

RESEARCH ARTICLE

Reproductive Strategies and Conservation Measures for Threatened Taxa, *Guaiacum officinale* L.

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Manuscript details:	ABSTRACT
<p>Date of publication 18.10.2014</p> <p>Available online on http://www.ijlsci.in</p> <p>ISSN: 2320-964X (Online) ISSN: 2320-7817 (Print)</p> <p>Editor: Dr. Arvind Chavhan</p> <p>Cite this article as: Kumbhare Shravan D, Kamble Rahul B and Chaturvedi Alka (2014) Reproductive Strategies and Conservation Measures for Threatened Taxa, <i>Guaiacum officinale</i> L. Int. J. of Life Sciences, 2014, Special Issue A2: 81-84.</p> <p>Acknowledgement: Authors are thankful to Authorities, Agricultural College, Nagpur for permitting study in MaharajbagZoo, Nagpur and Forest Department, Nagpur, Govt. of Maharashtra.</p> <p>Copyright: © Author(s), This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derives License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.</p>	<p><i>Guaiacum officinale</i> belonging to family Zygophyllaceae is native to the West Indies from the Greater Antilles through to Bonaire, and Aruba; also in Panama, Colombia and Venezuela. This tree was naturalized in India from long years back. The species is listed in Appendix II of CITES (Convention on International Trade of Endangered Species). <i>G. officinale</i> is one of two species yielding the true lignum vitae, the other being <i>Guaiacum sanctum</i>. There was no any work documented earlier on this important taxa <i>Guaiacum officinale</i>. So keeping this view in mind, the work on reproductive biology conducted which expose the behavior of its pistil and concluded as the reason behind less percentage of fruit formation. Besides this, <i>in vitro</i> practices with the help of plant tissue culture techniques were performed on <i>Guaiacum officinale</i> and achieved callus induction as best response on anthers as an explants with hormonal concentration, Kinetin (0.3mg/L) and 2, 4-D (1.5mg/L).</p> <p>Keywords: <i>Guaiacum officinale</i>, Conservation, threatened, reproductive biology, <i>in vitro</i> studies.</p> <p>INTRODUCTION</p> <p><i>Guaiacum officinale</i> L. is a slow-growing broad leaf, evergreen tree or shrub reaching 9-12 m in height. Leaves are thick. Each leaf is composed of 2 or 3 pairs of smooth, stalk less leaflets arranged on a slender mid-rib. Beautiful bright blue flowers grow in great profusion and almost cover the tree and remain for a long time. The flowers grow in clusters at the ends of the branches. Fruits are capsule and become yellow when ripped. Seeds are black colored with red colored aril.</p> <p><i>Guaiacum officinale</i> is under the endangered category of IUCN (International Union for Conservation of Nature) and the major reason for this is the overexploitation of the plant for various purposes (IUCN). Besides this other reasons can also affect the plant, whose study was not conducted previously. Tissue culture is widely accepted technique as one of the best tool to perform conservation practices for those species which difficult to reproduce in natural way. This study concentrates on reasons other than concluded by IUCN which responsible for endangerment of the taxa as well as <i>in vitro</i> studies with the help of plant tissue culture were also conducted.</p>

MATERIALS AND METHODS

Study area: The selected area for the survey; Nagpur (220.08 km²), the second capital of Maharashtra State, situated at 21°13'N longitude and 79°6'E latitude. It is also one of the greenest cities of India (Figure No.1). Climate of the city is characterized by minimum temperature in December (5°C to 7°C). Maximum temperature is during May (around 45°C). In general the climate is semi-arid with unimodal monsoon. On an average 1205mm rainfall is received during rainy season (Chaturvedi et al., 2003). The Nagpur district is quite rich and varied in its plant composition. According to Flora of Nagpur District (Ugemuge, 1986) there are 1136 plant species which fall under 679 genera and 142 families.

Study Method: The study has three major objectives, first is the finding the distribution of *Guaiaicum officinale* in the study area. Second most important is quantification of degree of threat to the plants and the third important objective is to find the reasons behind less productivity through the reproductive studies and to overcome these threats, *in vitro* practices with the help of plant tissue culture techniques. The basic methodology adopted for the study was field survey in which observations were recorded in different seasons.

Species threats have been analyzed at two levels, first at the habitat level and second at the species level. The habitat level analysis is holistic in approach whereas species level analysis is selective in nature (Srivastava, 2012). The methodology adopted for threat analysis included recording of field observations.

To understand the pollen viability as one of the tool for analysis of threat to selected trees, following method was applied.

(A) Reproductive Biology

To understand the reproductive biology of *Guaiaicum officinale*, following studies were conducted.

(a) Maceration of anthers: In this method the thin transverse section of the anther of *Guaiaicum officinale* was taken. The section was treated with mixture of acetic acid and ethyl alcohol (1:3 proportions) for 10 min. after withdrawing this mixture the sections placed in 1N HCl for 10 min at 60°C in oven. Then HCl was replaced with 1% methyl green stain in a basic P_H by adding Sodium bicarbonate (NaHCO₃). Then section was placed in 50% Glycerol and mount on the slide and observations were made.

(b) Pollen viability test: Pollen viability test is conducted with Evans blue (or T-1824 Make- Himedia) stain. The Evans blue stain used for its accuracy in penetration into non-viable cells (Shivanna and Rangaswamy; 1993). The mature anther is taken and its pollen grains were dusted on the drop of Evans blue stain taken on the glass slide and after some time observations were made.

(c) Pollen pistil interaction: This technique is used to find whether the stigma is receptive for pollen grains or not. The test is proceeded in the following manner. The pistil was fixed in the modified Carnoy's mixture (6:4:1, Absolute alcohol+ Chloroform+ Glacial Acetic Acid) for one hour and transfer to the water through a descending series of ethanol and finally few amount of the staining mixture at 45°C for 12 hours. Then the stained pistil was transferred to clearing and softening mixture and then hydrolyzed in the hot air oven at 58°C for 30 minutes. Material is then washed twice in lactic acid and mount in the mounting medium and observed under the microscope.

(B) Tissue culture of *G. officinale*

For *G. officinale*, anthers were used as the explants for tissue culture supplemented with the MS (Murashige and Skoog, 1962) medium with the hormonal concentration of Kinetin (0.3mg/L) and 2, 4-D (1.5mg/L).

RESULTS AND DISCUSSION

Only 4 individuals of *Guaiaicum officinale* were found, 3 of which present in Maharajbag and single found in the civil lines. All individuals are healthy and attract lots of pollinators with its beautiful attractive flowers. Loss of habitat and small fragments of habitat can only support small populations of plants and animals and small populations are more vulnerable to extinction. Minor fluctuations in climate, resources and other factors unremarkable and quickly corrected in large populations can be catastrophic in small, isolated populations. Thus fragmentation of habitat is an important cause of species extinction (Rosenzweig, 1995). The selected taxa threatened due to habitat loss and also in danger due to their overexploitation and are affected by their reproductive behavior. The work on reproductive biology conducted under the project expose the behavior of its pistil. The pistil is very less receptive to pollens and doesn't allow or make it very difficult for pollen grains to germinate on its stigmatic surface. Anthers do not possess any structural

problem and pollen development is not affected since, all the tissues of anther are well developed and functional. So, it is concluded as the reason was found

for very less fruit formation in *G. officinale* is its stigma unreceptively.

Table No. 1: Threats and Causes for low population of *Guaiacum officinale*.

Plant Name	Location	NoI	MH	MG	MC	PO	Threats and Causes of Rarity
<i>Guaiacum officinale</i>	Maharajbag Civil Lines	3 1	9	164	85	Butterflies Honey bees(<i>Apis mellifera</i>), Ants (<i>Crematogaster</i> species), Wasps	Habitat fragmentation Loss of habitat Very low percentage(20%) of fruit formation Unavailability of the seeds Very slow growing plant Overexploitation
(Note: NoI: Number of Individuals, MH: Mean Height (in meters), MG: Mean Girth (in centimeters), MC: Mean Canopy (in percentage), PO : Pollinators Observed)							

Table 2: Reproductive biology of *Guaiacum officinale*

Sr. No.	Test	Observation
1.	Maceration of Anthers	Anther tissues are well developed and functional
2.	Pollen viability test	53.71%
3.	Pollen Pistil interaction	Stigma is unreceptive or very less receptive
4.	Fruit setting	20%

Table 3: Anther culture of *Guaiacum officinale*

Tube no.	Type of explant	Duration					
		5 days	10 Days	15 days	20 days	25 days	30 days
1.	Anther	Swelling	Callus initiation	Callus growth	Callus growth	Callus growth	Compact <i>Calli</i>
2.	Anther	NR	NR	NR	NR	NR	NR
3.	Anther	NR	NR	NR	NR	NR	NR
4.	Anther	NR	NR	NR	NR	NR	NR
5.	Anther	Swelling	Callus initiation	Callus growth	Callus growth	Callus growth	Compact <i>Calli</i>
6.	Anther	NR	NR	NR	NR	NR	NR
7.	Anther	Swelling	Callus initiation	Callus growth	Callus growth	Callus growth	Compact <i>Calli</i>
8.	Anther	Swelling	Callus initiation	Callus growth	Callus growth	Callus growth	Compact <i>Calli</i>
9.	Anther	Swelling	Callus initiation	Callus growth	Callus growth	Callus growth	Compact <i>Calli</i>
10.	Anther	NR	NR	NR	NR	NR	NR

(Note: NR: No Response)

Table 4: Callus induction on MS medium In Anther explant of *Guaiacum officinale*

Type of Explant	No. of Explants inoculated	No. of Explants responded	% Response	Time Duration for Response
Anther	10	5	50%	5-6 days

Fruits of *Guaiacum* species dehisced to reveal fleshy, bright-red. This bright color comes from an aril that covers the seed. The aril inhibits germination (Alexander 1966) and must be removed before germination will occur. No study was found regarding the reproductive biology of *G. officinale*. No any literature on *in vitro* practices were found on *Guaiacum officinale* so, because of its rarity *in vitro* of *G. officinale* was conducted with tissue culture under this study which responded with good result (50%) when anther was taken as explant with hormonal concentration of Kinetin (0.3mg/L) and 2,4-D (1.5mg/L).

CONCLUSION

The total of only 4 individuals of *Guaiacum officinale* present in Nagpur city and these healthy attractive individuals possesses an average of 85% canopy. The study concluded that the taxa facing greatest threat not only because of habitat loss but its own reproductive strategies are also generating barriers for its propagation. No any major problem observed in study of pollinator interactions and anther structure and its function but the taxa bears great difficulty in stigmatic receptivity to pollens which can be a probable cause for very less number of fruit formation.

In the point of conservations measures, this study may help to plan future conservation strategies for these valuable endangered taxa with the help of plant tissue culture techniques. Loss of these individuals by any reason will disrupt the entire gene pool in diversity of plants in Nagpur city. Proper conservative measures should be taken for the survival of these plants.

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