Eco. Env. & Cons. 30 (May Suppl. Issue) : 2024; pp. (S470-S474)
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ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2024.v30i03s.083

High reproductive performance in a dioecious taxon irrespective of asynchronous flowering patterns: a case study of *Zanthoxylum armatum* DC.

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(Received 12 January, 2024; Accepted 7 March, 2024)

ABSTRACT

Zanthoxylum armatum, an immensely valuable medicinal plant of Himalaya has been assessed for its phenological and reproductive patterns. Being a plant with highly asynchronous flowering in both male and female plants, this species boasts of an adequate percentage of fruit set when analyzed in two ecologically different populations in the Jammu province of the UT of Jammu and Kashmir. Data from the year 2020 to 2023 reveals 77.08% and 83.47% fruit set in Sukrala and Dhandal populations respectively. Thus, the present study indicates the presence of a substitute pathway to sexual reproduction for fruit production in the species.

Key words: *Medicinal, Himalaya, Flowering, Asynchronous, Ecologically, fruit.*

Introduction

The forests of Himalaya boast high levels of species endemism and rich biodiversity, with phenological activity peaking distinctly during autumn, setting them apart from other temperate forests (Zobel and Singh, 1997). Despite this uniqueness, there have been few efforts to investigate the morphology, phenology, and reproductive strategies of dioecious broad-leaved tree species in this range. One such species, *Zanthoxylum armatum* of the Rutaceae family, holds significant medicinal and economic value but remains understudied in terms of its phenological behavior and reproductive strategies.

Known commonly as Indian prickly ash and referred to locally as tejphal (Hindi), tejowati (Sanskrit), and timur (Nepali), this plant plays vital roles in medicine, household applications, commerce, and ethnobotany. Its fruits, leaves, bark, and seeds are utilized for treating various ailments such as toothaches, headaches, fevers, diarrhea, dysentery, and tonsillitis (Phuyal *et al.*, 2018). Species is a rich source of diverse phytochemicals, including alkaloids, amides, lignals, and sterols (Medhi *et al*., 2013).

Z. armatum is a deciduous shrub or small tree thriving in well-drained alluvial and black soil. Its habitat ranges from the warmer valleys of the Himalayas in Jammu and Kashmir to Assam and the Khasi region (1,000 to 2,100 meters above sea level), to the Eastern Ghats in Orissa and Andhra Pradesh (at 1,200 meters), and to the lesser Himalayan regions in northeastern India, including the Naga Hills, Meghalaya, Mizoram, and Manipur (Kala *et al*., 2005). In its natural environment, it typically grows up to 6 meters in height, adorned with dense foliage and armed with branched flattened prickles. Its compound leaves, ranging from 4 to 20 centimeters in length, are imparipinnate, winged along the rachis, serrate with gland dots, and emit an aromatic fragrance reminiscent of lime and mint.

The species produces flowers on separate male and female plants. Male flowers form on axillary or terminal panicles, featuring 4-8 bright yellow anthers, while female flowers develop on dense panicles, characterized by green hues and 1-4 pistils. Ripe fruit follicles are typically reddish and measure 4 to 5 millimeters in diameter. The dried fruit carries an aroma present in the brown fruit wall (pericarp-shell), potentially inducing a numbing or anesthetic sensation on the tongue. Seeds are solitary, globose, shiny, and possess a bitter taste. Flowering predominantly occurs during March and April in the subtropical climes of UT of J&K, where the present study was carried out.

The escalating market demands, coupled with questionable collection methods and insufficient harvesting skills, have led to a significant decline in the population of *Z. armatum* in its natural habitat, consequently impeding its regeneration.

Despite extensive research into the pharmacological properties of Z. armatum (Singh and Singh, 2011; Phuyal *et al.*, 2018), limited information exists on its overall biology, with even fewer studies focusing on its sexual reproduction. Understanding the phenology and reproductive strategies of this species is crucial for devising conservation strategies. Hence, this study aims to elucidate the flowering phenology and reproductive behavior of *Z. armatum*.

Materials and Methods

Study site

The study was conducted in two populations belonging to two different districts of Jammu and Kashmir. Both the populations namely, Sukrala and Dhandal, belonging to Kathua and Udhampur district respectively vary in geographical as well as population aspect. The population of Sukrala was a pine forest and that of Dhandal was a fixed forest at the elevation of 1093 and 696 masl respectively.

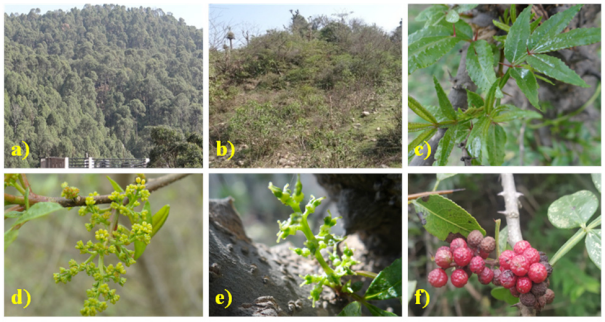

Fig.1. Study sites and floral morphology
(a) Sukrala, b) Dhandal, (c) Compound leaves with spines, (d) Male inflorescence, (e) Female inflorescence, (f) Mature fruits

Flowering phenology and Synchrony

To study the flowering phenology of both males and females on individual as well as population levels, 15 plants from each sex were selected. Observations from initiation of bud formation to initiation of fruit formation were recorded. Having a short phenophase of a few days, all observations recorded are in days. On an individual level, following observations on flowering phenology were recorded:

i) Duration : Number of days that each individual of both the sexes remains in flowering
ii) Date of first flowering : First date that an individual starts flowering

On population level, following observations were made

i) Total duration: Total number of days that the male/female population remains in flower

Table 1: Morphometric data of the vegetative and floral parts of *Zanthoxylum armatum* DC.

S.No.	Character	Male	Female
1	Height of the plant (m)	3.01±0.239*(1.9-3.9)**	2.23±0.191(1.3-3.1)
2	Length of compound leaf (cm)	7.22±0.828(4.3-12.4)	8.34±0.753(4.9-11.7)
3	Length of leaflet (cm)	0.69±0.058(0.4-1)	0.76±0.060(0.5-1.1)
4	Breadth of leaflet	0.67±0.071(0.4-1.1)	0.693±0.03(04-1)
5	Length of spine (cm)	0.68±0.008(0.4-0.9)	0.452±0.023(0.1-0.8)
6	Length of inflorescence (cm)	3.22±0.099(2.8-3.7)	3.55±0.082(29-3.78)
7	No. of inflorescence per branch	20.8±1.114(15-26)	18.6±1.147(13-25)
8	No. of flowers per inflorescence	39.5±1.393(13-53)	28.35±1.64(19-45)
9	No. of stamens per flower	5.8±0.249(4-7)	-
10	No. of pistils per flower	-	2.3±0.231(2-4)

ii) Mean duration : mean time that one sex remains in flower: corresponds to the length of each individual phenophase divided by the total number of individuals
iii) Peak bloom : days maximum for an individual floral display
iv) Index of synchrony of a given sex with its conspecific

$$Xi = \frac{\Sigma ij}{(N-1)fi}$$

Where, ij = number of days that both sexes male (i) and female (j) were in flowering synchronously
N = Number of individuals in a population
fi = Number of days that individual i is in bloom
When Xi = 1, perfect flowering synchrony occurs, or there is a complete overlap between the flowering periods of individuals i and j in the population; when Xi = 0 flowering does not occur synchronously, or there is no overlap in the flowering periods of those individuals. Intermediate values between 0 and 1 indicate partial flowering synchrony.

Reproductive Output

To study the reproductive output in *Z. armatum*, fruit set was recorded on open pollination, manual pollination and bagging of female inflorescences in both the populations.

Results

Flowering Phenology

The species started its phenophase in the beginning of February with male plants initiating flowering earlier than the females. Commencement of bud formation started in the 1st week of February in males, while it started a few weeks later, i.e. in 3rd week of February in females in Sukrala population whereas it started in 4th week in males and 2nd week in females in Dhandal population. Flowering among male individuals was more asynchronous than females. Even though bud initiation is almost at the same time among the individuals of a particular sex, male flowers show great irregularity in their flowering times on the same plant as well as between different plants. Females, however, flower almost synchronously (Table 2). Few insects i.e. *Calliphora sp.* and *Eristalis sp.* are amongst the common visitors on both male and female inflorescences. However, the frequency and duration of visitation is more on male inflorescences than their female counterparts.

Value of index of synchrony between the individuals of male and female plants corresponds to **0.125**, revealing very low level of flowering synchrony between the two sexes.

Anthers dehisce by longitudinal slits. Anther dehiscence in some flowers begins in last week of Feb in Sukrala population, a time at which female flowers have not yet attained maturity and stigma receptivity. Stigma usually becomes receptive by the 2nd week of March. Being a unisexual flower, receptivity was determined by observing pollen load and percentage pollen germination on stigmatic surface on different times and days. Similar case was observed in Dhandal population also (Fig. 2). Maximum stigma receptivity was observed between 11:30-13:00 hrs, which also corresponds to the period of maximum insect visitation. Anthesis and receptivity timeline has been shown in Fig. 3. Only 5-8 days of overlapping period was observed between anther dehiscence and stigma receptivity.

Reproductive Output

Fruits of *Zanthoxylum armatum* are bivalved capsules turning from green at young stage to red and finally to brown at ripening. Since number of carpels varies in a flower, therefore, a varying number of fruits (1-3) was observed per flower.

Data on fruit and seed set was recorded during the three year study (2020 to 2022) on open pollination, manual pollination and bagging of female inflorescences (Table 3). Fruit set recorded from all the pollination experiments was invariably higher in

Table 2. Time frame of phenological events in male and female plants of *Zanthoxylum armatum*

S. No.	Phenological event	Sukrala		Dhandal	
		Male	Female	Male	Female
1	Date of first flowering	17 Feb	02 Mar	25 Feb	10 Mar
2	Duration	20-29 days	22-29 days	20-28 days	22-29 days
3	Total duration	28-30 days	26-30 days	27-30 days	25-26 days
4	Mean duration	16.93 days	25.13 days	14.25 days	26.24 days
5	Peak bloom	03 Mar-19 Mar	12 Mar-29 Mar	16 Mar-30 Mar	25 Mar-09 Apr

Table 3. Data on fruit and seed set in *Zanthoxylum armatum*

Population/ Parameter	Open pollination		Manual pollination inflorescences		Bagging of female	
	Sukrala (28.35±1.64) (2.4±0.18)	Dhandal (27.94±1.39) (2.3±0.139)	S	D	S	D
Fruit set/ Inflorescence	20.62±2.95 (15-26)	18.46±2.33 (15-22)	15.77±0.71 (12-22)	13.77±0.68 (10-19)	10.46±2.22 (2-25)	8.08±1.49 (0-5)
% age fruit set/inflorescence	79.39	65.61	66.07	49.27	36.89	28.92
Fruit set/ Flower	1.86±0.18 (1-3)	1.93±0.16 (1-3)	1.5±0.14 (1-2)	1.64±0.16 (1-3)	1.26±0.98 (1-2)	1.12±0.25 (1-2)
%age fruit set/ flower	77.08	83.47	62.5	71.3	47.66	44.47

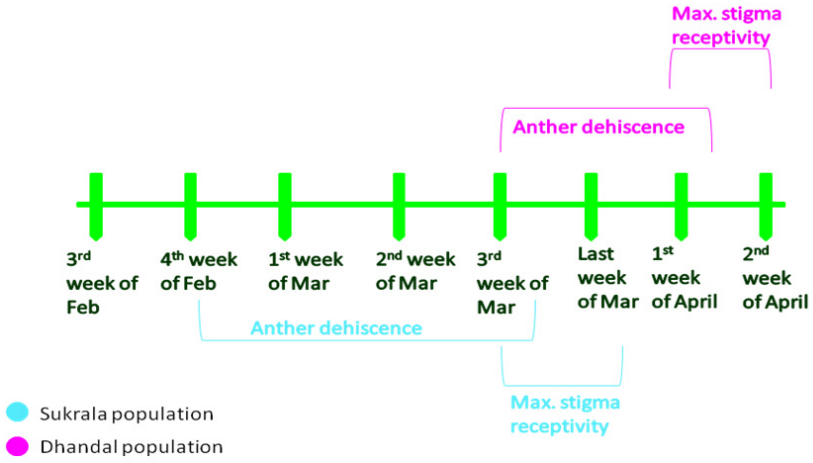

Fig. 2. Timeline of anther dehiscence and stigma receptivity in *Zanthoxylum armatum*

Sukrala population than Dhandal. Reproductive output in terms of fruit set was highest on open pollination, followed by manual pollination and the least in bagging of inflorescences, indicating the occurrence of apomixis in the species.

Discussion

The timing and intensity of flowering in male individuals often precede those of female individuals in *Z. armatum*. This pattern in dioecious taxa, supported by several studies (Bullock and Bawa, 1981; Otarola *et al*., 2013; Roca and Sazima, 2016), manifests over an extended period and results in a notably higher flower production rate in males compared to females (Delph et al., 1996; Murphy, 1998). This discrepancy in flowering intensity is attributed to the greater number of flowers per axil observed in male individuals, a trait commonly seen in dioecious species (Bawa, 1983). Similar observations have been made in related species like *D. sericea* (Santos and Sano, 2004; Foresto *et al.*, 2018) and across the entire Ebenaceae family (Wallnofer, 2001).

In the context of successful sexual reproduction, synchronization of flowering between male and female plants is crucial. However, our study revealed a relatively brief eight-day period of overlapping peak bloom, indicating low synchrony between male and female flowering events. Intriguingly, despite this asynchrony, fruit set was observed even when female flowers attained stigma receptivity after the dehiscence of male flowers.

While synchronous flowering is considered a primary requirement for successful fertilization in plants, the observed low synchrony between male and female flowering in *Z. armatum* did not seem to significantly affect its reproductive behavior, as evidenced by the adequate percentage of fruit set. These findings raise the possibility of an alternative method of fruit formation in *Z. armatum*, one that bypasses the traditional sexual pathway.

A growing demand of *Z. armatum* has caused a

serious reduction in native population as a consequence of over harvesting and deforestation. As a result, target species has been reported as a "Endangered" species as per International Union for Conservation of Nature (IUCN) criteria (Samant *et al*., 2007). Natural regeneration mainly takes place through seeds. Given these insights, there arises an urgent need to delve into the developmental pathway of fruit formation in *Z. armatum* and devise reproductive propagation protocols aimed at enhancing the multiplication and conservation of this valuable plant species.

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