

Reproductive ecology of an endangered monocarpic herbaceous perennial, *Ferula jaeschkeana* Vatke

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Abstract: For successful cultivation and conservation of valuable medicinal plants, a detailed knowledge of their reproductive biology is essential. The floral organization, breeding system and pollination ecology of *Ferula jaeschkeana* Vatke, being unclear, were investigated for the first time with a conservation perspective. Our investigation revealed the andromonoecious nature of *Ferula jaeschkeana*. The pollen-ovule ratio of the species suggests that the species is an outbreeder. The pollen grains were prolate in shape with few striations. A bagging experiment designed to check the nature of the breeding system and mode of pollination revealed the occurrence of a mixed-mating system with both xenogamous and geitonogamous modes of pollination with no indication of agamospermy. The self-incompatibility rate values suggested that the species is self-compatible. Our observations also indicate the occurrence of ambophily in the species, with both insects (entomophily) and wind (anemophily) acting as the agents of pollination. The formation of aborted ovules resulted in poor fruit set in upper branches. Some of these fruits were hollow or with aborted seeds. In lower branches, completely nonfunctional female structures were present and thus no fruit set was observed. The endangered status and restricted distribution of this species may be partly due to the monocarpy, abortion of ovules, formation of lower nonfunctional central umbels, low seed viability and harsh environmental conditions negatively impacting reproduction in natural populations. Our results present a detailed account on reproductive biology of this valuable medicinal plant species, which may in turn help in its conservation and management.

Key words: Ambophily; breeding; conservation; endangered; *Ferula jaeschkeana*; pollination.

Handling Editor: Christina Alba

Introduction

In order to understand the nature of species adaptation and reproductive success of plants, detailed knowledge of the reproductive biology is necessary (Anderson *et al.* 2006). Studies on mating systems and pollination biology can detect vulnerable points in the reproductive process, allowing for the design of appropriate measures for

the conservation of threatened taxa (Melia *et al.* 2012; Ortiz 2014). The main reason for the failure of various species recovery plans, especially for rare plants, is the lack of basic biological data (Boersma *et al.* 2001; Heywood & Iriondo 2003). Knowledge of reproductive biology is thus critical to the effective management of rare and endangered species (Gross *et al.* 2003; Moza & Bhatnagar 2007; Tandon *et al.* 2003; Weller 1994).

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The present study was conducted from 2012–2014 to explore the reproductive biology of *Ferula jaeschkeana* Vatke (Apiaceae), an important endangered medicinal herb of the Kashmir Valley (India) locally known as “Yang” or “Krandel”. In Jammu and Kashmir, the species was found to grow in disjunct populations on dry sunny slopes because of its susceptibility to cattle grazing, local usage and other anthropogenic activities. *Ferula jaeschkeana*, being a vulnerable species (Chawla *et al.* 2012; Kumar *et al.* 2011; Pant & Pant 2011), needs immediate conservation and protection (Yaqoob & Nawchoo 2015a, b). The oleo-resin gum of *Ferula jaeschkeana* is used in folk medicine for the treatment of tumors, chronic wounds and ulcers. The latex is used to cure gastric problems and the powder prepared from the dried parts is used to cure chest pain (Yaqoob *et al.* 2016). Owing to its immense medicinal and traditional importance and very little information available on its distribution, reproductive biology and meiotic behavior, the present study was devised for the first time to understand the reproductive biology of this valuable species, to collect baseline information on its biology and to understand potential deficiencies in its reproduction.

Material and methods

Ferula jaeschkeana is a herbaceous plant growing 1–2.5 m tall, with reddish-brown stems. A transplanted population of *Ferula jaeschkeana* at KUBG (Kashmir University Botanical Garden) and four natural populations at Dachigam, Drang, Betab valley-Pahalgam and Gulmarg were selected for the present study. The coordinates and the altitude of the sites are 34° 7' 57.17" N, 74° 50' 15.19" E, 1595 m.a.s.l. (KUBG), 34° 8' 9.99" N, 75° 2' 52.40" E, 1900 m.a.s.l. (Dachigam), 34° 2' 8.70" N, 74° 25' 4.57" E, 2235 m.a.s.l. (Drang), 34° 3' 15.17" N, 75° 21' 49.83" E, 2405 m.a.s.l. (Betab valley-Pahalgam) and 34° 3' 40.2" N, 74° 23' 15.95" E, 2590 m.a.s.l. (Gulmarg).

Floral organization

Measurements were made on plants growing both in the transplant garden and the natural habitats. Photographs were taken using a Canon A810 camera. Fifteen mature individuals were selected randomly and tagged from each population in order to observe the inflorescence pattern and organization of umbels in the species. A detailed study was carried out on the flower

structure and shape and size of floral parts using a stereo zoom microscope at 1× magnification (Zeiss Discovery V8). Macro images of various plant traits were captured with the camera, saved digitally, and examined through stereo zoom microscope. The quantitative floral traits (see Table 1 for a list) were measured using graph paper with a grid size of 1 mm.

Pollen morphology

Morphological studies of pollen grains were carried out under both light microscope (Olympus, OIC) as well as scanning electron microscope (Hitachi, S3000 H). The polar and equatorial axis diameters of each pollen grain were measured at 40× magnification using an ocular and stage micrometer. The volume of pollen grain was calculated by following Zhang *et al.* (2009) formula as follows:

$$V = \pi PE^2/6$$

where P is the polar axis diameter and E is the equatorial axis diameter.

Pollen fertility and *in vitro* pollen germination

To estimate pollen fertility we collected 15 fresh floral buds with dehiscing anthers and squashed the anthers in 1 % acetocarmine (Singh 2003). The slides were then observed under light microscope. The healthy and plump, darkly stained pollen grains with regular margins were recorded as fertile, whereas lightly stained and shriveled pollen grains were considered as sterile. Counts of fertile and sterile pollen grains were carried out from randomly chosen microscopic fields at magnification of 10×. Percentage pollen fertility was calculated as follows:

$$\% \text{ pollen fertility} = \frac{\text{Number of fertile pollen grains}}{\text{Total number of pollen grains observed}} \times 100$$

In vitro pollen germination was first carried out with a nutrient medium following the method of Brewbaker & Kwack (1963). However, Brewbaker and Kwack's medium did not stimulate pollen germination in *F. jaeschkeana* so different concentrations and proportions of the nutrients were prepared (Table 3). The anthers from open flowers were squashed in cavity blocks and the cultures were incubated in laboratory conditions at 22 ± 2 °C for 6–12 hours. The slides were observed under a light microscope and the pollen grains with pollen tube length more than their diameter were

considered germinated. The percentage pollen germination was calculated as:

$$\% \text{ pollen germination} = \frac{\text{Number of pollen grains germinated}}{\text{Total number of pollen grains observed}} \times 100$$

Pollen-ovule ratio and stigma receptivity

Fifteen mature flowers about to open were collected at random from each study site for estimating pollen-ovule ratio. Pollen quantity was estimated by squashing one anther (several times) in ten drops of distilled water in a cavity block and shaken with a glass rod. After shaking, the pollen count was made in one drop of water and it was repeated several times. The following equation was followed to calculate the number of pollen per flower:

$$\begin{aligned} p \times q &= r \\ r \times s &= t \end{aligned}$$

where p is the mean pollen count per drop of water, q is the number of water drops taken initially in which one anther was squashed, r is the mean number of pollen per anther, s is the number of anthers per flower and t is the total count per flower.

The number of ovules per ovary was counted by dissecting the ovary longitudinally and viewing it under stereo microscope. Outcrossing species predominantly produce a considerably higher number of pollen grains than ovules (Cruden 1977). The pollen-ovule ratio (P/O) was calculated following Cruden's (1977) method as follows:

$$P/O = \frac{\text{Pollen count per anther} \times \text{No. of anthers per flower}}{\text{Number of ovules per flower}}$$

To determine the stigma receptivity of the species, pollen load over the entire stigma and pollen germination on stigmas were assessed in all the populations. The stigmas were then observed under light microscope (with 10 \times , 40 \times and 100 \times magnifications) and those stigmas carrying the germinating pollen grains were recorded as receptive. The data presented on stigma receptivity is the overall mean across the populations.

Pollination mechanism

The mechanism of pollination was studied in the species by recording the foraging behavior of various insects visiting the flowers from 11:00–14:30 on 10 sample dates. The floral visitors were trapped, anaesthetized with chloroform and observed under stereo microscope for pollen load on their body parts. Representative specimens of the

insects were collected and identified by the Head, Laboratory of Entomology, Department of Zoology, University of Kashmir. To determine the role of wind in pollination, 15 randomly scattered, glycerine-smeared slides were tied vertically to plants at each study site and any pollen present on the slides was recorded.

The following pollination indices were calculated:

Foraging behavior (FB ; *sensu* Sajjad *et al.* 2009): FB is the time stayed by an individual insect species per inflorescence per visit, counted using a stop watch.

Insect visiting efficiency (IVE ; *sensu* Bingham & Orthner, 1998): IVE was calculated as follows:

$$IVE = \frac{\text{Number of flowers visited by insect in one visit}}{\text{Total number of flowers available}}$$

Foraging speed (F_s ; *sensu* Pando *et al.* 2011): F_s is the number of flowers visited by a pollinator per minute. It was calculated as follows:

$$F_s = (F_i/d_i) \times 60$$

where ' d_i ' is the time(s) given by stopwatch and ' F_i ' is the number of flowers visited during ' d_i '.

Index of visitation rate (IVR ; *sensu* Talavera *et al.* 2001): IVR used to measure the visitation rate in a relative way, by taking into account both frequency of visits and activity rate. It was calculated as follows:

$$IVR = F \times AR$$

Where 'F' is number of individuals belonging to a visiting-insect category relative to the total number of insects included in the census, and 'AR' is the activity rate, i.e., the mean number of flowers that a visiting-insect category visited per minute.

Visitor visitation frequency (V_F ; *sensu* Cosacov *et al.* 2008): V_F is the visitor number per flower per hour. It was calculated as follows:

$$V_F = V / (F T)$$

where 'V' is the total number of visits to flowers, 'F' is the total number of flowers in the patch and T is the observation time in hours.

We used linear regression analysis to determine the correlation between various pollination indices.

Nature of breeding system

The nature of the breeding system present in the species was examined by controlled pollinations and bagging experiments. In each experiment,

fifteen plants were randomly selected at each study site and were assessed to determine the breeding mechanisms. The experiments consisted of the following: (1) Inflorescences were tagged and allowed to be pollinated naturally. (2) Central and lateral umbels were bagged together to examine the ability of different umbels to cross-pollinate. (3) Central umbels were bagged to allow crossing between umbellules (geitonogamy). (4) Central umbellules were bagged to examine selfing. (5) Emasculated central umbellules with decapitated stigmas were bagged to check for apomictic fruit development.

The various reproductive indices calculated include:

Self-incompatibility rate (*ISI*; *sensu* Ruiz-Zapata & Arroyo 1978): *ISI* was used to measure self-incompatibility of the plant species.

$$ISI = (\text{self-fruit set}) / (\text{cross-fruit set})$$

where 'self-fruit set' and 'cross-fruit set' are data obtained from controlled pollination experiments. *ISI* ≥ 1 indicates self-compatibility; *ISI* values ranging from 0.2-1 indicate partial self-compatibility, *ISI* < 0.2 indicates mostly self-incompatible; and *ISI* = 0 indicates total self-incompatibility.

Selfing rate (*S*; *sensu* Charlesworth & Charlesworth 1987): *S* was used to estimate the frequency of self-pollination.

$$S = (P_x - P_o) / (P_x - P_s)$$

where '*P_x*' are seeds resulting from cross-pollination, '*P_o*' are seeds resulting from open pollination and '*P_s*' are seeds resulting from self-pollination.

Pollen limitation index (*PL*; *sensu* Tamura & Kudo 2000) expressed as:

$$PL = 1 - C/X$$

where *C* and *X* represent the seed set of control plants i.e., the flowers which were left open and pollen-supplemented plants respectively.

Pre-emergent reproductive success (*PERS*; *sensu* Wiens *et al.* 1987): *PERS* was used to measure the number of ovules that complete development and survive to enter the environment.

$$PERS = (\text{fruit} / \text{flower}) \times (\text{seed} / \text{ovule})$$

Seed biology

Fruit weight was calculated by weighing 100 randomly selected fruits from each population (repeated 10 times), following Agarwal & Dadlani (1988):

$$\text{Average fruit weight} = \frac{\text{Weight of 'N' fruits}}{N}$$

After four weeks of bagging, fruit set was calculated following Lubbers & Christensen (1986):

$$\% \text{ Fruit set} = \frac{\text{Total number of fruits produced}}{\text{Number of bisexual flowers borne on the plant}} \times 100$$

Fruitset was determined in unbagged plants following the same method.

Seed viability was determined using the Tetrazolium test (Baezo & Vallejo 2006). Three replicates of twenty seeds each from each site were placed on a moist filter paper for 24 hours at room temperature and cut longitudinally to expose the embryos. Then the seeds were soaked in 1 % aqueous solution of 2, 3, 5 triphenyl tetrazolium chloride (TZ) and were kept in dark at 30 °C for 3-4 hours. Seeds with embryos stained dark red were evaluated as viable. The hollow seeds or those with reduced embryos (colourless) were counted as non-viable

Data analysis

The data were statistically analyzed by one-way analysis of variance (ANOVA) using SPSS 16.0 software. The comparison of the means among different populations was done with the Tukey's test at *P* < 0.05. All the values given in the present work are means \pm SD.

Results

Floral organization

Ferula jaeschkeana is a monocarpic species with annual and biennial plants that remain vegetative; flowers are produced by perennial plants only. *Ferula jaeschkeana* is andromonoecious i.e., it produces hermaphrodite (perfect) flowers along with functionally male flowers. The inflorescence is a compound umbel with central and lateral umbels. Each branch constitutes a central/terminal umbel (bisexual flowers) and 1-4 lateral umbels (male flowers only). The umbels are 3 - 10 cm across; terminal umbels are sessile or sub-sessile and lateral umbels long-pedunculate. Each branch has 8-15 umbellule's per umbel. Each umbellule bears 15-20 flowers (Fig. 1). The species produces a large number of flowers that are showy with nectaries present at the base of ovary. The top of the ovary often forms a stylopodium. The flower is yellow in colour, pentamerous with a large stylopodium which produces nectar. Petals are elliptic, long and are five in number. The apex is incurved and acuminate. Anthers are five, dorsifixed, introse and bithecous with longitudinal dehiscence. The gynoecium remains embedded in the stylopodium. The gynoecium is bicarpellary

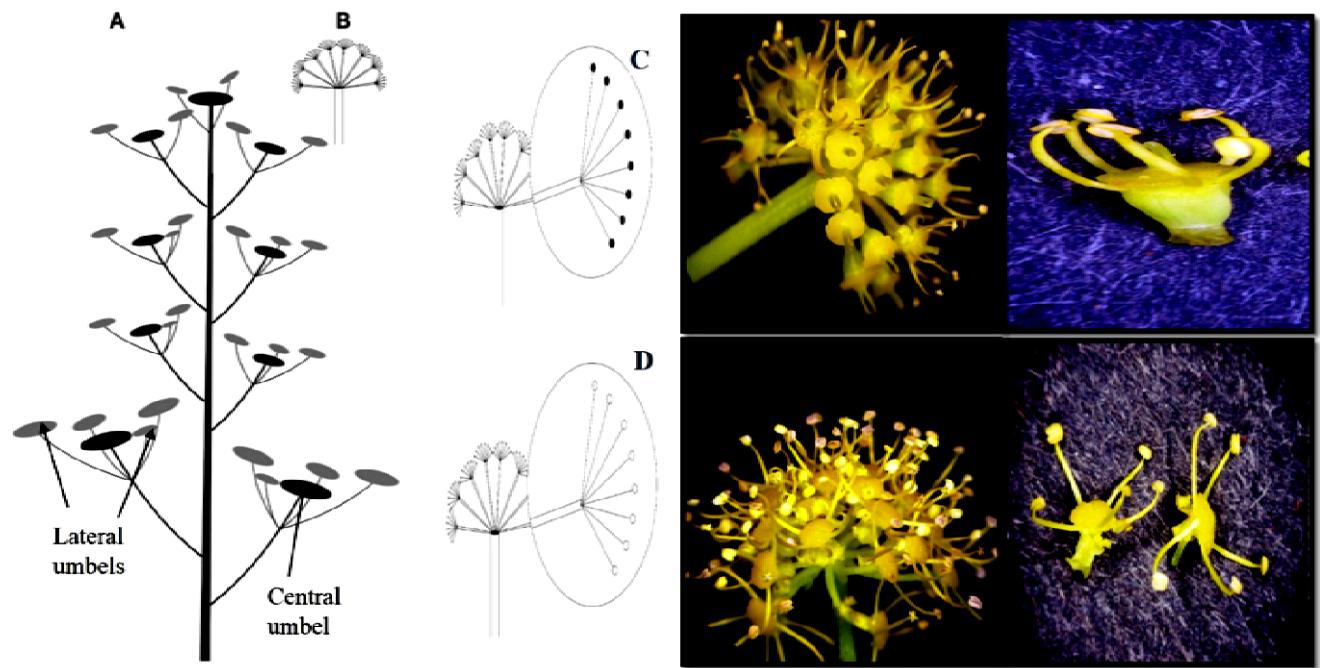


Fig. 1. Floral architecture in *Ferula jaeschkeana* A. Arrangement of umbels and their position within the inflorescence B. Each umbel consists of umbellules bearing a large number of closely packed flowers C. Central umbellules consisting of hermaphrodite flowers D. Lateral umbellules consisting of staminate/male flowers only.

syncarpous, having a single pendulous ovule in each locule with axile placentation. There are two long styles with capitate stigmas. The quantitative floral traits are summarized in Table 1.

Table 1. Quantitative floral traits of *Ferula jaeschkeana* averaging over all populations.

Traits (mm)	Mean \pm SD
Petal length	1.53 \pm 0.35
Petal width	1.03 \pm 0.14
Filament length	3.07 \pm 0.45
Anther length	0.83 \pm 0.17
Anther width	0.63 \pm 0.14
Style length	1.70 \pm 0.27
Ovule length	0.55 \pm 0.05
Ovule width	0.27 \pm 0.02

Pollen morphology

The scanning electron microscope as well as light microscopic studies revealed that the pollen grains are prolate in shape, 3-colporate and with the exine more or less smooth with few striations (Fig. 2). The polar diameter is $33.4 \pm \text{SD } 2.36 \mu\text{m}$ and the equatorial diameter is $17.26 \pm \text{SD } 1.6 \mu\text{m}$ with a P/E ratio equal to $1.93 \pm \text{SD } 1.47$. In polar

view, the shape of pollen grains is 3-lobed round, while in the equatorial view, the pollen grains appear ellipsoidal in shape. The pollen volume of the species was $123.60 \mu\text{m}^3$.

Pollen fertility and in vitro pollen germination

The species produces an enormous quantity of pollen grains but a large fraction of these are sterile (Fig. 2). The pollen fertility test reveals that the plants from different populations yield 63.43 - 75.93 % fertile pollen grains, with the lowest fertility recorded in the plants under transplanted conditions at KUBG. The data on pollen fertility are summarized in Table 2.

In vitro studies of pollen germination revealed that the nutrient medium (Table 3) was effective in inducing germination of pollen grains and the highest percentage germination ($15.2 \pm \text{SD } 1.02$) was recorded for the M6 medium (Table 3).

Pollen-ovule ratio and stigma receptivity

In *Ferula jaeschkeana*, each flower produces two ovules while the pollen production per flower ranges from 13485 to 14710 pollen grains across the study sites. Thus, the pollen-ovule ratio on average ranges from 6742.50 to 7355 per flower across the selected study sites (Fig. 3).



Fig. 2. Pollen morphology of *Ferula jaeschkeana* A. B. Scanning Electron Microscope (SEM) of pollen grains C. Light microscopic view of pollen grains D. Fertile and sterile pollen grains.

Table 2. Pollen fertility of *Ferula jaeschkeana* in different populations.

Population	Total no. of pollen scanned (mean \pm SD)	No. of fertile pollen grains (mean \pm SD)	Percentage fertility (%)
KUBG	2672.28 \pm 28.1	1695.14 \pm 13.11	63.43 \pm 1.96a
Dachigam	2842.24 \pm 42.16	1902.32 \pm 11.81	66.93 \pm 0.93ab
Drang	2497.08 \pm 32.72	1712.81 \pm 9.13	68.59 \pm 0.70bc
Betab valley - Pahalgam	2572.82 \pm 26.11	1853.08 \pm 14.63	72.02 \pm 0.97cd
Gulmarg	2583.21 \pm 36.19	1961.55 \pm 10.25	75.93 \pm 3.06d
F value			22.13
LSD \leq 0.05*			2.61

Means labelled with the different small letters indicate that they significantly differ from each other among different populations.

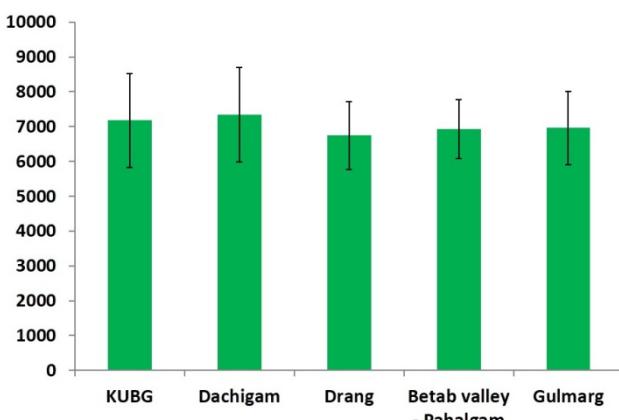
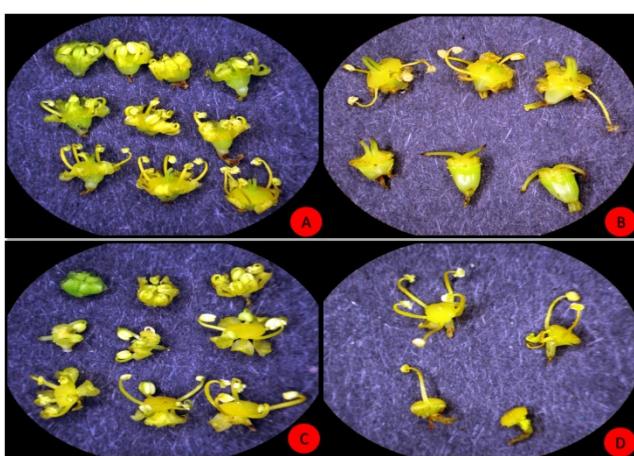
We observed temporal separation of sexual function within hermaphroditic flowers (dichogamy). The anthers mature early (protandry) followed by the female phase (Fig. 4). The stigmas emerge and become receptive within 6-7 days.

Anthesis of flowers is slightly asynchronous, ensuring the availability of pollen to the plants for a longer duration. Within an umbellule, anthesis starts from the periphery and proceeds centripetally, while within the plant, it starts from the

Table 3. *In vitro* pollen germination of *Ferula jaeschkeana*.

Label	Ratio of different nutrients					Percent germination (mean \pm SD)
	20% Sucrose	400 ppm Boric acid	600 ppm Calcium nitrate	400 ppm Magnesium sulphate	400 ppm Potassium nitrate	
M1	1	1	0	0	0	0
M2	1	1	1	0	0	0
M3	1	1	1	1	0	0
M4	1	1	1	1	1	0
M5	1	1	1	0	1	$2.39 \pm 0.45a$
M6	2	2	1	0	1	$15.2 \pm 1.02b$
M7	2	2	1	1	1	$4.76 \pm 0.31c$
M8	2	2	2	2	1	0
F value						310.97
LSD $\leq 0.05^*$						1.061

Means labelled with different small letters indicate that treatments significantly differ from each other.

**Fig. 3.** Pollen-ovule ratio of different populations of *Ferula jaeschkeana*.**Fig. 4.** Different developmental stages of a flower A. B. Bisexual flowers C. D. Male flowers.

central umbels (bisexual) followed by lateral umbels (male) (Fig. 5). In a plant, anthesis occurred acropetally i.e. anthesis starts from the lower umbels in order of maturity followed by the umbels towards the top of the plant. With the progression of anthesis, the number of pollen grains on the stigmas start to increase. The maximum number of germinated pollen grains on receptive stigmas was recorded on days 8 and 9, indicating the peak receptivity of stigmas on that particular day. The stigma becomes highly receptive when the angle between the styles with respect to stylopodium is 80 - 100°. On the 11th and 12th days of anthesis, the stigmatic surface dries up, which marks the end of receptivity.

Pollination mechanism

Insect foraging starts early in the morning on a normal sunny day. The process of visitation is at its peak during 11:00-14:30 hours and gradually decreases in the later hours. The insect group with the maximum pollen load and which was considered the major pollinator was identified as *Apis* sp. However, in addition to the *Apis* sp. some other insects (*Allograpta* sp., *Vespa* sp., *Musca domestica*, *Formicidae* sp., *Stomoxy* sp. and Beetles) were also observed to visit the plant species (Table 4).

The data revealed that the *Apis* sp. stayed for the maximum duration per flower as well as per bout. On the basis of insect visiting efficiency ($IVE = 0.19 \pm SD 0.08$), the *Apis* sp. seems to be the major pollinator and the *Allograpta* sp., an infrequent visitor ($IVE = 0.09 \pm SD 0.03$). Due to

Table 4. Some important visitors of *Ferula jaeschkeana* in different populations.

Insect Visitor	Population				
	KUBG	Dachi-gam	Drang	Betab valley - Pahalgam	Gulmarg
<i>Apis</i> sp.					
(Hymenoptera -Apidae)	+	+	+	+	+
<i>Musca domestica</i> (Diptera- Muscidae)	+	+	+	-	-
<i>Allograpta</i> sp.	+	+	+	-	-
<i>Vespula</i> sp. (Hymenoptera - Vespadae)	+	-	-	-	-

(+ = present, - = absent)

cold and inclement weather conditions in high-altitude populations (Betab Valley-Pahalgam and Gulmarg), discontinuous visitation of pollinators was observed and thus pollination by insects was less. The wind has been found to be a viable method for pollination, especially in Betab Valley - Pahalgam and Gulmarg populations, indicating ambophily (anemophily and entomophily) in this species. Some airborne pollen gathered on the glass slides supports the effectiveness of wind pollination.

The mean number of umbellules per branch visited by bees did not vary greatly among different sites, ranging from $7.99 \pm SD 2.32$ at Gulmarg to $11.92 \pm SD 2.95$ at KUBG. The mean number of flowers visited per umbellule ranged from $3.1 \pm SD 1.04$ at Gulmarg to $4.3 \pm SD 2.31$ at Dachigam. The time spent per flower at KUBG, Dachigam, Drang, Betab valley - Pahalgam and Gulmarg was $9.26 \pm SD 4.57$, $9.12 \pm SD 4.41$, $8.64 \pm SD 3.54$, $8.12 \pm SD 2.56$ and $7.91 \pm SD 2.50$ s, respectively.

Comparative pollination indices at different study sites of *Ferula jaeschkeana* are given in Table 5. A highly significant positive correlation was observed between foraging behavior and the number of flowers visited per umbel per visit ($P < 0.0001$, $R^2 = 0.9992$) and between foraging speed and visitation frequency ($P = 0.003$, $R^2 = 0.9631$). A significant negative correlation was observed between foraging speed and foraging behavior ($P = 0.010$, $R^2 = 0.9205$) and between visitation

frequency and the foraging behavior ($p = 0.015$, $R^2 = 0.8945$) (Fig. 6).

Nature of the breeding system

The percentage of fruit set by different bagging experiments is shown in Table 6. The bagged single flowers of central umbellules did not produce any fruits and no apomictic seed development was observed in the species. The bagging experiment revealed that both xenogamous and geitonogamous modes of pollination operate in the species. The values of different reproductive indices are shown in Table 7. Controlled pollination experiments revealed that the species exhibited more cross-fruit set as compared to self-fruit set. Late curling of stylar branches was observed, which aids the stigmas in coming in close contact with the anthers. The staminal movements were also observed in the species and we found that the anthers come in close contact with the stigma (Fig. 7).

Seed biology

The fruits of *Ferula jaeschkeana* are reddish brown and are $2.06 \pm SD 0.28$ cm long and $0.97 \pm SD 0.15$ cm wide averaging across all sites. The fruit shape is ellipsoid, with an average fruit weight of $5.53 \pm SD 0.22$ g across all sites. The fruits are flattened with lateral wings. On average, the species produces $20.50 \pm SD 4.38$ mericarps per umbellule at Gulmarg, $23.75 \pm SD 3.76$ at Betab Valley-Pahalgam, $26.01 \pm SD 4.63$ at Drang, $31.80 \pm SD 5.43$ at Dachigam and $30.81 \pm SD 3.98$ at KUBG. This low fruit set amounts to $64.61 \pm SD 0.33$ %, $64.18 \pm SD 3.18$ %, $66.35 \pm SD 0.94$ %, $70.04 \pm SD 2.05$ % and $72.66 \pm SD 2.67$ % fruits at Gulmarg, Betab Valley-Pahalgam, Drang, Dachigam and KUBG respectively in upper branches. Thus, the species has a low percentage of fruit set ($68 \pm SD 4$ %) due to the formation of aborted ovules in upper branches (Fig. 8). Some of these fruits are hollow or with aborted seeds. During seed set, a large number of beetles cover the fruits. The larvae of the beetles feed on the embryos of the seeds and pores are visible on the fruits. These fruits finally become hollow which leads to a further reduction in the reproductive output. It was also found that there are completely nonfunctional female structures in central umbels of the lower branches (Fig. 9). The percentage of such branches varies from 11.11 % to 40.01 % per plant.

The viability of seeds collected from different sites varied between $55.34 \pm SD 4.05$ % to $64.68 \pm SD 2.25$ % (Fig 10). The embryos of seeds after the

Table 5. Comparison of different pollination indices (mean \pm SD) of *Apis* sp. at different sites.

Population	FB (min)	IVE	F _S	IVR	V _F
KUBG	7.54 \pm 0.44a	0.193 \pm 0.08a	6.47 \pm 0.5a	420.17 \pm 3.01a	0.162 \pm 0.003a
Dachigam	7.34 \pm 0.55a	0.188 \pm 0.00a	6.57 \pm 0.07ab	529.55 \pm 11.04b	0.126 \pm 0.01a
Drang	5.05 \pm 0.05b	0.194 \pm 0.02a	6.94 \pm 0.25b	524.91 \pm 5.0b	0.25 \pm 0.04b
Betab valley - Pahalgam	3.93 \pm 0.30c	0.183 \pm 0.01a	7.38 \pm 0.39bc	656.3 \pm 5.48c	0.474 \pm 0.03c
Gulmarg	3.26 \pm 0.27c	0.193 \pm 0.00a	7.90 \pm 0.3c	790.0 \pm 9.53d	0.60 \pm 0.00d
Fvalue	85.67	0.04	9.36	1095.48	209.95
LSD \leq 0.05*	0.539	0.059	0.497	11.01	0.036

Means labelled with different small letters within each of the pollination indices indicate that populations significantly differ.



Fig. 5. Chronology of different stages of anthesis in an inflorescence A. B. Anthesis of flowers in central umbels shown by arrows C. Anthesis of flowers in lateral umbels and complete dehiscence of anthers in central umbels (shown by the arrow) D. Central umbellule with receptive stigmas E. F. End of receptivity in the central umbel.

one or two year's storage upon treating with TZ were not stained red and thus were evaluated as being non-viable.

Discussion

Floral organization

Many of the *Ferula* species are classified as monocarpic plants (Molamohammadi & Masood-Reza 1989; Zargari 1991). We observed that *Ferula jaeschkeana* produces hermaphrodite (perfect)

flowers along with male flowers. This is in agreement with the suggestions by Lovett-Doust & Lovett-Doust (1982) and Schlessman et al. (2004), who reported that the plants in Apiaceae are andromonoecious, i.e., produce hermaphrodite (perfect) flowers along with functionally male ones.

Pollen morphology

During the present investigation, it was observed that the pollen grains are 3-colporate and the exine is more or less smooth with few striations,

Table 6. Bagging experiments revealing fruit set (mean \pm SD) in different populations of *Ferula jaeschkeana*.

Experiment	Percentage fruit set					F value	LSD $\leq 0.05^*$
	KUBG	Dachigam	Drang	Betab valley – Pahalgam	Gulmarg		
No bagging	72.66 \pm 2.67a	70.04 \pm 2.05ab	66.35 \pm 0.94bc	64.18 \pm 3.18c	64.61 \pm 0.33bc	8.98	3.136
Controlled crossing	69.57 \pm 0.42a	62.49 \pm 0.11b	61.25 \pm 0.24b	58.14 \pm 0.05c	55.30 \pm 1.17d	264.3	0.848
Central and lateral umbels	52.50 \pm 1.50a	47.99 \pm 2.60ab	48.02 \pm 1.97ab	43.67 \pm 1.32b	43.46 \pm 0.50b	14.03	2.553
Bagging	38.66 \pm 2.30a	33.3 \pm 1.29b	30.69 \pm 0.20bc	28.31 \pm 2.32cd	26.20 \pm 1.2d	25.33	2.46
Single flower	0	0	0	0	0	-	-
Controlled selfing	59.45 \pm 1.55a	57.22 \pm 2.22ab	54.88 \pm 0.88bc	51.16 \pm 1.17cd	49.74 \pm 0.75d	24.49	2.09
Apomixis	0	0	0	0	0	-	-

Means labelled with different small letters within each of the bagging treatments indicate that populations significantly differ.

Table 7. Comparison of reproductive indices (mean \pm SD) at different sites of *Ferula jaeschkeana*.

Population	PL	PERS	ISI	S
KUBG	-0.044 \pm 0.008a	0.411 \pm 0.02a	0.88 \pm 0.01a	-1.20 \pm 0.20a
Dachigam	-0.120 \pm 0.010b	0.41 \pm 0.01a	0.87 \pm 0.02a	-0.86 \pm 0.07b
Drang	-0.083 \pm 0.005c	0.43 \pm 0.10a	0.89 \pm 0.03a	-0.80 \pm 0.05b
Betab valley - Pahalgam	-0.106 \pm 0.015bc	0.49 \pm 0.05a	0.91 \pm 0.07a	-1.43 \pm 0.09a
Gulmarg	-0.168 \pm 0.017d	0.525 \pm 0.04a	0.856 \pm 0.02a	-0.30 \pm 0.005c
F value	42.07	2.591	0.825	49.66
LSD $\leq 0.05^*$	0.018	0.082	0.057	0.156

*Least significance difference.

Means labelled with the different small letters within each of the reproductive indices indicate that populations significantly differ.

which is in conformity with Perveen & Qaiser (2006). During the present study, the *P/E* ratio was found to be $1.93 \pm$ SD 1.47 indicating prolate shape of pollen grains which is in congruence with Erdtman (1943). The relatively smooth pollen grains with few striations reveal both wind as well as insect pollination of the species. It is reported that reticulate or echinulate pollen grains reflect biotic pollination (entomophily) and smooth pollen grains reflect abiotic pollination (wind or water) (Tanaka *et al.* 2004; Lumaga *et al.* 2006), which conforms to our observations of the pollination mechanisms.

Pollen-ovule ratio and stigma receptivity

The higher pollen-ovule ratio suggests that the species is outbreeding. Our studies are in

agreement with Lindsey (1982), who also reported a high pollen-ovule ratio in some members of the family Apiaceae (e.g., *Thapsium barbinodec* with 22000:1, *Thapsium trifoliatum* with 11200:1 and *Zizea trifoliata* with 14500:1).

Asynchronous anthesis of flowers, stigma receptivity, pollen shedding and anther dehiscence ensure a supply of pollen for a longer duration, which aids in efficient pollination. The temporal differences in the development of male and female function is quite advantageous for the species because it not only makes pollen available for long periods but also attracts pollinators for a long duration, thereby encouraging outcrossing (Griffin *et al.* 2000; Wafai *et al.* 2005). Asynchronous flowering within populations reduces intraspecific competition for

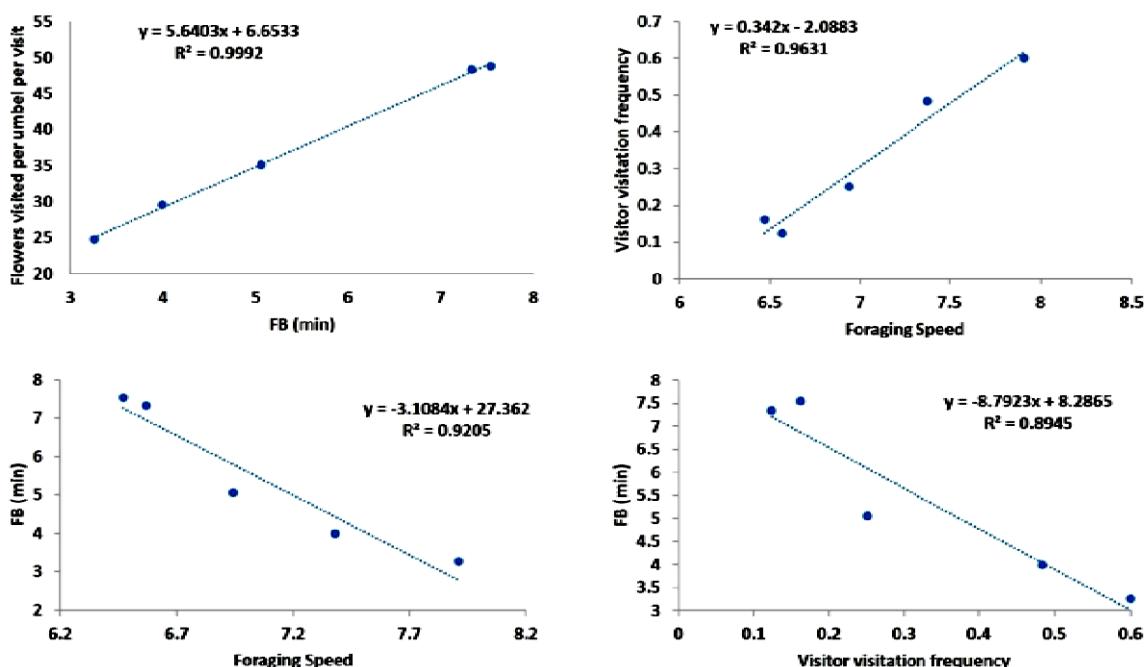


Fig. 6. Correlation between different pollination indices in *Ferula jaeschkeana*.

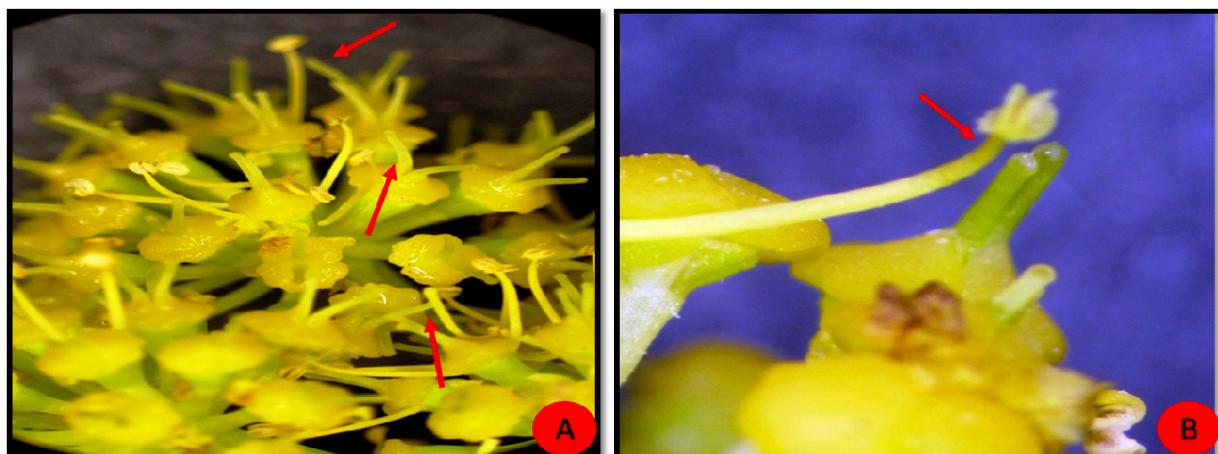


Fig. 7. Movements in *Ferula jaeschkeana* A. Stylar movements B. Staminal movements.

pollinators and enhances the outbreeding potential of the species (Pors & Werner 1989; Rogstad 1994).

Pollination mechanism

Being protandrous, *Ferula jaeschkeana* is mainly cross-pollinated and depends heavily on external pollinating agents. The present investigation revealed that the species produces a large number of flowers that are showy, with nectaries present at the base of the ovary. Ashman (2003) and Irwin and Strauss (2005) reported that plants with increased floral display usually receive higher

numbers of visits by pollinators. Nectaries play an important role in attracting the pollinators (Harborne 1993).

The present study revealed the process of insect visitation is at its peak during 11:00-14:30 hours and gradually decreases in the later hours. Our studies are in agreement with Lindsey (1979) who reported that in *Ziziea trifoliata*, *Thapsium trifoliatum* and *Thapsium barbinodec*, anthesis is diurnal and peaks between 11:00 and 13:00 hours daily.

The present observations and field surveys reveal that pollination in *Ferula jaeschkeana* is

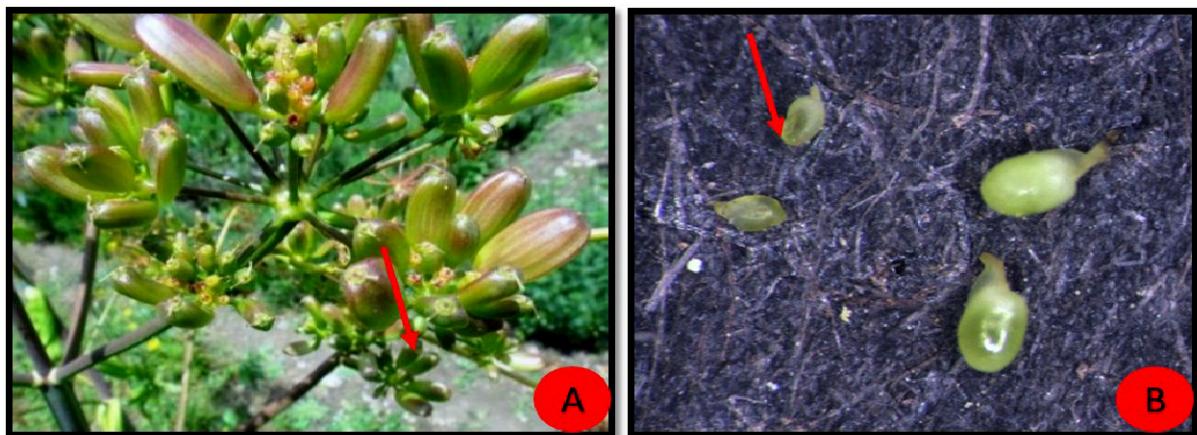


Fig. 8. Low fruit set and aborted ovules in *Ferula jaeschkeana* A. Sterile (shown by the arrow) and normal fruits B. Aborted (shown by the arrow) and normal ovules.

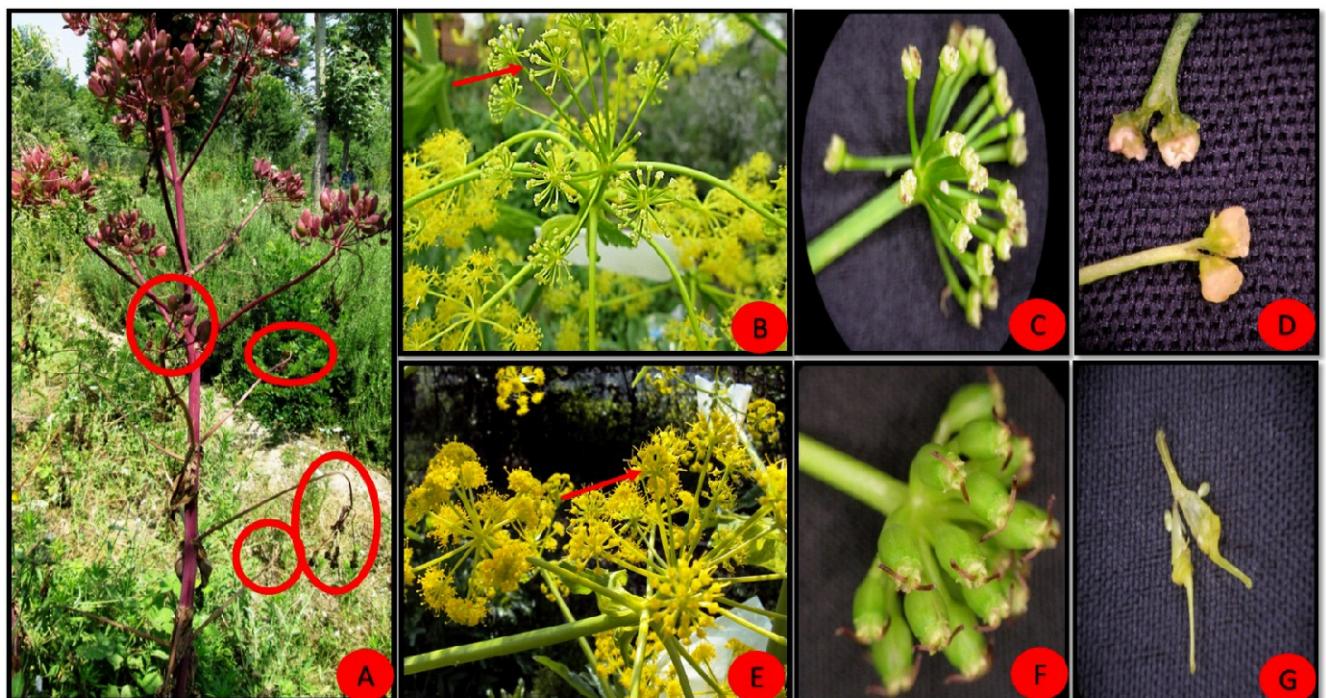


Fig. 9. Nonfunctional female structures in central umbels of lower branches A. B. Aborted central umbel (shown by the rings and arrow), C. D. Stereo zoom view of aborted central umbel. E. Normal central umbel. F. G. Stereo zoom view of normal central umbel.

carried out by insects belonging to the Hymenoptera (*Apis* sp.). However, in addition to the *Apis* sp., some other insects were also observed to visit the plant species. The plants of the family Apiaceae are pollinated by members of Diptera (Zych 2007), beetles (Lamborn & Ollerton 2000), and bees or other representatives of the Hymenoptera (Davila & Wardle 2008), including ants (Carvalheiro *et al.* 2008).

It was observed that at higher altitudes, *F. jaeschkeana* was also pollinated by wind. Our studies are in agreement with Carlquist (1974) and Ehrendorfer (1979) who reported that inclement weather conditions and scarcity of pollinators at higher altitudes favour wind pollination, which is more effective than animal pollination in promoting pollen dispersal for successful crossing.

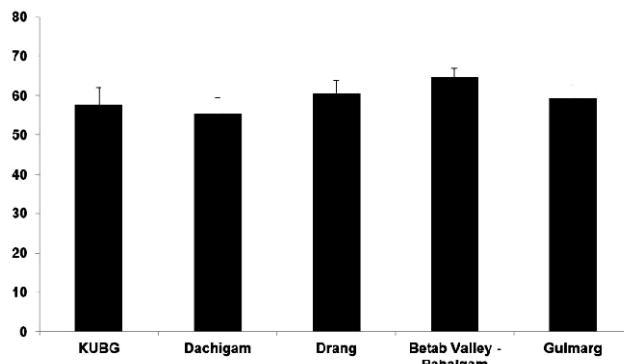


Fig 10. Percentage seed viability of the selected populations of *Ferula jaeschkeana*.

Breeding system

The bagging experiment revealed that the seeds always develop as a result of sexual reproduction and the species uses both xenogamous and geitonogamous modes of pollination. It has also been reported that members of the family Apiaceae exhibit diverse breeding systems, ranging from complete selfing to obligatory outcrossing (Koul *et al.* 1993). Our studies are in agreement with Bell (1971) and Schlessman *et al.* (2004) who reported overlap of sexual phases in some taxa in addition to dichogamy in most of the members of family Apiaceae. Lindsey (1982) reported that in *Zizia trifoliata*, *Thapsium trifoliatum* and *Thaspium barbinodec* of family Apiaceae strong outcrossing occurs as a result of separation of male and female functions within and between inflorescences. However, according to him, a low percentage of mechanical self-pollination is possible and geitonogamous pollinations between flowers of non-synchronous flowering stalks may account for significant amounts of selfing within populations.

Our study revealed that the species exhibits a moderate pre-emergent reproductive success (*PERS*), suggesting that a high number of ovules do not complete development. The self-incompatibility rate (*ISI*) values also are consistent with the self-compatible nature of the species. During the present study, the pollen limitation (*PL*) index was found to be negative. A negative value means that naturally pollinated flowers received better pollen than pollen-supplemented plants. The negative value of selfing rate (*S*) also indicates that open cross results in a higher seed set as compared to controlled self or cross pollination.

The stylar and staminal movements observed in the species favour geitonogamy. The pheno-

menon of late curling of stylar branches so that the stigmatic surfaces touch the anthers or contact pollen deposited on non-stigmatic areas of the style was also reported in *Hibiscus laevis* (Klips & Snow 1997) and *Viola pubescens* (Culley 2002). The movement of male essential parts (stamens) towards the exerted stigma was also reported in *Aquilegia canadensis* (Eckert & Schaeffer 1998), *Collinsia verna* (Kalisz *et al.* 1999) and many species in the tribe Collinsieae (Armbruster *et al.* 2002).

Seed biology

The species shows an overall low percentage of fruit set compared to the number of bisexual flowers borne on a plant due to the formation of aborted ovules. Stone *et al.* (1995) also reported that reduced pollen fertility can reduce seed production and could have an important effect on plant fertility and consequently on population performance. The abortion of ovules in *Ferula jaeschkeana* may be due to inter-ovary competition for resources. Many-flowered inflorescences increase pollinator attr-action (Cruzan *et al.* 1988), but as pollination takes place, inter-ovary competition for resources can dramatically affect fruit and seed set (Holtsford 1985; Stephenson 1980). It was also observed that the lower flowering branches of *Ferula jaeschkeana* are prone to herbivory and therefore, to minimize loss, the plant may have developed a strategy to allocate fewer resources towards these branches.

Conclusion

Ferula jaeschkeana is a monocarpic andromonoecious herbaceous perennial plant with annual and biennial plants that remain vegetative. We investigated the floral biology, breeding system and pollination ecology of the species. The bagging experiment revealed a mixed-mating system using both xenogamous and geitonogamous modes of pollination operative in the species and with no indication of agamospermy. The staminal and stylar movements favouring geitonogamy were also observed in the species that may in turn provide reproductive assurance for the population maintenance. Breeding indices suggested the self-compatible nature of the species. Our observations indicate occurrence of ambophilous in the species, with both insects (entomophily) and wind (anemophily) acting as the agents of pollination. The abortion of ovules resulted in poor fruit set in upper

branches. Some of these fruits were hollow or with aborted seeds. In lower branches, completely nonfunctional female structures were present and no fruit set was observed in these branches. Monocarpy, low pollen fertility, abortion of ovules, formation of lower nonfunctional central umbels and low seed viability are various phenomena observed during the present investigation. These may be major factors responsible for the restricted distribution of the species. This information is very useful for planning to introduce the species into cultivation and strategies for addressing conservation and management.

Acknowledgement

The first author is grateful to Council of Scientific and Industrial Research (CSIR) for providing financial assistance as JRF.

Conflict of interest The authors declare no conflict of interest.

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(Received on 27.12.2014 and accepted after revisions, on 24.06.2015)