

Reproductive biology of *Gymnema sylvestre* and *Tylophora indica* (Asclepiadaceae) in Central India

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Abstract

In *Gymnema sylvestre* (Asclepiadaceae), a large quantity of nectar attracts and floral visitors are small bees (*Melipona* sp.), bumble bees (*Xylocopa* sp.), wasp (*Vespa* sp.) and honey bees (*Apis dorsata*). While, *Polistic orientalis* visited with low frequency in floral attract. Small bees (*Xylocopa* sp) and wasp (*Vespa* sp.) in large numbers forage the flowers between 10.00-13.30 hours. They come in contact with the dehiscent pollinia and collect pollen which is transferred on the pollen sac on the other flowers. In *Tylophora indica* (Asclepiadaceae) in vitro pollen germination in different sucrose solutions was compared to each other. Generally germination rates were better than control (1%). When the averages were compared, 10% sucrose was better (35.82%) than others. Germination rate of pollen tube increased when the percentage of sucrose increased. Gradual increase in the sucrose concentration (>10 %) percentage solution resulted in the germination percentage fall.

Key words: *Apis dorsata*, Asclepiadaceae, *Gymnema sylvestre*, Reproductive biology, Threatened and *Tylophora indica*.

Significance Statement

The study intended to study of reproductive biology of threatened medicinal plants from central India. This includes different phenological aspects like, leafing; budding, blooming, fruiting, etc. The reproductive biology of plants (*Gymnema sylvestre* and *Tylophora indica*) indicates the study of floral and pollination biology. Studies on the phenology of species are important to understand long term adaptations of the species to prevailing environmental conditions for reproductive success. Floral phenology includes all the events that occur from the opening of the flower until the flower remains functional (able to donate pollen and/or receive pollen). The period for which the flowers remain functional (longevity/ life span) is generally indicated by the freshness of the flower. Seed biology is another important aspect of reproductive biology which has relevance to conservation. Pollinators/visitors were active during daytime i.e. 07.00-16.00 hours and in fine weather they actively visited and spend 20-45 seconds per flower. However, during the rainy season insect activities on a rainy day were less than on a sunny day. Hymenoptera (bees, wasps and ants), Lepidoptera (butterflies and moths), Coleoptera (beetles) and Diptera (flies) are the major orders of insects involved in pollination.

Introduction

Studies on reproductive biology and breeding systems of rare plant species are indispensable for successful conservation efforts [1]. Many rare species become threatened because of habitat destruction and habitat fragmentation, both of which may reduce population size and the abundance and effectiveness of pollinators [2]. Without effective pollinators, many species of flowering plant may go extinct. The science of pollination ecology and floral biology has, however now been mainstreamed in biodiversity conservation. In 1990s, most of the Agriculturists around the world were concerned about the worldwide decline of pollinator diversity. They prompted policymaker at the Fifth Meeting of the Conference of Parties (COP) of the Convention on Biological Diversity (CBD) to establish an International initiative for the Conservation and Sustainable use of pollinators (also referred to as the International Pollinators Initiative, or IPI) in 2000. Fifth Meeting of the Conference of Parties (COP) considered this to be a crosscutting initiative within the programme of work on agricultural biodiversity to promote coordinated action worldwide, and so requested the development of a Plan of Action for the IPI. In seed plants, pollination is a prerequisite for fruit and seed development. It is the basis of genetic exchange between plants and recombination within plants. Pollination initiates many changes in the pistil and ovary. Many of the changes induced by pollination are observed in the lower part of the style and ovary. Post pollination changes in the pattern of RNA and protein synthesis and in the activity of several enzymes occur in the lower part of the style and ovary before the arrival of the pollen tube [3]. These changes are specific to self and cross-pollination in self-incompatible taxa. In angiosperms, the pollination mechanism is typically developed in three phases: release of pollen from anther, transfer of pollen from anther to stigma and finally successful placement of the pollen on the receptive stigma surface, followed by germination of pollen grain, which begins the next phase of fertilization. Each of the three phases shows great diversity [4]. Three pollinating agents (two abiotic, and one biotic i.e. many species of animals) are involved in effecting pollination. Plants in general were classified based on their floral biology as adapted to self-and cross-pollination, either by wind or by animals, a majority of these being insects [5]. Kolreuter first reported the role of insects as pollinators in 1763. It was estimated that in nature 5% of flower self-pollinated and 95% are cross-pollinated, out of which 10% depends upon wind and 85% upon the insects. The insects of the family apidae are the most reliable agents for pollination. Among honeybees are particularly important pollinators as they are capable of carrying pollen and nectar with which they feed their immature stages and in the process, the plants visited by them are benefited. About 10,00,000 kinds of flowers in this universe are assumed depending on floral visitation like, honeybees and other insects [6]. Obtaining adequate pollination in crops that must be pollinated by insects has become a problem in our agricultural economy [6].

Materials and Methods

Study area

The present investigations were carried out during the period 2012-2014 in Gwalior district (lies between 26°11'43.5" latitude North to 78°10'25.0" East longitude) representing the northern most part of Madhya Pradesh. The average elevation is about 197 meters above the sea level and spread over an area of 5214.00 sq km in the Chambal river valley. The development of the particular vegetation in any area is largely dependent on various meteorological factors like temperature, rainfall, relative humidity, etc. The variation of meteorological factor over Gwalior is remarkable.

The study has been carried out in the following manner:

Gymnema sylvestre (Retz.) R. Br. ex Schult. and *Tylophora indica* (Burm.f.) are medicinally important and threatened plant species were selected from certain study sites at and around Gwalior (Madhya Pradesh). The above species have been categorized under different threat categories as per IUCN criteria as worked out by Jain (2008).

Experiment details

Important aspects such as floral biology, floral morphology, pollen viability, *in-vitro* pollen germination was carried out in sucrose with different concentrations [7]. Observations on pollinators were conducted over 10-15 days at the study sites during growing season of different medicinal plants as per Dafni's methods [8]. Flower visitors were recorded during the peak flowering time. Very close observation was made to find out the pollinator-stigma contact. Flowers were observed from 0700 hours to 1900 hours during the blooming period. All observations were made from a fixed location from which 20-40 flowers could be monitored. Pollination efficiency of different pollinating insects was recorded by making observations on pollen load on their body parts under a microscope according to the given by [9]. Drop method was used for pollen count by counting the average ten drops (50% glycerine) in total volume of 50 ml [10]. Pollen tube growth in pistil, floral visitors behavior and pollination of plant species were undertaken for the investigations during 2012-2014.

Results and Discussion

1. *Gymnema sylvestre* (Retz.) R. Br. ex Schult (Gurmar)

Floral biology

In *Gymnema sylvestre*, flowers were small (5.895 ± 0.04 mm long), yellow, in axillary and lateral umbel in cymes with inflorescences 1.405 ± 0.01 cm long, born in extra-axillary position in corymbose cymes. Calyx pubescent, five lobed, obtuse, ovate and ciliate (1.5 ± 0.08 mm long). Corolla pale yellow, campanulate, 2.85 ± 0.06 mm long, pistil length 0.71 ± 0.04 mm long, about equaling the lobes; lobes five, ovate-deltoid, spreading and glabrous. Corona corolline, of 5, fleshy processes inserted on the corolla tube, alternate with its lobes, free at the short deltoid tip, which are protruding out of the mouth of corolla tube. Gynostegium was 1.90 ± 0.05 mm long. Anther connective produced into a membranous tip, pollinia (16.85 ± 0.15 μ m x 10 long), style apex thick, sub hemispherical, much exerted beyond the anthers and follicles were paired carpels 2, unilocular; locules many ovuled. Result indicate that fruit set percentage in open pollinated flower was 12.42 ± 2.15 percent.

Phenology

In *Gymnema sylvestre*, all these phenophases showed almost a similar trend for three consecutive years (2012, 2013 and 2014) within each phenophase. Mean date of the leaf emergence showed a peak in second week of May and leaf maturity started in first week of August. However, flowering starts from the end of July and remains in full bloom till second week of August and continues for about 4-5 week till end of August. The fruit become mature in mid September and disperse by the end of November.

Anthesis

Anthesis and anther dehiscence time showed almost a similar trend for three consecutive years (2012, 2013 and 2014). In *G. sylvestre*, total number of flowers per inflorescence was 11.3 ± 3.4 , 10 ± 4.1 and 8.3 ± 4.1 in year 2012, 2013 and 2014 respectively. Anthesis occurred between 07.00 am- 06.00 pm, 07.30 am- 05.30 pm and 06.30 am- 06.00 pm and anther dehiscence between 12.30am - 02.30pm, 12.00 am- 02.00 pm and 11.30 am- 02.00 in year 2012, 2013 and 2014 respectively. Honey bees and small bees in large numbers forage the flowers between 10.00 am -12.00noon. They come in contact with the dehiscid anthers and collect pollen which is transferred on pollen sac of other flowers. On the other hand, bumble bees and wasp also visited flowers in after noon hours (12.00 noon – 2.30 pm) but they are nectar robbers.

Changes in structural features of anthers and stigma were observed in different time periods after the bud opening. At the earlier stage stigma is non receptivity. Pollen viability was highest during this phase (54.55 %). In the next phase the stigma becomes receptive and pollen receptivity goes on decreasing. Little pollen remains available in the anthers during this phase. In the drooping stage (3rd day) no pollen was available in the anther and they dried up, stigma became non receptive.

Pollen germination

In vitro pollen germination, in order to determine the viability and germination potential of pollen grains of *Gymnema sylvestre*, pollen grain were cultured in different concentrations of sucrose (1% to 15%). Germination was obtained in all these concentrations but, the best result in terms of pollen germination percentage was between 7.5-10 % concentrations of sucrose solution i.e. 32.18 % and 33.33 % respectively. Percentage of pollen germination (%) and length of pollen tube (μ m) under different concentration of sucrose solution. By increasing sucrose solution concentration, pollen germination percentage decreases was observed. Within first one hour pollen tube length was increased up to 2.14 μ m (10x) with 7.5 % sucrose solution. Moreover, at the end of 3 hours, best germination and least bursting was observed with 7.5% sucrose solution. The concentration of sucrose solution required for pollen germination and pollen tube growth showed variation with concentration and time duration.

Pollination biology

In *Gymnema sylvestre*, a large quantity of nectar attractants and floral visitors are small bees (*Melipona* sp.), bumble bees (*Xylocopa* sp.), wasp (*Vespa* sp.) and honey bees (*Apis dorsata*). They come in contact with the dehiscid pollinia and collect

pollen which is transferred on the pollen sac on the other flowers. Small bee and bumble bee represent high visiting frequency. It was 10.00 am- 03.00 pm small bee (*Melipona sp.*) and 11.30 am- 03.30 pm bumblebee (*Xylocopa sp.*) and total time spent on each flower was 3-8 second and 2-10 second respectively. Similarly, wasp and honey bee represent intermediate visiting frequency. The foraging time recorded was 10.30 am- 03.30 pm in case of wasp and 12.00 noon-02.30 pm in case of honey bee). The time spent by visitors on each flower was 3-6 second in wasp and 6-19 second in honey bee.

Floral visitors

The recorded visitor species included three bees (60 %) and two wasps (40%). All the floral visitors were observed to collect nectar. Floral visitor generality (in terms of numbers of flower visited) was significantly higher in year 2014 compared with 2012 and 2013. Conversely, in *Gymnema sylvestre* generality (in average time spent per flower) in honey bee was significantly lower at 2012 i.e. 9.5 ± 0.8 second/minute than in 2013, 9.5 ± 1.0 second/minute and in 2014, 12.8 ± 0.8 second/ minute. However, regular floral visitors like honey bees (*Apis indica*), wasp (*Vesps sp.*), beetle (*Coleoptera sp.*) and bumble bee (*Xylocopa sp.*) were simultaneously increasing in the average time spent per flower (in second) during the visitors time from the year 2012-2014.

2. *Tylophora indica* (Burm. f.) Merr. (Dumbel)

Floral biology

In *Tylophora indica*, flowers were greenish-yellow outside, purplish within, 0.70 ± 0.01 cm long, in many flowered umbels (dichasial cyme) with inflorescence length 6.28 ± 0.10 cm long. The number of primary branches of flowers per inflorescence was found to be 9.00 ± 0.32 . Flowers numerous, borne in terminal or auxiliary, long-stocked clusters with present of 64.55 ± 2.88 flower per stem recorded. Fruit a follicle, fusiform, divaricate, striate, seed ovate, elongated into a coma, silky hairs at one end. Result indicate that fruit set percentage in open pollinated flower was $13.83 \pm 2.13\%$, number of seed per fruit was 15.00 ± 0.31 and length of calyx and corolla was recorded 0.29 ± 0.01 and 0.50 ± 0.01 respectively. Pollinium orientation has been used as a diagnostic character in *T. indica*, pollinia were slightly erect (39.65 ± 0.61 μm x10 long), shape of pollinial sacs (9.90 ± 0.25 μm x10 long) were more or less oval but globular shape was found, that is why this type of pollinia was recognized as erect pollinia.

Phenology

In *Tylophora indica*, mean date of the leaf emergence showed a peak in first week of April while leaf maturity started in second week of July. However, flowering starts from the end of June and remains in full bloom till second week of July and continues for about 6-7 week till third week of August. The fruit become mature in second week of September and disperse by the end of September.

Anthesis

Tylophora indica, was found in flowering stage during August-September at different location of Gwalior district. Total numbers of flower per inflorescence prodred are $7.3 \pm 3.1.8$, 7.5 ± 1.4 and 7.5 ± 1.4 in year 2012, 2013 and 2014 respectively. Anthesis occurred between 10.00 am- 04.00 pm, 08.00 am- 04.00 pm and 09.00 am- 05.00 pm and anther dehiscence time between 09.30 am- 11.30 pm, 10.00 am- 01.00 pm and 10.00am- 12.00 noon in year 2012, 2013 and 2014 respectively. Wasp, small bee and flies were main pollen visitors of the species. There were 20.7 ± 14 %, 18.8 ± 12 % and 20.4 ± 11.4 % fruit set in open pollinated flowers in year 2012, 2013 and 2014 respectively.

The stigma receptivity at the early stage of flowering (flower opening) was very low which increased in the later hours of the day and becomes moderate on second day. The pollen viability was found to be maximum during the early phase of flower. Zero stigma receptivity was observed on third day.

Pollen germination

In vitro pollen germination effects of different concentration of sucrose solutions on the germination rate of *Tylophora indica*. Different sucrose solutions were compared to each others. Generally germination rates were better than control (1%). When the averages are compared, 10% sucrose was better (35.82%) than others. Germination rate of pollen tube increased when the percentage of sucrose increased. Gradual increase in the sucrose concentration (>10 %) percentage solution resulted fall in the germination percentage.

Pollination biology

In *Tylophora indica*, a large quantity of nectar attractants and floral visitors were wasp (*Polistic orientalis* and *Polistic sp.*), bumble bees (*Xylocopa sp.*) and flies. While, *Polistic orientalis* visited in low frequency in floral attract. Small bees (*Melipona sp.*) and flies in large numbers forage the flowers between 11.00 am-02.30 pm and 09.30 am-01.00 pm. They come in contact with the dehisced pollinia and collect pollen which is transferred on the pollen sac on the other flowers. Similarly wasp (*Polistic orientalis*) showed less visiting frequency and foraging time between 12.30 am- 02.00 pm and time spent for each flower as 9-11 second.

Floral visitors

Total time spent (second/ minute) per flower in the visitation dataset varied because of the flower visitor diversity, duration of flowering season and floral abundance within the habitat. Floral visitors like, wasp (*Vespa orientalis*), small bees (*Melipona sp.*) and flies (*Diptera*) were simultaneously decreased in the average time spent per flower (in second) during the visitors time (second/minute) from the year 2012-2014. However, wasp represents the high number of visitation time spent per flower i.e. 27.3 ± 5.0 , 33 ± 4.7 and 22.42 ± 3.7 second /minute in 2012, 2013 and 2014 respectively.

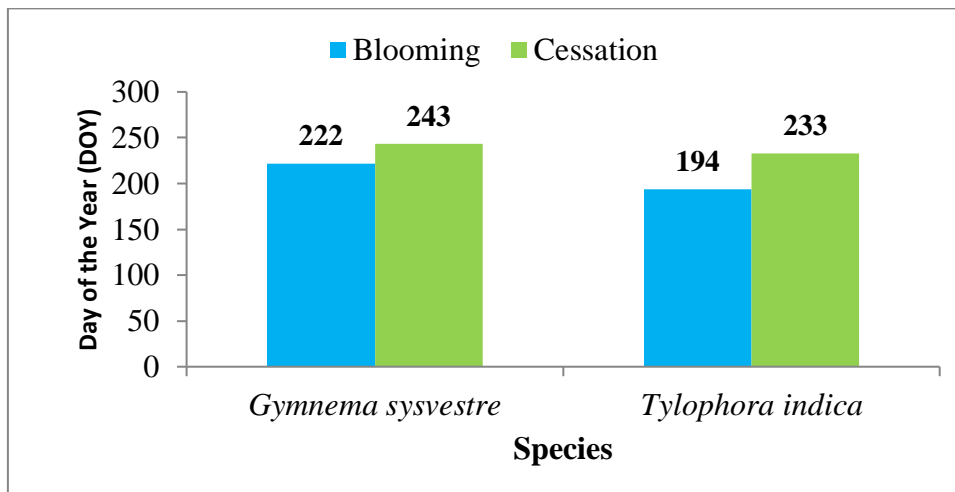


Figure 1. Floral phenology of two selected species

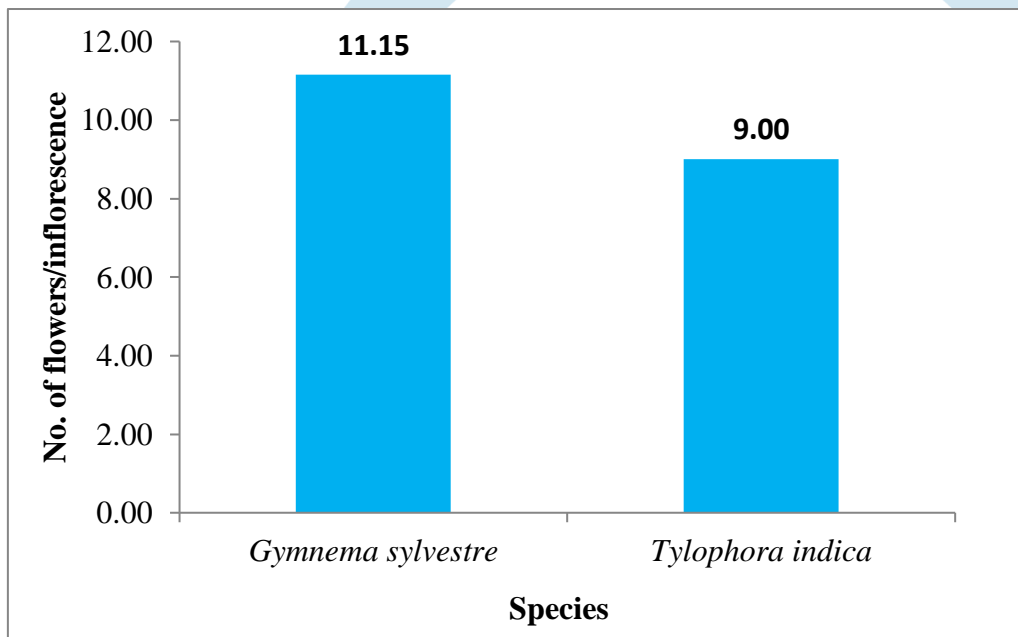


Figure 2. Number of flower per inflorescence in investigated plants

Pollen production and pollen ovule ratio (P/O) was found selected plant species. The average number of pollen per flower is 1203 in *Gymnema sylvestre* and 1990 in *Tylophora indica* was found. However, it was observed that highest P/O ratio (17:01) was found in *Tylophora indica*.

Table 1. Pollen production and pollen-ovule (P/O) ratio of investigated plants

S. No.	Name of plants	<i>Gymnema sylvestre</i>	<i>Tylophora indica</i>
1	Average no. of anthers/flower	5	5
2	Average no. of pollen/anther	241	398
3	Average no. of pollen/flower	1203	1990
4	Average no. of ovule/flower	121	116
5	Pollen-ovule ratio	10:01	17:01

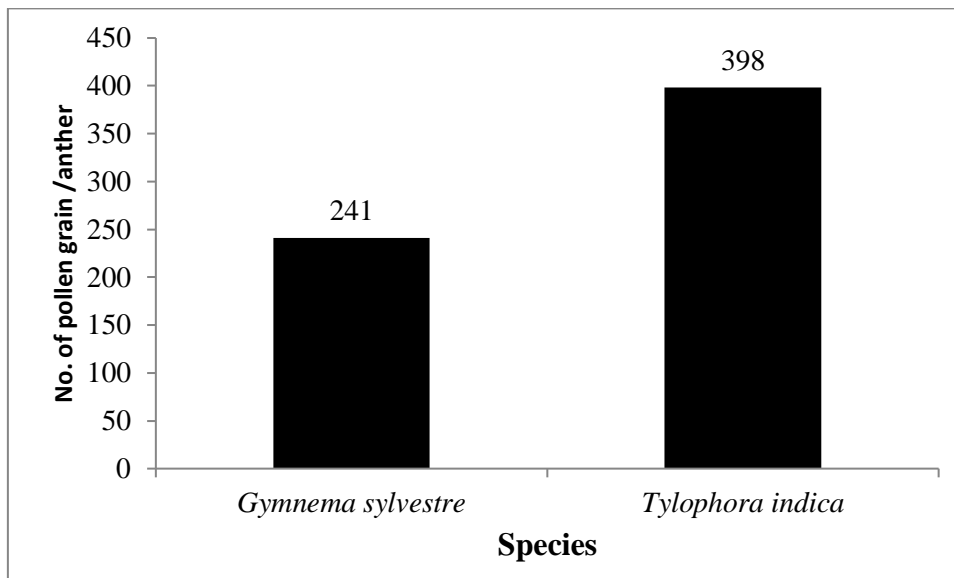


Figure 3. Average number of pollen per anther in investigated plants

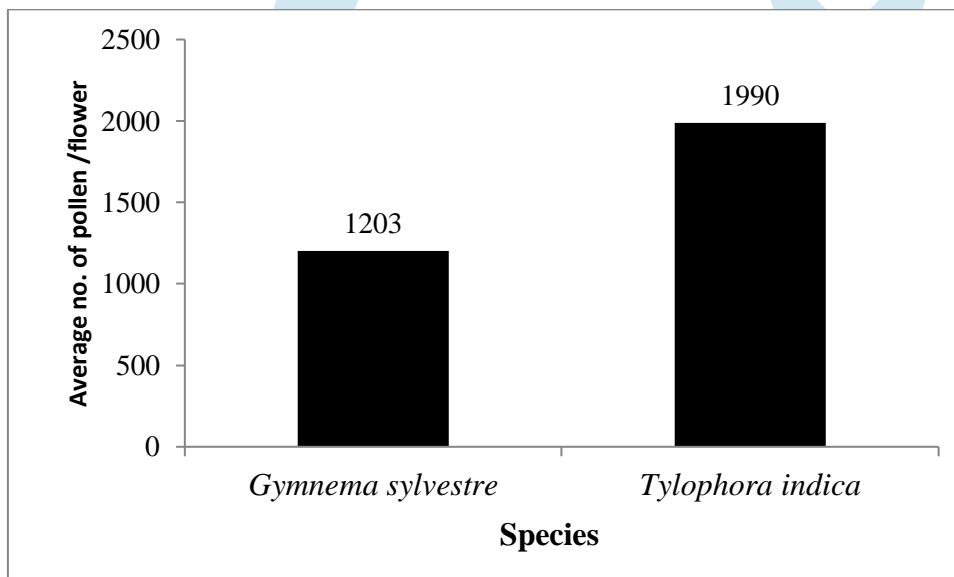


Figure 4. Average number of pollen per flower in investigated plants

The most frequent visitors to these plants were *Apis indica* (Hymenoptera), *Apis dorsata* (Hymenoptera), *Melipona sp.* (Hymenoptera), *Pieris brassicae* (Lepidoptera), *Xylocopa sp.* (Hymenoptera), *Vespa orientalis* (Hymenoptera), *Vespa sp.* (Hymenoptera), *Camponotus campestris* (Hymenoptera), *Trathile hepilae* (Diptera), *Papilio desmoleus* (Lepidoptera), *Limenistris sp.* (Lepidoptera) and fly (Diptera) were also recorded. The number of visitors was found to be highest in *Gymnema sylvestre* 5 and *Tylophora indica* is 4.

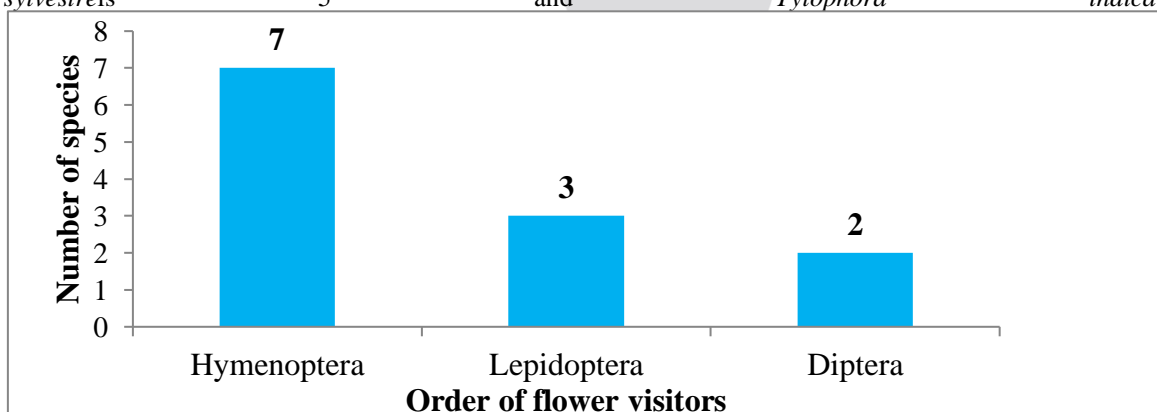


Figure 5. Flower visitors of investigated plants

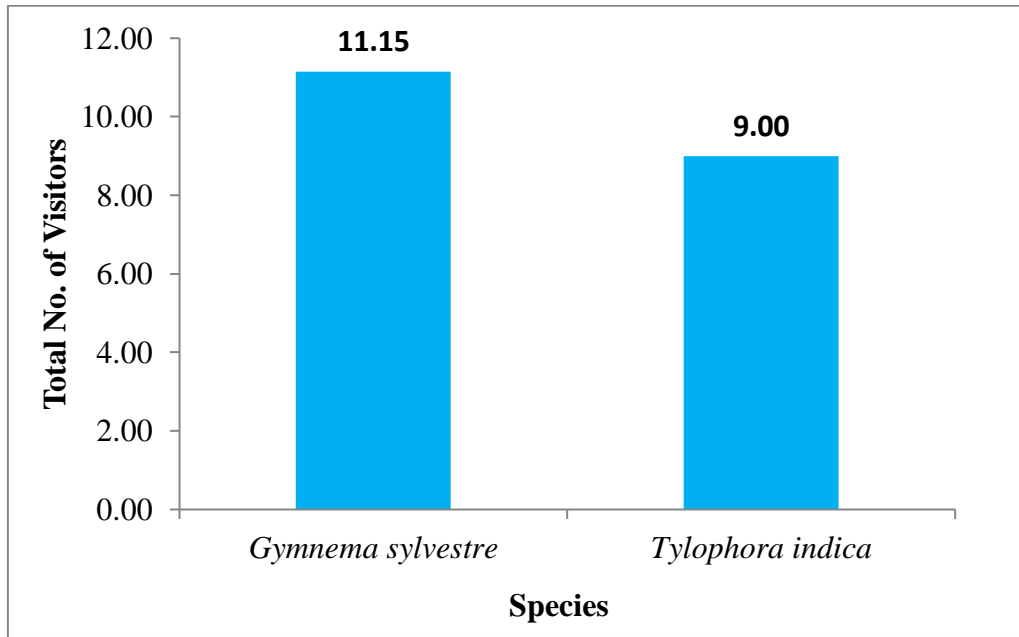


Figure 6. Total number of visitors/pollinators present in investigated plants

Small bees (*Melipona sp.*) and bumble bees (*Xylocopa sp.*) were found to be the main visitors in *Gymnema sylvestre* and *Tylophora indica*. Time spent (seconds) by the visitors/pollinators of selected plant species was recorded and it was found that *Camponotus campestris* spent maximum time (s) with an average of 106.16 second on flower.

The reproductive season and the anthesis of two species were observed in their respective seasons. The pollen germination percentage was calculated for the species and found to be highest (11.15%) in *Gymnema sylvestre* and (9.00 %) in *Tylophora indica*.

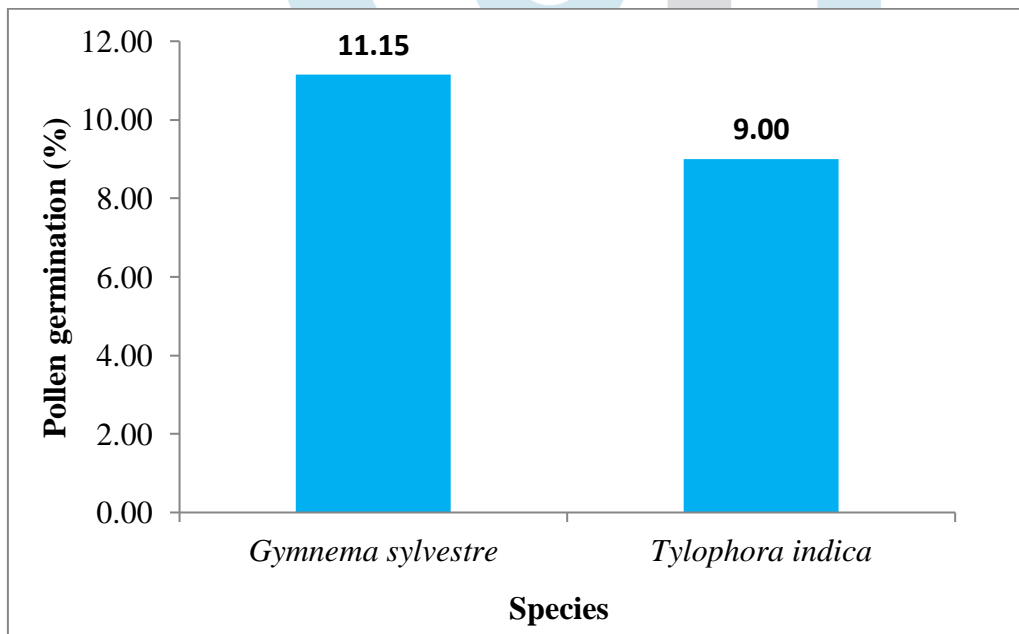


Figure 7. Pollen germination percentage in investigated plants

Discussion

Gymnema sylvestre and *Tylophora indica* are medicinally important species in the Central region of India and have been widely studied in recent years. *Tylophora indica* (Burm.f) Merr. (Asclepidaceae) is a threatened medicinal plant found in restricted localities in India [11]. The reproductive biology of these plants was studied to determine the main pollination mechanism and pollen restriction of these species [12]. This study was conducted, including observations on phenology and floral characters of flowers, pollinator visitations and pollinator behavior. Pollen restriction and reproductive success were assessed by fruit and seed-set [13-14]. On comparing the floral phenological characters of these species it was found that *Tylophora indica* has longer blooming duration (39 days), flowering peak and number of flowers per inflorescence (21.8). *Gymnema sylvestre* showed shorter

blooming period (21 days) when compared with other plant species. The lowest number (9) of flowers per inflorescence was observed in *T. indica*.

Tylophora indica showed precise time of flowering and fruit formation. Among different parameters, photoperiodic stimuli play an important role in the initiation of flowering which marks the end of vegetative phase. In the present investigation flowering starts from the end of June and remains in full bloom till second week of July and continues for about 6-7 week till third week of August [15]. The fruit become mature in second week of September and disperse by the end of September. Anthesis and pollen production is essential to the study of pollination, developing a functional model for forecasting pollen concentrations and to understand more about the ecological background of pollen dispersal [16-17].

Conclusion

Anthesis time occurred between 07.00am - 06.00pm, 07.30am - 05.30pm and 06.30am - 06.00pm and anther dehiscence between 12.30am - 02.30pm, 12.00am - 02.00pm and 11.30am - 02.00pm in year 2012, 2013 and 2014 respectively. Pollen were cultured in different concentrations of sucrose from 1% to 15%. Germination was obtained at all these concentrations but, pollen germination between 7.5-10 % concentrations of sucrose solution was highest 32.18 % and 33.33 % respectively. In *Gymnema sylvestre*, a large quantity of nectar attracts and floral visitors are small bees (*Melipona* sp.), bumble bees (*Xylocopa* sp.), wasp (*Vespa* sp.) and honey bees (*Apis dorsata*). While, *Polistic orientalis* visited with low frequency in floral attract. Small bees (*Xylocopa* sp) and wasp (*Vespa* sp.) in large numbers forage the flowers between 10.00-13.30 hours. They come in contact with the dehiscent pollinia and collect pollen which is transferred on the pollen sac on the other flowers. In *Tylophora indica*, in vitro pollen germination in different sucrose solutions was compared to each other. Generally germination rates were better than control (1%). When the averages were compared, 10% sucrose was better (35.82%) than others. Germination rate of pollen tube increased when the percentage of sucrose increased. Gradual increase in the sucrose concentration (>10 %) percentage solution resulted in the germination percentage fall. A large quantity of nectar attracts and floral visitors observed were wasp (*Polistic orientalis*), wasp (*Polistic* sp.), bumble bees (*Xylocopa* sp.) and flies. While, *Polistic orientalis* visited with low frequency in floral attract. Small bees (*Xylocopa* sp) and flies in large numbers forage the flowers between 11.00am - 02.30pm and 09.30am - 01.00pm. They come in contact with the dehiscent pollinia and collect pollen which is transferred on the pollen sac on the other flowers.

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