plants. India: Lucknow. 1989:1-100.
52. Tiwari P, Mishra BN, Sangwan NS. Phytochemical and pharmacological properties of *Gymnema sylvestre*: an important medicinal plant. Biomed Res. Int. 2014;6:1-18.
53. Vijay A. Effect of season and PGR on rooting in Gudmar (*Gymnema sylvestre*). Int. J. Agric. Stat. Sci. 2016;12(1):273-275.
54. Warrier PK. Indian medicinal plants: a compendium of 500 species. Indian Medicinal Plants. Vol. 1-5. Orient Longman Ltd., Madras. 1995.
55. World Health Organization. WHO guidelines on good agricultural and collection practices [GACP] for medicinal plants. World Health Organization Geneva; 2003 Dec 16.

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