

Ephedra foliata: Only Gymnosperm from Rajasthan - Need for conservation and propagation

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ABSTRACT

Among Genus *Ephedra* species only *Ephedra foliata* is reported in Rajasthan. It belongs to family Ephedraceae and it is distributed in temperate and sub-tropical area of world. It has several traditional uses for treatment in Chinese and other medicine. It contains alkaloids Ephedrine and pseudo ephedrine, which are of great importance. It is propagated by seed but due to low seed germination, micropagation is used from mass propagation. *Ephedra foliata* is a mild CNS stimulant & also have cardio vascular effect.

Keywords: *Ephedra foliata*; Ephedraceae; Germination percentage; Mean germination time; Average germination value.

1. Introduction

Ephedra is one of the largest genera of the Ephedraceae family which is distributed in arid and semi-arid regions of the world. The *Ephedra* genus (Ephedraceae family) is one of the oldest medicinal plants known to human kind and consists of 69 species mainly distributed in semi-arid environments through both the palearctic and Nearctic realms, although some species are distributed through few neotropical countries (Hollander et al., 2010). The species grouped in this genus are among the few gymnosperms adapted to arid environments. They are perennial and dioeciously and several species are rarely found shrubs and more commonly, vines (example *Ephedra equisetina* (Buyw) (Ickert et al., 2016).

Among 69 species of *Ephedra* genus, four subspecies and two accepted varieties are also reported which are widely distributed in arid and semi-arid regions of Asia, Europe, Northern Africa (saha), South western, North America and South America. Among these 13 species occur in North America (Hollander et al., 2010; Ickert et al., 2016 and Bolinder et al., 2016). Diversification of North American *Ephedra* species may be due to the organogenesis and climatic changes documented for these region related to the expansion of arid lands in compare to adaptations to new climatic condition (Loera et al., 2012). In central and Northern Mexico there are 8 species of *Ephedra* viz. *E. antisiphilitica* Berland ex. C. A. Mey, *E. aspera* Engelm exs. Watson, *E. californica* S. watson, *E. compacta* Rose, *E. nevadensis* S. watson, *E. pendunculata* Engelm ex S. watson, *E. californica* S. watson, *E. compacta* Rose, *E. nevadensis* S. watson, *E. pendunculata* Engelm ex. S. watson. *E. torreyana* S. watson and *E. trifurca* Torr ex. S. watson.

It has been suggested that *Ephedra* was perhaps a more genus in the cretaceous and early tertiary in a period from the early middle Oligocene to the Miocene (Huang and Price 2003) in the lower cretaceous (Yang et. al. 2005), in the early cretaceous (Rydin et al., 2004; Wang and Zheng 2010). And after the cretaceous tertiary boundary (Rydin et al., 2010).

The altitudinal range of the genus varies from depression below sea level (Death Valley in California and the Dead Sea area) to about 5000 meter in the Andes of Ecuador and to 5300 meter in the Himalayas (Ickert et al., 2016).

Ephedra foliata Boiss ex. C.A. Mey belongs to family Ephedraceae. It is commonly known as "Unthphog" and Shrubby horse tail is widely distributed in desert of Africa, Arabian Peninsula and India.

In India it is found in arid and semi-arid region of the north western part (Lodha et al., 2014). It is the only gymnosperm that grows sporadically on sand hills in arid and semi-arid area in the Thar Desert of India. At present *E. foliata* is considered to be a threatened species in India (IJCN 2017; Meena et al., 2019). The plants are woody climber with highly reduced scale like foliage leaves arranged in a decussate pattern on nodes. The branches and seeds of this plant are an important component of the diet of Camel, Sheep, Goat and Insect species serving in the nutrient poor arid desert of Rajasthan. It also acts as a major soil binder (Singh et al., 2007).

Ephedra foliata is known by various synonyms viz. *Ephedra aitchisonii* (Stapf), *Ephedra alte Beandis*, *Ephedra asparagoides* Griff, *Ephedra ciliata aitch*, *Ephedra foliata variety aitchisonii* stapf, *Ephedra variety ciliate* (Fisch and C.A. Mey) (stapf) *Ephedra foliata variety polylepis* (Boiss and Hausskn) stapf, *Ephedra kokanica* Regel, *Ephedra penduncularis* Boiss, *Ephedra polyepis* Boiss and Hausskn, and *Ephedra rollandii* Maire (GBIF-<http://www.gbif.org/species/2653400/synonyms>). *Ephedra foliata* is known by various common names in Arabic as Alanda warakia, fedr waraki and Al-kuwodat-warak and in English as Shrubby horsetail.

1.1. Study objective: Study objective was to explore current situation and various efforts used or needed for its conservation and propagation.

1.2. Distribution: *Ephedra* genus is indigenous to the temperate and subtropical latitudes of Europe, Asia, and America and grows especially in northern and western China, Northern India and Spain. In the United States, *Ephedra* plant grows along the Rocky Mountains (Ebadi 2007).

Ephedra foliata is native to North Africa and South West Asia. It is distributed in Asia (Afghanistan, India, Punjab, Iran, Iraq, Jordan, Palestine, Kuwait, Qatar, Baharin, Saudi Arabic, United Arab Emirates, Oman and Yemen) and in Africa (Algeria, Egypt, Mauritania, Morocco, Somalia, Djibouti and Ethiopia (Bell and Baehman 2011; Miller and Moris 2004; Dobignard and Chatelain 2011).

1.3. Traditional Uses: The Chinese dispensatory written in 1569 mentions that *Ephedra* species were valuable as an antipyretic, diaphoretic, circulatory stimulant and seductive for cough, However *Ephedra* has been used in traditional Chinese medicine to treat allergies, asthma, lung congestion, chill, cold, Hay fever, coughs, edema, fever, flu, Headache and nasal in Russia for respiratory disorders and rheumatism for many centuries. The Native Americans and Spaniards of the South western United States and *Ephedra* for various medicinal purposes, especially venereal disease (Al snafi 2017; Bell and Bachman 2011). An active principle was isolated by Yamanashi in 1885. In 1887 Nagai obtained the alkaloid in pure formed and named it "Ephedrine". Pharmacological investigation indicated that the drug was toxic; mydriatic and Sympathomimetic (Ebadi 2007).

The species is known to contain the alkaloids Ephedrine and pseudo ephedrine, which are of great importance for their biological and pharmacological potential (Ghiasvand et al., 2019). It is used to treat bronchial asthma, hypersensitivity, fever, influenza, chills, cold, cerebral pains, nasal blockage and other respectively problems

(Elhadef et al., 2020). It possess antimicrobial, antioxidant, Antidiabetic, Hepato protective and cardio vascular activity (Al-Snafi. 2017).

1.4. Propagation: Plant is conventionally propagated through seeds. The percentage seed germination and establishment is low due to biotic stressors like high ambient temperature and soil alkalinity which are common severe environment condition of the region. Pre dispersal seed predation and post-dispersal seed predation by insects group, rodents and other burrowing animals is a serious issue in respectively recruitment of new *E. foliata* population (Singh et al., 2007). Beside this anthropogenic activities also have impact on the dwindling population of *E. foliata* (Singh 2004; Lodha et al., 2014).

1.5. Arbuscular mycorrhizal fungi and Drought stress mitigation in *Ephedra foliata*: Arbuscular mycorrhizal fungi (AMF) are one of the most important drive of soil ecosystem dynamics. AMF have the potential to improve plant growth and development by modulating keep hormonal pathway which usual in decreasing the advance impact of abiotic stress such as drought. In a pot experiment it was found that non-inoculated AMF, *E. foliata* plants exhibit –induced growth in response to drought stress with a concomitant lowering of chlorophyll pigments, relative to non-stressed and AMF inoculated plant.

AMF inoculated *E. foliata* showed improved nitrogen metabolism by positively regulating nitrate and nitrite reductase activity which results in greater ammonium availability for the synthesis of amino acids. AMF inoculation was found to increase antioxidant enzyme activity, ascorbic acid contents and reduction in glutathione level. This result in significant amelioration of oxidative damage to plant membrane by restricting the excess generation of reactive oxygen species (ROS) such as hydrogen peroxide, creator content of proline, glucose and total soluble protein in AMF-inoculated plants provided other benefits to *E. foliata* plants and their ability to withstand drought stress and also evident by a greater level of sucrose phosphate synthetase activity. AMF significantly enhances essential nutrients uptake like K, Mg, and Ca. Thus AMF inoculation of *E. foliata* plants significantly reduced the dexterous effect of drought stress by up regulating the antioxidant defense system, synthesis of osmolytes and maintaining phytohormone level.

1.6. Micro propagation: As its seed germination is problematic this micro propagation is an efficient method for mass propagation. In fact this method is perfect method for mass propagation of threatened plants via direct and indirect organogenesis under in vitro condition for ex-situ conservation, genetic improvement and commercial application without any seasonal limitations.

In vitro propagation of *E. foliata* was reported by various researchers (Lodha et al., 2014; Lodha et al., 2014 b). In order to developed efficient protocol with economical and practical application indirect organogenesis was done using intermodal explants. Maximum callus inclusion (80.3 percent) was achieved on Murashige and Skoogs (MS medium) supplemented with 0.5 mg per liter, 2,4-dichlorophenoxyacetic acid +0.5 mg/liter kinetin and additives (50 mg/liter ascorbic acid and 25 mg/liter citric acid). It was found that maximum shoot regeneration was achieved on MS medium containing 6 mg/liter kinetin followed by 5 mg/liter 6-Benzeyeamino purine. MS medium half strength with 3 mg/liter ortho-naphthaline acetic acid resulted in the highest rooting percentage (32%). Among

all 60 percent of the plantlets survived during acclimatization and were successfully transferred for enhanced bioactive constituent and germplasm conservation. In vitro propagation of female *Ephedra foliata* Boiss and Kotschy ex Boiss does made using micro propagation (Lodha et al., 2014).

In India Thar Desert *Ephedra foliata* is an ecological and economically important threatened Gymnosperm using nodal explants of mature female plants a method for micropagation of *E. foliata* has been developed. Maximum bud break (90%) of the explants was obtained on MS medium supplemented with 1.5 mg/liter of benzyl adenine (BA) + addition. Explants produce 5.3 ± 0.40 shoot from single node with 3.25 ± 0.29 cm length. The multiplication of shoots in culture was affected by salt composition of media, type and concentration of plant growth regulators (PGRs) and their interaction; time of transfer of the culture. Maximum number of shoots (26.3 per culture vessel) was regenerated on MS medium modified reducing concentration of nitrate to half with the supplemented and under ex vitro on soilrite moistened with one fourth strength of MS mean salts treating shoot base with 500 mg/liter IBA for 5 minute. These were hardened in green house (Lodha et al., 2014).

1.7. Chemical constituents: *Ephedra foliata* produces ephedrine and pseudoephedrine (Ramawat 2010; Khanna and Uddin 1976). The total alkaloids contents of *Ephedra foliata* were 0.04-0.2 percent (Bahernik et al., 2000).

Callus of *E. foliata* produce 0.1% ephedrine and pseudoephedrine under white light. However in blue and red light exposes to culture tissue produce elevated chemicals and it increased the production by three folds (Shukla 1980). In *Ephedra foliata* total phenolic contents and total flavonoids was found in 52.6 mg Gallic acid equivalents/g dry weight and total flavonoids contents was 25 mg/g dry weight (Ibragic and Sofic 2015).

1.8. Hepatoprotective effect: *Ephedra foliata* as hepatoprotective was studied in Wistar albino rats. Liver injury in rats by using carbon tetrachloride was induced. The biochemical parameters; serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphates (ALP) and total bilirubin were estimated as reflection of the liver condition. The hepatoprotective effect offered by *Ephedra foliata* (whole plant) crude extract at 500 mg/kg doses, was found to be significant in all parameters studied with 42.6, 39.5, 21.2 and 46.2 percent reduction in SGOT, SGPT, ALP and bilirubin, respectively.

Ephedrine stimulated both alpha and beta receptors. It is due to partly direct action on the receptors and partly indirectly by neither releasing nor adrenaline from its tissue stores. It is also a mild CNS stimulant. It has following pharmacological effects: 1. Cardiovascular effects, 2. Bronchodilation, 3. Nasal decongestion, 4. Mydriasis, 5. Nocturnal enuresis, 6. Spinal anesthesia, 7. CNS stimulation, 8. Weight lost effect, etc.

2. Material and Methods

Seeds of *Ephedra foliata* were collected and after proper cleaning and drying they were stored for further analysis. Seed length, width and thickness were recorded for 100 seeds of each seed lot.

3. Result

Only one sample of seed was found and data regarding its seed length and width were taken. Very few seed were found on plants and their Mean seed length was 6.49 and mean seed width 3.19.

Table 1. Seed size of *Ephedra foliata*

Place of collection	Date of collection	Seed Analysis	
		Mean length (mm)	Mean width (mm)
Nagpur	05-03-2023	6.49	3.19

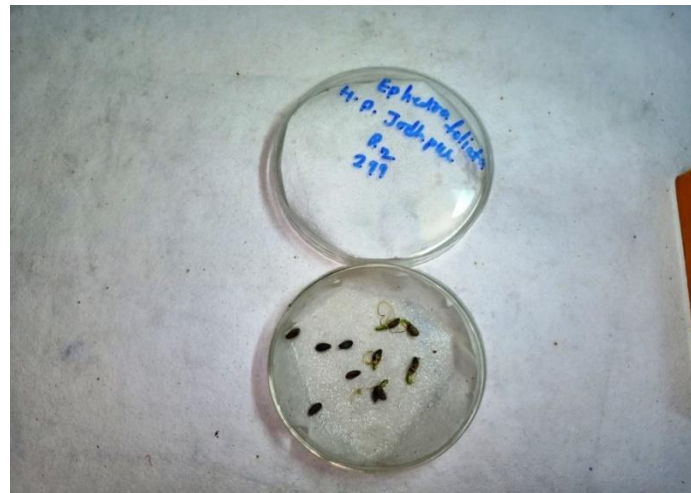


Figure 1. Seed germination of *Ephedra foliata*



Figure 2. Seeds of *Ephedra foliata*

4. Conclusion

Ephedra foliata is an important species which is nearly rare in Rajasthan. There is need to conserve and propagate the species as well developed method for mass propagation. There is also need to carry out further studies on its uses in different aspects.

5. Future Recommendation

The plant is very rare in Rajasthan and its seed availability is very poor. There is an urgent need to conserve and propagate plant for biodiversity conservation of this only Gymnosperm available in the area.

Declarations

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Competing Interests Statement

The authors declare no competing financial, professional, or personal interests.

Consent for publication

The authors declare that they consented to the publication of this study.

Authors' contributions

All the authors took part in the literature review, analysis, and manuscript writing equally.

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