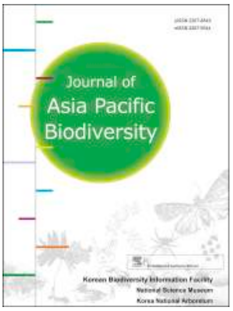

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Original article

# Sexual dimorphism and pollination mechanism of *Zanthoxylum armatum*, a vulnerable medicinally important dioecious tree

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## ABSTRACT

Among dioecious species, the differential strategy of resource allocation between male and female plants is depicted in their life-history traits and reproductive features. This is classified as sexual dimorphism. The strategies of resource allocation along with the habitat of these species pose an impact on their pollination mechanism and reproductive success. *Zanthoxylum armatum* DC (Rutaceae) is a small tree with wide economic and medicinal value. The species is naturally distributed in tropical parts of India and other countries. Due to overexploitation of natural populations in India, the species is rapidly declining. To restore the population of the species in the wild and establish commercial plantations, detailed knowledge of its reproductive biology is essential. Thus, the present study was conducted in natural population of *Z. armatum* growing in Union territory of Jammu and Kashmir, India. The study revealed sex biased allocation and sexual dimorphism both in vegetative and reproductive traits. Such traits include more height and higher number of shoots, inflorescences and flowers in male plants as compared to female plants. The natural populations of *Z. armatum* exhibit male-biased sex ratio and such biasness appears to be the outcome of resource allocation strategies and other demographic characteristics. Species possibly exhibit presence of ambophilous suite, i.e., a combination of wind and insect pollination in floral features. However, pollen dispersal by wind is up to limited distance and thus wind may or may not be effective in fruit-set.

**Keywords:** Dioecy, Floral biology, Entomophily, Sexual dimorphism, sex-ratio

**Running Title:** Reproductive Biology of Zanthoxylum armatum

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## Introduction

Dioecy has been reported in nearly 7% of the genera and 43% of the families of angiosperms (Renner, 2014). The dioecious species are known to exhibit differential strategies of resource allocation between male and female plants which is depicted in their life-history traits (vegetative growth, plant size) and reproductive traits (phenology, floral traits, flower production, flower size, etc.). In dioecious taxa, male and female individuals may differ both in the timing of flowering, and the amount of resource that are allocated to vegetative growth and sexual reproduction (Putwain and Harper, 1972; Delph, 1999; Barrett, 2010). This differential strategy and timing of resource allocation leads to sexual dimorphism (Agren, 1988; Ramsey and Schemske, 2002; Mangla et al., 2020).

The male plants tend to allocate more resources for their vegetative growth, and exhibit early and frequent flowering than their female counterparts (Puixeu et al., 2019). In contrast, the female plants allocate more resources for production of fruits and thus have less flowers and lower vegetative growth (Sakai et al., 1995; Harris and Pannell, 2008; Vaughton and Ramsey, 2011). The differences in resource allocation between sexes may be more pronounced among perennial dioecious taxa (Vamosi and Vamosi, 2004, Ohya et al., 2017). Since the female plants need more resources for their survival, their mortality rates are generally higher than male plants (Puixeu et al. 2019; Mangla et al., 2020). Thus, less female plants can be observed in natural populations, leading to male-biased sex ratio. A comprehensive study by Field et al., (2013), based on the sex ratio of 243 angiosperm species belonging to 61 families has indicated that male-biased sex ratios in natural populations are frequent among dioecious taxa. Such biasness in sex ratio is favored by certain ecological factors viz, nutrient availability, moisture, light as well as genetic factors like gender determination mechanism (Espírito Santo et al., 2003; Yakimowski and Barrett, 2014).

The sexual dimorphism and male-biasness caused by variable resource allocation strategy between the sexes usually results in unique pollination requirements for successful fruit and seed set (Obeso, 2002; Barrett and Hough, 2013). It is variously opined that dioecy is closely associated with wind pollination. However, the prevailing habitat conditions and the availability of pollinators (mostly generalist insects) may act as driving forces for shift towards entomophilies (Culley et al., 2002; Vamosi et al. 2003; Freidman and Barrett, 2008) in tropical woody dioecious taxa. The closed canopy in tropical forest poses a hindrance on wind to carry pollen far from the source (Bullock, 1985; Thomas and La Frankie, 1993; Renner and Ricklefs, 1995; Vamosi et al., 2003). Nonetheless, due to insect availability, the dioecious taxa may offer rewards to them in form of pollen and/ or nectar and thus exhibit a combination of both wind and insect pollination. This dual mode of pollination i.e., ambophily is seen as a mechanism to ensure fruit set (Bawa, 1980; Goodwillie, 1999; Mangla and Tandon, 2011). In such cases, the sex biased allocation and sexual dimorphism becomes more pronounced in the male plants as they produce more floral attractants and rewards (number of flowers per plant, scents, colour, nectar, pollen) than their female counterparts (Hessey and Pannell, 2011; Puixeu et al., 2019), leading to better attraction of the insects and effective pollen dispersal (Kaplan and Mulcahy, 1971; Sharma, 1991; Hamal, 2015; Sharma, 2015).

The genus *Zanthoxylum* (family: Rutaceae) is disturbed in several pantropical regions of the world, though several species are also present in the temperate zone of eastern Asia and North America (Kubitzki et al., 2011). The genus is interesting in view of the fact that certain species viz. *Z.setulosum*, *Z. fagara , Z, ailanthoides* demonstrate wind/insect pollination while others viz. *Z. americanum and Z. simulans* exhibit apomixis (Munter et al., 2018). In India about 13 species have been reported (Sikarwar et al., 2023). The present study was conducted on *Zanthoxylum armatum* DC. a woody, evergreen dioecious species commonly known as Indian prickly ash or toothache tree. The species *Z. armatum* is restricted in its distribution to countries of Southeast Asia (including India, China, Nepal, Bhutan, Pakistan, Bangladesh, Nepal, Vietnam, Indonesia, Cambodia, Thailand, and Malaysia) (Phuyal et al., 2019). In India, it grows naturally in the Union territory of Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Orissa, Khasi hills, Assam and Andhra Pradesh at an altitudinal range of 600-2100 m (Kumar et al., 2010; Pant and Pant, 2011; Singh et al., 2011; Paul et al., 2018). The plant holds important pharmaceutical and biological properties as the fruits and seeds are used in folk medicines as well as by pharmaceutical companies to produce medicines to treat arthritis, dental disorders, asthma, bronchitis, dyspepsia etc. (Phuyal et al., 2019). Due to low regeneration and overexploitation, the natural populations of *Z. armatum* are depleting rapidly, and according to the International Union for Conservation of Nature, the species is given the status of endangered to Vulnerable in various part of India (Kumar et al., 2010; Pant and Pant, 2011; Sikarwar et al., 2023). Thus, the present study was conducted to study reproductive biology (including details on phenology, floral biology, sex ratio, reproductive traits and pollination) of the species in natural populations. Being a dioecious taxon differential resource allocation strategy between sexes and incidences of the sexual dimorphism were also investigated.

## Material and methods

### *Species and Study sites*

The present study was conducted in the Union territory of Jammu and Kashmir, India. The intensive field investigations were carried out in three populations namely: (1) Sukrala: N-32º 38.478 and E-075º35.299, 1073m above sea level (asl), (2) Bhaddu: N-32º38.479 and E-075º35.300, 691m asl, and (3) Dhandal: N-32º38.057 and E-075º35.464, 696m asl; were marked for present study. The plants of the species were growing as understory shrub to small trees in the pine-forests at Sukrala, along with other mixed thorny vegetation (*Berberis lyceum* Royle., *Carissa opaca* Stapf., *Flacourtia indica* Merr. etc.) while at Bhaddu plants of the species were growing understory of the Oak forest (*Quercus leucotrichophola* A. camus). The population at Dhandal was in mixed forest with slopy terrain. The mean annual temperature of the study site is 22ºC. The study area receives an average annual rainfall of about 174.1 mm (India Meteorological Department, Srinagar, J&K).

### *Sex ratio*

Assessment of sex ratio was carried in the marked populations during the flowering season (first week of February-last week of March) for three consecutive years (2014-2016). The sex ratios in the populations were determined by using the line transects method. Transects (n=15 to 20) of 30m were drawn randomly in each population. All plants of each sex all along the transect were included in calculations. Sex of the plant was determined by studying morphology of randomly collected flowers. The proportion of male to female plants was computed from the data obtained from line-transect study and compared using a Chi square goodness of fit test (Zar, 1996) to access the deviation from expected 1:1 sex ratio.

### *Phenology, vegetative traits and floral biology*

Phenoevents comprising leaf senescence and flushing, onset and duration of flowering and fruiting were recorded on the marked plants (n=10 each sex) in each population. The height, number of branches (first order), and size of prickles were recorded for the marked male and female plants to study the sex biased differences in vegetative traits.

During the period of full bloom, number of inflorescences produced on the male and female plants (n= 5 each sex) were counted manually. Further, mature inflorescences were marked randomly on the male and female plants to count the number of flowers per inflorescence. For floral anthesis and anther dehiscence, flowers (n=30), both on male and female plants were observed at regular intervals on different days between 0600h-1800h. Anther dehiscence was also studied in lab under the stereo zoom microscope (SMZ Nikon, Japan) in the flowers of different ages starting from one day before flower opening. For male flowers, the length of sepals, petals, and stamen, and for female flowers, length of sepals, petals, and gynoecium were measured using ocular stage micrometer. Number of anthers per male flowers (n=100) the number of carpels per female flowers (n=100) and number of ovules per carpel (n=100) was recorded from randomly collected flowers under stereo zoom microscope.

The detailed morpho-anatomical features were studied by scanning electron microscopy and sectioning of glycol methcrylate embedded male and female flowers. For the same, the flowers were randomly collected from the marked plants and fixed in Karnovsky's fixative. Further protocol followed for scanning electron microscopy was as described by Gupta et al. (1998). The process of resin sectioning and staining was followed as described by Feder and O'Brien (1968).

For estimation of pollen production per male flower, anther (n = 30) was squashed in a drop of 1% acetocarmine on a clean glass slide. The slide was scanned under the compound microscope to count the number of pollen grains. This number multiplied by the number of anthers per male flower yielded pollen production per male flower. To count the mean ovule production in female flowers, carpels (n=50) were gently dissected in a drop of 1% acetocarmine and ovule number was counted to estimate the number of ovules per carpel. The number multiplied by the number of carpels per female flower yielded ovule production per female flower. From the mean values of pollen and ovule production, pollen: ovule ratio was calculated (Shivanna and Tandon 2014)

The data was analyzed through ANOVA with post hoc Tukey's test, by considering population as fixed factor, and season traits (vegetative and reproductive traits), and sex as variable. The variations between study seasons were not significant. The variation between study populations was also not significant for vegetative and reproductive traits among the different reproductive seasons. Hence, data was pooled and comparison between sexes was done with t-test. The statistical package for the social science (SPSS. 25) was used for analysis. The mean values are presented with standard error.

### *Pollination biology*

#### *Anemophily*

To determine the role of wind pollination in *Z. armatum*, sticky slides method was employed (Kearns and Inouye, 1993). The 1×1 $cm^2$ areas were marked on the glass slides (of size

7.5×2.5 cm$^2$) and smeared with Mayer's albumen (equal volume of egg white and glycerol). These slides were hung on T-shaped wooden stands and kept at fixed distances (0, 1, 2, 3, 4, 6, 8, and 10 m) from the male plants. The slides were kept in early morning (~0600 h) on the day of experiment and collected after 24 hrs. At each point, 3-5 smeared slides were kept. The experiment was conducted during the peak time of flowering (15th to 30th march 2014-16) in each population and was repeated thrice. To count pollen grains on sticky slides, a slide with pollen grains of the species was kept for reference. The data was obtained from a total of 45 slides in the study period.

### *Entomophily*

During the peak flowering period, the male (n=30) and female (n=20) plants were marked and observations were made for insect visitation, followed by their peak activity period in 24 hrs. For night-time observations, hand-held battery-operated torch was used. Finding the absence of nocturnal foragers on the plants (of both genders), observations was subsequently kept confined between 0500 and 1800 hours. Between the duration, insects were observed for their foraging behavior and flower-handling time (using stopwatch). For the correct identification, insects were trapped using a net and preserved in a glass jar, lined with cotton soaked in ethyl acetate. The collected insects (n=10, of each type) were observed under the stereo zoom microscope, in order to check the deposition of pollen on their body parts. Individually, the insects were washed in 70% ethanol and number of pollen grains of the species, obtained from the body of the insect, was counted, and the total pollen load was determined (Dafni and Calder, 1987; Kearns and Inouye, 1993; Dafni, 2005).

### *Fruit set*

Mature female flowers (n=100) were tagged in the field for observing fruit set on open pollination. These were monitored at regular interval of 24-hrs for 10 days to check fruit set/initiation. Percentage fruit set at the level of infructescence was calculated as:

$$\frac{\text{Number of fruits formed}}{\text{Number of flowers} \times \text{average number of carpels/flower}}$$

## Results

### *Sex ratio*

In the Dhandal population, a total of 41 plants (25 male and 16 female) were found at a minimum distance of 8m. Sex ratio was male-biased (1.5 ♂: 1 ♀). While in the population of Sukrala, a total of 50 plants were present out of which, 24 were male, 25 were female and one was in vegetative phase. Thus, the sex ratio resides near 1♂: 1♀. Some male and female plants were growing side by side; the distance between some of them being less than 2m, even their branches were overlapping. In the Bhaddu population only 26 plants (16 male and 10 female) were found. The minimum and maximum distance recorded between plants was 10m and 22m respectively. Thus, the male plants out number female plants in this population with the ratio being 1.6 ♂: 1♀. Overall, the male-biasness in sex ratio was statistically significant as observed by Chi square goodness of fit test ($\chi 2 = 5.4$, p= 0.019, $p < 0.05$.) when all populations taken together (n=116: 65 male plants, 51 female plants).

*Phenology, vegetative traits and floral biology*

In the marked populations, the plants start to shed their leaves when the mean temperature reached 15°C or below (in the month of December). Flowering initiates in second-third week of February and the canopy of the species was nearly leafless during flowering Peak flowering was observed during second week of March. There was variability in the timing and pattern of flowering among different sexes and at population level. Generally, male flowers appeared prior to the female flowers by 5-10 days in the populations. However, male and female plants at Sukrala population showed synchronous flowering. By the end of March, leaf flush was observed in both sexes. The fruit initiation was observed by mid-April and mature fruits were present on female trees during months of July-August.

*Vegetative traits*

The difference in mean height of male and female plants was significant (F (1,55) =21.2, R2=0.43, p<0.00)amongst all the populations (Table 1), male and female plants of Bhaddu being the tallest among all. The mean height of male trees in Dhandal, Sukrala and Bhaddu was: 4.72±1.55m, 3.26±0.77m and 6.55±3.69m respectively, while mean height of female trees in these populations was 2.56±0.52m, 2.53±0.77m and 3.24±1.22m respectively. The differences in mean height of male and female plants were significant amongst all the populations (Table 1).

The mean number of branches produced by a male plant was higher (652.3±36.57) as compared to the female plants (370.35±22.81). The prickles present on the male plants were also longer than those on the female plants (Table 1).

*Floral Biology*

*Inflorescence and flowers*

The flowers on both the male and female plants of *Z. armatum*, were borne in dense terminal or sparse axillary panicles that arise in alternate manner from the axil of leaves or from stem axis directly (Figure 1A, E). The mean number of inflorescences (33466.67±1383) produced on a male plant was higher and almost two-fold to the number in female plants (15200.00±304). On an average, a male inflorescence bears 36.6±1.9 flowers, while a female inflorescence produces 34.03±1.9 flowers (Table 1). The flowers (both male and female) were small, monochlamydous and have 6-8 reduced perianth lobes of about 1mm length. The average size of a male and a female flower was 3.5±0.14mm and 3.69±0.10mm respectively (Table 1).

*Male flower*: In male flowers, a fleshy and lobed cushion like structure 'receptacle' was present in the centre. The stamens were borne on margins of this receptacle. On an average 5.61±0.16 (4-8) stamens are borne per flower (Figure 1B). Mature anthers were bright yellow in colour. Average pollen production per flower in the species was 32,976±379 (Table 1). It takes an average of 31 days for a male inflorescence to dehisce anthers of all its flowers, while the individual flower takes approximately 14-16 days from its differentiation until the stage of dehiscence. Interestingly, a male flower takes approximately 7 days to dehisce its anthers completely as the pollen release was gradual.

Scanning electron micrographs of the receptacle of the male flower revealed the presence of numerous nectaries, surrounded with kidney shaped cells i.e., nectarostomata (Almeida et. al, 2013; Wist and Davis, 2006). The mean number of nectarostomata on the surface of a nectary disc was found to be 53.4±0.8 (Figure 1C, D).

*Female flower*: The female flowers of *Z. armatum* were green and showed the presence of apocarpous gynoecium with 1-4 carpels. Each carpel consists of an ovary, a short style, and dry bulbous and papillate stigma. A short thick gynophore (~1.25mm in length) was also present (Figure 1F). The mean number of carpels per flower was 2.13. Invariably, in flowers dissected from all the three populations, the number of ovules per carpel was 2. The walls of carpel also showed traces of secretions and presence of nectarstomata, though the number was less (18.2±0.9) than that of male flowers. The calculated pollen: ovule ratio for *Z. armatum* was 7799.08:1.

### *Stigmatic movements in female flowers*

In a female inflorescence, there were remarkable differences in maturity of female flowers. On close examination of an inflorescence, differential stigmatic movement and hence different maturity levels of female flowers were recognized. In a young flower, the stigma and style of individual carpel were straight and apprised. At maturity, the stigmas of neighboring carpels start to bend to their opposite sides. In case of pollination failure, the stigma and upper part of the style of adjacent pistils gradually diverge to form an angle of $180^0$. The maximum distance between two stigmas measured at this point was 2.17mm. Thereafter, unpollinated carpels degenerated and fall off. Followed by successful pollination, the colour of female flower gradually changes from green to yellowish-green, the ovary swells and showed fruit initiation (Figure 1F-G).

### *Pollination*

#### *Anemophily*

To check wind assisted pollen dispersal, the sticky slides were observed under compound microscope for pollen grains. Fewer pollen grains were observed on the slides at several point distances, and also the number of pollen grains on the slides (per $cm^2$) decreased as the distance from the male plants was increased. The maximum distance at which pollen were observed on these slides was 3m, beyond which (i.e., at 4, 6, 8m) slides did not show presence of pollen grains. The mean pollen load at 1m, 2m and 3m distance was 171±25.7, 65±19.22 and 10.25±3.21, respectively (Figure 2).

#### *Entomophily*

Flowers of *Z. armatum* are briskly visited by several insects during the flowering period. Their frequency and visitations were found to be variable at different times of the day (Table 2). The most frequent visitor of the male flowers was *Episyrphus balteatus* (commonly known as marmalade hoverfly) resembling superficially to brown honey bees (Figure 3A). During peak flowering, it constitutes more than 50% of the total insect visitors (Table 2) and visit female flowers

(Figure 3A-C). Other insect that visit both male and female flowers of the species only during peak blooming period was blue flies belonging to genus *Calliphora* (3D-E). Among other less frequent insect visitors were the *Sarcophaga sp.*, small flies, some bugs and rarely some wasps. Of all the insects, *Episyrphus balteatus*, *Calliphora sp.* and small hover flies were common to both male and pistillate plants and were present in every population. Of these three insects, pollen load was found only on *Episyrphus balteatus* and *Calliphora*, making them legitimate pollinators (Table 2).

### *Fruit set*

Mature fruit in *Z. armatum* can be seen on the female plants approximately after 5 weeks of flowering (by second-third week of March). Percentage fruit set per infructescence in the three populations was: 65.38% (Sukrala), 62.76% (Bhaddu) and 56.03% (Dhandal) with a cumulative average of about 61%. Fruit is a bivalve capsule, which remains green in young stages and turns red to brown at maturity.

## Discussion

The present study documents the presence of sexual dimorphism and biased sex allocation in vegetative and reproductive traits of *Z. armatum*, along with a male-biased sex ratio in natural populations of the species. It also suggests the existence of insect pollination and limited wind-assisted pollen dispersal.

Male plants of *Z. armatum* exhibit sexual dimorphism in vegetative traits, including greater height, higher number of branches and thorns, and a denser canopy compared to female plants. Reproductive traits too show male-biased dimorphism, with males producing significantly more inflorescences and flowers per inflorescence. Such contrasting strategies of reproduction highlight gender related differences in reproductive allocation in the species (e.g. in *Lithraea molleoides*; Galfrascoli and Calvino, 2020). Female plants, bearing the metabolic cost of fruit and seed production, require more resources, leading to reduced growth, higher mortality in xeric conditions, and delayed and less frequent flowering (Obeso, 1997; Nicotra, 1998; Charlesworth and Charlesworth, 1981; Ataroff and Schwarzkopf, 1992; Obeso, 2002; Schultz, 2009; Clark and Clark, 1987; Antos and Allen, 1999). In contrast, male plants, with lower reproductive investment, grow stronger and produce more flowers, giving them a competitive advantage in resource-limited environments, resulting in a male-biased population (Llyod and Webb, 1977; Eppley, 2001). These findings align with previous studies on dioecious taxa, including *Fuchsia thymifolia*, *Oemleria* *cerasiformis*, and *Myrica esculenta* (Allen and Antos, 1993; Khanduri et al., 2019). Contrary observations were noted in *Z. ailanthoides*, where female-biased reproductive allocation and sexual dimorphism were reported (Matsuyama and Sakimoto, 2010). A recent study by Londoño-Echeverri et al. (2021) has also reported sexual dimorphism in *Z. magnifructum* from tropical dry forest in Colombia.

Dioecious species usually exhibit 1:1 primary sex ratio, but actual sex ratios vary in a population due to differential reproductive costs, resource allocation, and environmental factors (Barrett and Hough, 2013; Field et al., 2013; Pannell et al., 2014; Mangla et al., 2020). Thus, natural populations become either male-biased or female-biased. The natural population of species investigated in the present study also showed male biasness. The present results are in consonance of notion that the dioecious taxa growing in xeric conditions and tropical climates are known to exhibit male-biased populations (Renner and Rickelfs, 1995; Vamosi et al., 2003; Soza et al., 2012).

The floral biology of *Z. armatum* includes traits favoring both wind (anemophily) and insect (entomophily) pollination. The anemophilous floral traits in the species include reduced perianth in flowers, profuse flowering in male plants, high pollen production, dry-papillate type of stigma, reduced ovule number and high pollen-ovule ratio . Additionally, the flowering in the sparse leaf plant canopy provides a chance for better dispersal of the pollen grains. The typical suite of insect pollination is presence of nectar, and yellow anthers. In addition, change in colour of carpels and stigmatic movement also seems to be the strategies which assist insects to distinguish pollinated vs. unpollinated flowers. The present study demonstrated both wind and insect pollination; however the relative contribution of both the modes is not firmly established due to some anthropogenic factors viz. monkey menace and heavy exploitation of wood. In addition, more vegetative growth in terms of height among male plants aids in effective dispersal by wind. Also, prior anthesis in male flowers and gradual pollen dispersal are aids to anemophily (Friedman and Barrett, 2008; 2009; Mangla and Tandon, 2014). However, it is notable that the wind dispersed pollen reaches to a maximum distance of 3m only. This can be chiefly due to sticky pollen grains, and wind may not be effective to carry such pollens to a greater distance as the species grows as understory tree.  Low wind velocity and vegetation hindrance in tropical forests may pose a risk of reproductive failure for diclinous species relying solely on wind pollination (Bawa, 1980; Renner and Ricklefs, 1995; Vamosi et al., 2003). Consequently, dioecious taxa may show an evolutionary shift towards entomophily due to demographic factors and availability of insects (Culley et al., 2002; Vamosi et al., 2003). In this context, *Z. armatum* exhibits floral traits supporting both wind and insect pollination, suggesting this evolutionary shift, as wind-assisted pollen dispersal appears limited and may not significantly contribute to fruit set. This observation contrasts/supports previous studies in the genus Zanthoxylum, where either winds (*Z. setulosum*, *Z. fagara*; Bullock, 1994) or insect pollination *(Z. ailanthoides*; Abe, 2006) has been reported. The limitation of wind-assisted short distance pollen dispersal in *Z. armatum* must have been overcome by insect pollination through generalist pollinators. This is in consonance with other diclinous taxa like *Sagittaria sp.*, *Plantago* etc. where generalist insect carried out pollination (Glaettli and Barrett, 2008; Sharma et al., 1993).

The remarkable role of sexual dimorphism can also be recorded and highlighted in the lieu of pollination mechanism and reproductive success of the species. On an average, an infructescence produces about 61% fruits, demonstrating the importance of insects for fruit set, as wind carries pollen only up to 3 meters. In the Sukrala population, where male and female trees were growing in this range; the fruit set was highest among the studied populations. This observation does not allow us to neglect the role of anemophily in fruit-set. In Dhandal and Bhaddu, the inter tree distance was much greater and limitation of distance seems to be overcome by *Episyphrus balteatus* (brown bee) and *Calliphora sp.* (blue bee) as these insects are known to travel greater distances (Raymond et al., 2013). In zoophilous unisexual flowers, larger male floral display ensures maximum visitation of pollinators, which provide better mating opportunity. For example, in *Thymelaea hirsuta* the yellow anthers help in attracting pollinators (Eckhart, 1999; Caporali et al., 2006). Likewise, in male flowers of *Z. armatum*, bright yellow anthers at anthesis, en-masse blooming and condensed inflorescence on male trees serve as cues for insect pollinators. This suggests that sexual dimorphism in vegetative and reproductive traits of *Z. armatum* may be associated with the different modes of pollination in the species.

From the present study it is concluded that the natural populations of *Z. armatum* are male-biased and exhibit sex biased allocation and sexual dimorphism in vegetative and reproductive traits. The male-biased sex ratio in natural populations appears to be the outcome of disparities in resource allocation strategies between sexes and other demographic characteristics but further investigations are needed. Species is also unique in combining sexual dimorphism with sex biased allocation and ambophilous suite, i.e., a combination of wind and insect pollination. Further studies may be directed to decipher the eco-evolutionary state of ambophily as well as underlying genetic and physiological machinery resulting in dimorphism in the taxon.

## Conflict of Interests

Authors declare no conflict of interests.

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## Figure legends

**Figure 1.** Floral biology features of *Z. armatum*. A. A young male inflorescence. A male flower is marked with arrow showing yellow anthers. B. A male flower showing receptacle (r) and stamens (an: anther, f: filament). C. Scanning electron micrograph showing nectarostomata (arrow) present on the receptacle of male flowers. D. vs: vasculature the part of transverse section of receptacle showing the nectarostomata (arrow). E. A female inflorescence. A female flower is marked with arrow F. The different stages (0-3) of female flowers exhibiting stigmatic movement.0-carpels where style (st) and stigma (sg) are apprised; 1-3 shows the increasing distance and curvature between styles of adjacent carpels of a female flower (ov: ovary) G. The ovaries of two carpels of a female flowery showing fruit initiation. <scale bars: 7 mm (A); 2mm (B); 100µm (C); 400 µm (D); 8 mm (E); 2 mm (F-G)>

**Figure 2.** Graph showing the pollen grains density (per cm$^2$) as observed on stick slides. The wind mediated pollen flow in *Z. armatum* was restricted upto 3 monly. No pollen grains were further observed at further distances i.e. 4, 6, 8, 10 m at each point distance the mean values are presented with standard error.

**Figure 3.** Pollinators of *Z. armatum* A. Intense visitation and foraging activity of *Episyphrus balteatus* (arrows) at the time of peak flowering in male plants. B. An *Episyphrus balteatus* sitting on receptacle and foraging the male flowers. Pollen load can be observed on their legs (arrow). C. An *E. balteatus* on female flowers. D and E. *Calliphora* sp. visiting the male and female flowers respectively. The pollen load (arrow) can be seen sticking to the hairs present on the body of insect.

## Table caption

**Table 1.** Comparative summary of vegetative and reproductive traits in *Z. armatum* highlighting the presence of sexual dimorphism. The variation between sexes for vegetative and reproductive traits is significant. However, variation between the populations was not significant (except height) for number of branches, size of thorn, and reproductive traits (flower production etc.) as well as between the different reproductive seasons. Hence, the data was pooled gender-wise.

**Table 2.** Summary of insects foraging activity, mean flower handling time, mean pollen load on pollinators body parts during their visit to male and female flowers.

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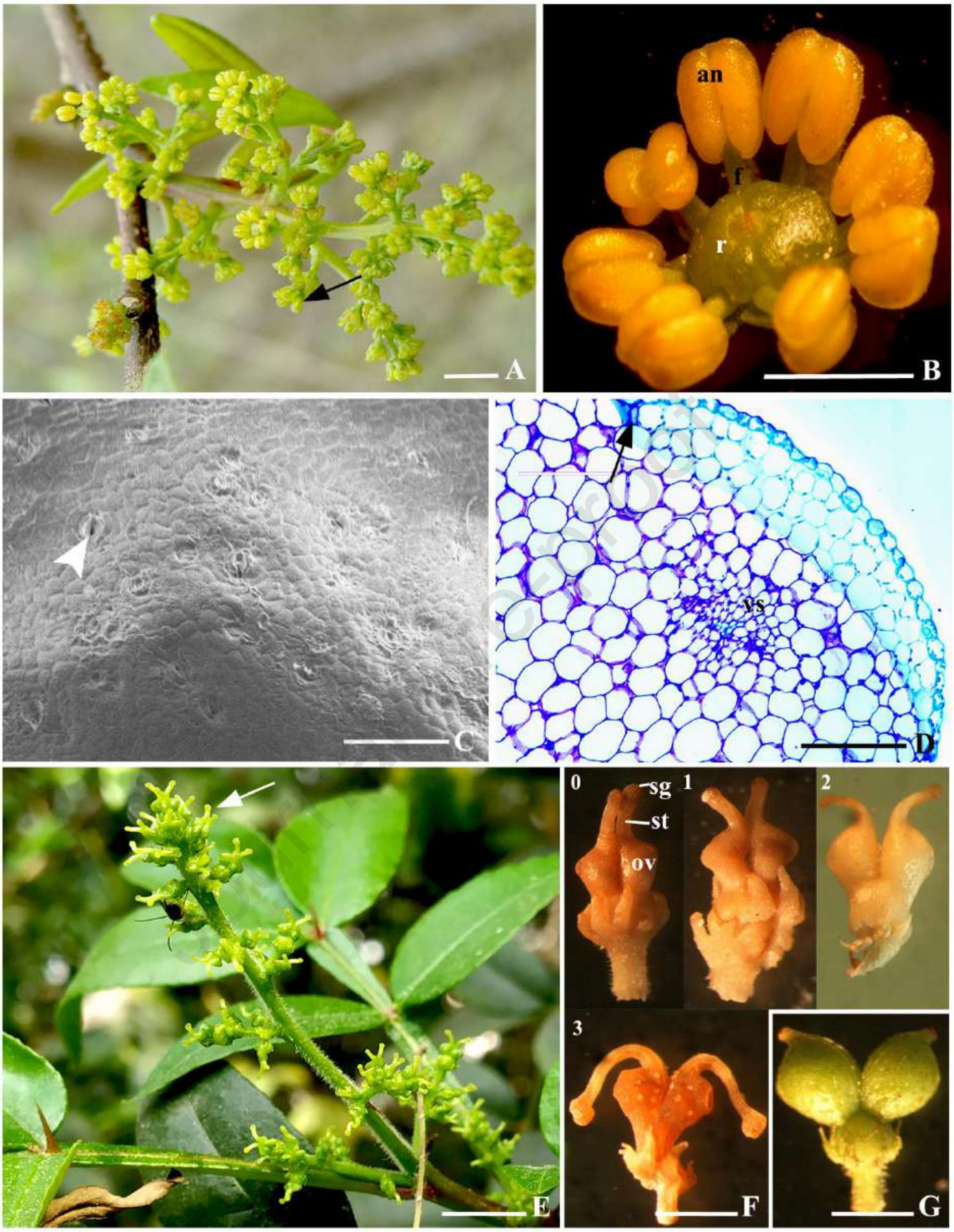
| **Trait** | | **Male plants** | **Female Plants** |
|---|---|---|---|
| ***Vegetative traits*** | | | |
| Height (in meter) | Dhandal | 4.72±1.55 | 2.56±0.52 |
| | Sukrala | 3.26±0.77 | 2.53±0.77 |
| | Bhaddu | 6.55±3.69 | 3.24±1.22 |
| Number of branches | | 652.3±36.57 | 370.35±22.81 |
| Thorn size (in cm) | | 0.64±0.042 | 0.42±0.045 |
| ***Reproductive traits*** | | | |
| Number of inflorescences | | 33466.67±1383 | 15200.00±304 |
| Number of flowers per inflorescence | | 36.6±1.9 | 34.03±1.9 |
| Size of flower (in mm) | | 3.5±0.14 | 3.69±0.10 |
| Number of anthers | | 5.61±0.16 | - |
| Pollen production per flower | | 32,976±379 | - |
| Number of carpels | | - | 2-4 |
| Number of ovules | | - | 4-6 |

**Table 2.** Summary of insects foraging activity, mean flower handling time, mean pollen load on pollinators body parts during their visit to male and female flowers.

| **Pollinators** | ***Episyphrus balteatus*** **( Marmalade hoverfly /Brown bee)** | | ***Calliphora* sp. (Blue bee)** | |
|---|---|---|---|---|
| | Male plants | Female plants | Male plants | Female plants |
| **Beginning of visitation** | 10-11th March | 15-16th March | 16-17th March | 24-25th March |
| **Peak foraging activity** | 18-21st March | 26-27th March | 18-21st March | 26-27th March |
| **Number of Trees scanned (30 mins.)** | 15 | 10 | 15 | 10 |
| **Number of insects visiting the plant** | 155.7±20.51 | 42.33±9.05 | 15.2±2.37 | 6.25±1.625 |
| **Mean Flower handling time (minutes) by insect** | 1.98±0.15 | 1.14±0.19 | 1.17±0.15 | 1.04±0.094 |
| **Mean duration on the tree (hours)** | 2.76±0.182 | 1.57±0.212 | 1.05±0.156 | 0.88±0.139 |
| **Mean pollen load on insect body (n=10)** | 212±11.4 | | 121±5.5 | |



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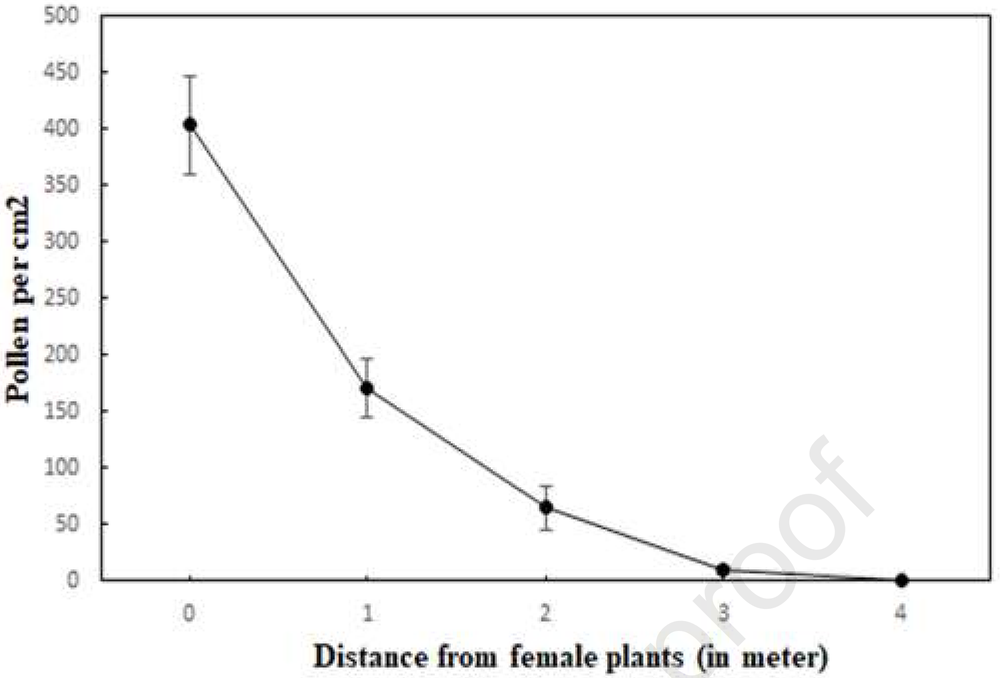

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**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: