



RESEARCH ARTICLE

Pollination ecology and flowering rhythm of *Gloriosa superba* L.: Behavioural insights into principal pollinators

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Abstract

Gloriosa superba L. (Glory lily), is an endangered medicinal plant valued for its high colchicine content. Understanding the floral biology and pollination ecology of *G. superba* is crucial for identifying factors limiting seed setting. This study was carried out at Dr. YS Parmar University of Horticulture and Forestry, Solan, Himachal Pradesh, India, during 2021-2022, focusing on *G. superba*'s floral biology and pollinator activity from anthesis to senescence. The scarlet red flowers were born on pedicels, with tepals measuring 6.69×1.45 cm tepal and a mean weight of 1.72 ± 0.04 mg. Pollen viability was highest on the one day after anthesis and stigma receptivity peaked on the day of anthesis. Among nine flower stages, nectar volume and nectar sugar concentrations were maximum at 6th and 7th stages, respectively. Three kinds of insect floral visitors viz., allotrophic, hemitrophic and eutrophic from 6 orders, 14 families and 16 genera were recorded. The Shannon, Simpson, dominance and Margalef richness indices during 2021 and 2022 were 2.58, 0.16, 0.83 and 1.3 and 2.15, 0.22, 0.83 and 1.22, respectively. Fruit set (86.45 ± 0.21 %), pod length (7.24 ± 0.23 cm) and colchicine percentage were highest in hand pollination. However, test weight (2.02 ± 0.01 g), germination percentage (77.98 ± 0.26 %) and colchicine percentage in seed under honeybee pollination were statistically similar to blower-assisted pollination.

Keywords

foraging behaviour seed colchicine content; glory lily; insect diversity; nectar studies

Introduction

Gloriosa superba L. commonly known as Malabar glory lily, is a commercially valuable medicinal plant of the family Liliaceae. It contains the alkaloid colchicine in its seeds, stems, leaves and tubers (1, 2). The species is distributed across tropical regions, from the North-West Himalayas to Assam and the Deccan Peninsula, at elevations up to 2120 m (3-5). The tubers contain 2-5 times higher colchicine content than seeds compared to (6). Traditionally, its seeds and tubers were used in treating gout, rheumatism, cholera, typhus, leprosy, colic and skin diseases, haemorrhoids, impotence, gonorrhoea and chronic ulcers (7-10). During the 1980s, *G. superba* faced near-extinction due to indiscriminate harvesting and continuous over-exploitation of tubers from natural habitats by local population as well as pharmaceutical companies and has been declared as an 'endangered' species by IUCN Red Data Book (5, 11-13).

Despite this, low seed germination with poor viability is also one of the factors responsible for its diminishing population.

Studying floral biology, pollination ecology and pollinators' behaviour is crucial for crop improvement, as it provides insights into floral morphology, pollination mechanisms and the adaptation of flower visitors (14, 15). The plant-pollinator relationship plays a key role in community structure and can influence the spatial distribution, species richness and abundance (16–19). The flower of *G. superba* is characterized by its large size, vivid and gradient tepals, claw-like shape and nectar spurs. Due to its unique floral structure, both self- and cross-pollination is challenging, requiring external agents such as biotic (insects) or abiotic (wind) factors. Previous research on *G. superba* has primarily focused on pollen studies, butterfly pollinators, reproductive biology, seed set, colchicine content, seed characteristics and germination ability (20–24). However, little attention has been given to the foraging behaviour of insect visitors, particularly non-butterfly pollinators, across different times of the day. Understanding floral biology, pollination mechanism, diversity of pollinators and their foraging behaviour is extremely important for management of this species for pollination. Information is lacking on nectar characteristics, pollinator diversity, foraging behaviour and pollination efficiency of *G. superba* in the Northwestern Himalayan region and across India. In this study, we aimed to assess the diversity and foraging behaviour of key insect visitors on *G. superba*.

Materials and Methods

The research was conducted during July–November 2021 and July–November 2022 at Medicinal and Aromatic Farm, Department of Forest Products, Dr. YSPUHF, Nauni at an altitude of 1270 meters amsl (30° 51'44.7444" N, 77° 10'9.1488" E) Solan, Himachal Pradesh, India. The climate is sub-temperate, with an average annual rainfall of 1250 mm and average annual temperature ranges from 11.0 to 25.9 °C. The floral events were determined by randomly selecting fully opened flowers (n=10) and floral characteristics namely measurement of flower spread, sepal length, petal length, tepal length, stamen length, pistil length, number of stamens, stamen basal gap and weight of flower were recorded in the laboratory. Stigma receptivity and pollen viability were determined using acetocarmine method (23, 25). The flowers selected for nectar collection were caged a day prior to collection to prevent nectar robbing by insects. From 800 to 1000 hours, nectar was directly extracted using micropipettes from the flower base into an Eppendorf tube and stored at 15 °C for further analysis (26). Changes in nectar volume and nectar sugar concentration at different floral stages, quantitative analysis of dry nectar sugars and qualitative estimation of nectar sugars were conducted using HPLC-ELSD (27, 28).

Using a standard protocol, the diversity of insect floral visitors was recorded using scan sampling and sweep net capture methods (29, 30). All the specimens were identified by using published keys (31). The foraging behaviour such as foraging rate, foraging speed, loose pollen grains of

important insect pollinators was also studied and these parameters were recorded at different day intervals throughout the flowering period. Statistical analysis was conducted using mean values for nectar parameters, yield parameters, foraging rate, foraging speed and loose pollen grains. ANOVA was used to compare mean values. A Randomized Block Design (RBD) factorial was used to analyze foraging behaviour using OPSTAT.

Results and Discussion

The flowering period commences from second fortnight of July to September with ripening of pods in the month of October. There were nine flower stages were observed in *G. superba* ranging from stage-1 to stage-9, which included pre-pollination (stage 1 to stage 5), nectar accumulation (stage 4 to stage 8) and post pollination stages (after stage 8) (Fig. 1). The scarlet red flowers were solitary in position having average floral characters viz., the pedicel length, tepal length, tepal breadth, filament length, anther length, stamen basal gap and weight of flower were recorded as 10.32 ± 0.14 cm, 6.69 ± 0.11 cm, 1.45 ± 0.05 cm, 4.62 ± 0.17 cm, 1.26 ± 0.03 cm, 0.48 ± 0.07 mm and 1.72 ± 0.04 g, respectively. The anther dehiscence was observed at the stage when longitudinal splitting of anthers started and average pollen viability ranged from 45.79 to 88.19 % and 46.67 to 88.61 % during year 2021 and 2022, respectively and significant maximum viable pollens were recorded on the first day after anthesis and pollen viability decreased thereafter (Fig. 2). The stigma remained receptive for four days i.e., one day prior to anthesis, on the day of anthesis, one day after anthesis and two days after anthesis and average stigma receptivity ranged from 39 to 80 % maximizing on the day of anthesis and minimizing two days after anthesis (Fig. 2). Similar observations on floral characters and pollen viability have been reported (26, 32, 33).

In *G. superba*, nectar production began after stage 4 and peaked at stage 6, then dropped with seed set until it halted entirely at stage 9. From stage 5 to stage 8, nectar secretion ranged between 4.13 ± 0.28 and $33.04 \pm \mu\text{l}/\text{flower}$ in 2021 and between 3.78 ± 0.33 and $32.10 \pm 0.54 \mu\text{l}/\text{flower}$ in 2022. Significantly maximum and minimum nectar accumulation was observed at stage-6 and stage-8 respectively. and after stage 8, nectar accumulation ceased. Further, the sugar concentration of nectar ranged from 52.57 ± 0.75 to 78.04 ± 0.33 % during 2021 and 52.14 ± 0.72 to 77.91 ± 0.36 % during 2022. Quantitative analysis of dry nectar sugars revealed that significant maximum dry nectar sugars were present at stage 6 ($94.70 \pm 0.85 \mu\text{g}/\text{flower}$) followed by stage 7 ($86.07 \pm 0.28 \mu\text{g}/\text{flower}$) and stage 8 ($81.07 \pm 0.34 \mu\text{g}/\text{flower}$) during 2021 and similar trend was observed during 2022 also with 94.70 ± 0.85 , 86.07 ± 0.28 and $81.07 \pm 0.34 \mu\text{g}/\text{flower}$ at stage 6, 7 and 8 respectively. The qualitative analysis revealed that nectar comprises of sugars namely glucose, sucrose, fructose and raffinose. The present findings on nectar volume and nectar sugar concentrations of *G. superba* at Nauni (Solan) are higher than the previous observations (24, 34). The results may be variable due to the dry climatic conditions of their study area.

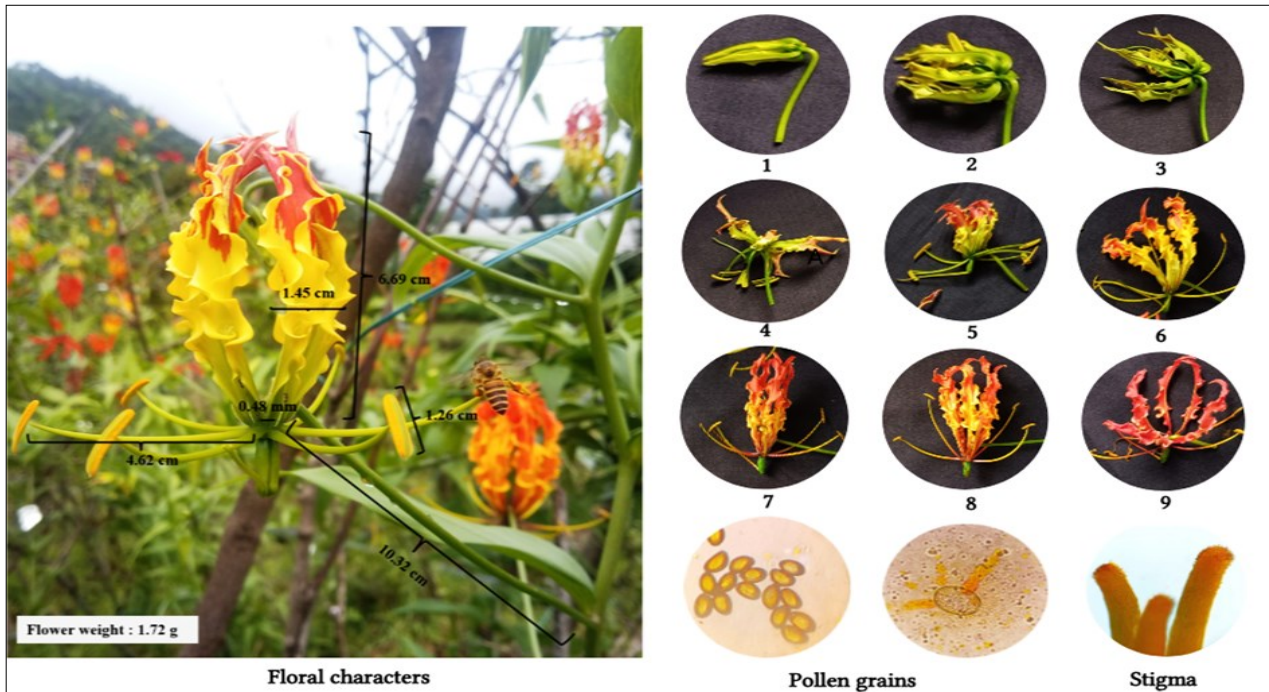


Fig. 1. Floral characters and developmental stages of *G. superba* flowers including bud opening (Stages 2-4), nectar secretion (Stages 4-7) and pollination/post-pollination phases (Stages 8-9).

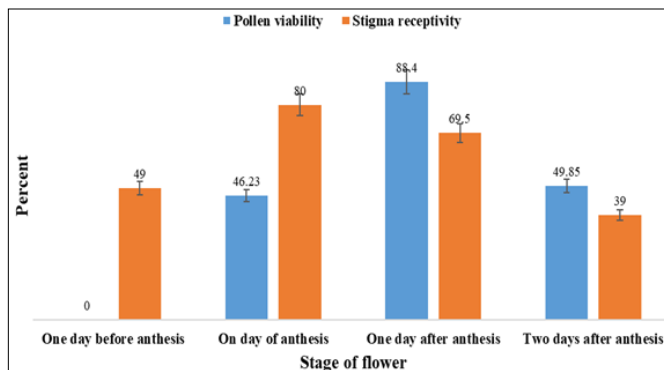


Fig. 2. Pollen viability and stigma receptivity at different floral stages in glory lily.

The allotrophic, hemitrophic and eutrophic types of insect visitors were recorded on glory lily from 6 orders, 14 families and 16 genera. Among all, hymenopterans were most dominant insect visitors followed by dipterans, lepidopterans, coleopterans, orthopterans and hemipterans (Table 1, Fig. 3). Majority of the insects were reported during both years except

the insects of Vespidae and Pieridae families whose presence was not recorded during 2021 while during 2022, all insects were present except some lepidopterans such as *P. bianor* and *E. core*. The diversity indices viz., Shannon, Simpson, dominance and Margalef richness indices during 2021 and 2022 were 2.58, 0.16, 0.83 and 1.3 and 2.15, 0.22, 0.83 and 1.22 respectively. During both years, maximum relative abundance of insect visitors was recorded during morning and noon hours (800 to 1400 hours) and their abundance decreased thereafter (Table 2). Maximum *B. haemorrhoidalis* population was recorded during 800-1000 hours only. During 2021, no population of *Vespa* spp. was found visiting glory lily flowers but in 2022, considerable population of *Vespa* spp. was recorded. The change in incidence of wasps may occurred due to fluctuations in weather parameters.

Earlier studies of pollinator diversity in glory lily suggested that butterflies particularly from Pieridae and Papilionidae, families may act as best pollinators for glory lily due to their large size and wing pollination mechanism

Table 1. Insect floral visitors on glory lily flowers

Order	Family	Name of the species	2021	2022	
Hymenoptera	Apidae	<i>Apis mellifera</i> Linnaeus 1758	+	+	
		<i>Apis cerana</i> Fabricius 1793	+	+	
		<i>Bombus haemorrhoidalis</i> Smith 1852	+	+	
	Vespidae	<i>Vespa magnifica</i> Smith 1852	-	+	
		<i>Vespa velutina</i> Lepeletier 1836	-	+	
		<i>Vespa</i> spp.	-	+	
		Small ants (<i>Lasius</i> sp.)	+	+	
	Formicidae	Large ants (<i>Camponotus</i> sp.)	+	+	
		Syrphidae	<i>Eristalis tenax</i> Linnaeus 1758	+	+
		Muscidae	<i>Musca</i> spp.	+	+
Diptera	Calliphoridae	<i>Calliphora</i> sp.	+	+	
	Tephritidae	<i>Bactrocera zahadi</i> Mahmood 1999	-	+	
Coleoptera	Coccinellidae	<i>Coccinella septempunctata</i> Linnaeus 1758	+	+	
	Meloidae	<i>Mylabris pustulata</i> Thunberg 1821	+	+	
	Pieridae	<i>Pieris brassicae</i> Linnaeus 1758	-	+	
Lepidoptera	Papilionidae	<i>Papilio bianor</i> Cramer 1777	+	-	
	Nymphalidae	<i>Euploea core</i> Cramer 1780	+	-	
Orthoptera	Pyrgomorphidae	<i>Aularches miliaris</i> Linnaeus 1758	+	+	
Hemiptera	Pentatomidae	<i>Erthesina fullo</i> Thunberg 1783	+	+	

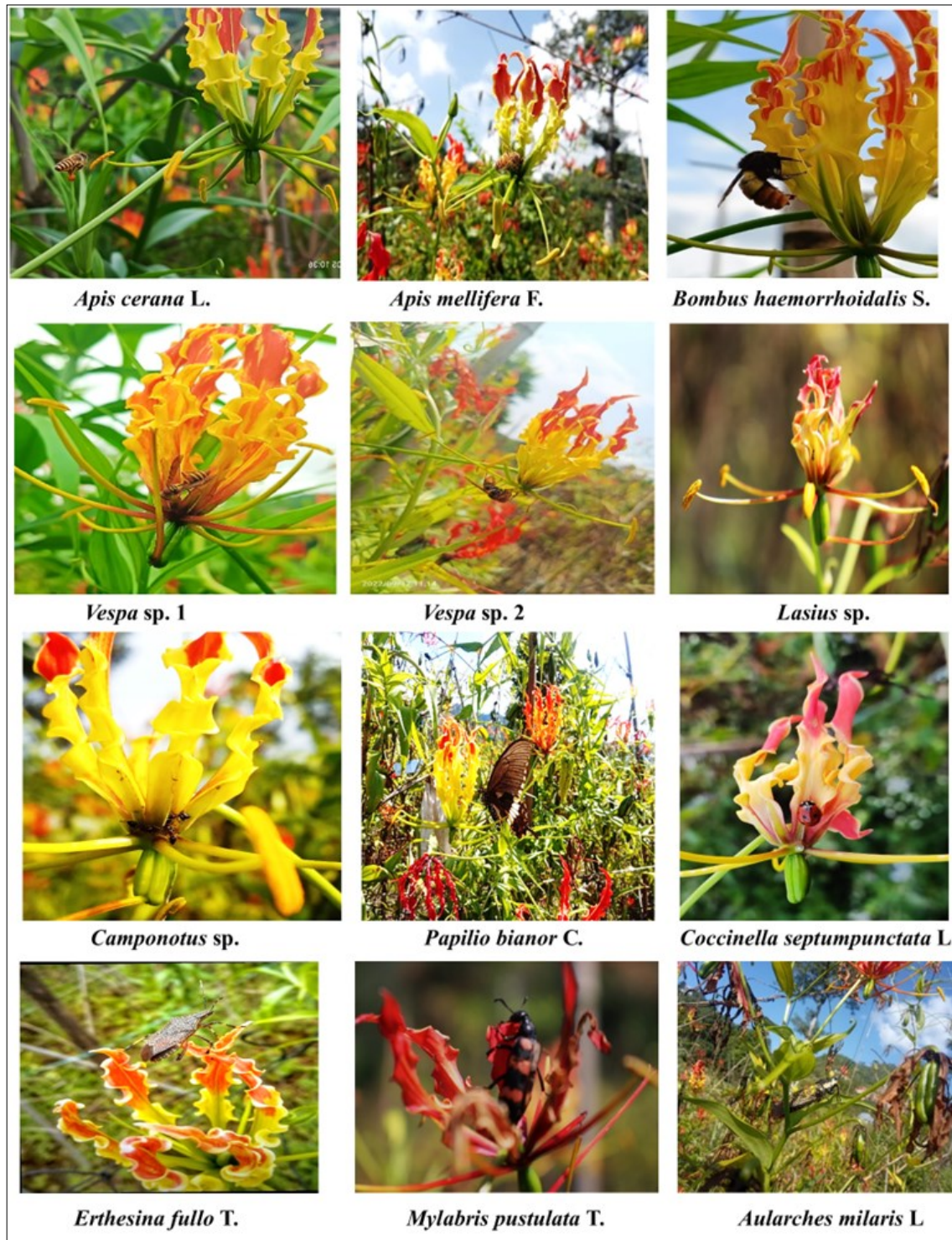


Fig. 3. Diversity of insect species observed on *Gloriosa superba* L.

Table 2. Relative abundance of insect visitors on *G. superba* during 2021 and 2022

Insects	Relative abundance of insect visitors (Number/m ² in 5 minutes)							
	2021				2022			
	0900h	1300h	1700h	Mean	0900h	1300h	1700h	Mean
AC	6.53 (2.74)	4.23 (2.28)	1.20 (1.47)	3.99 (2.16)	7.10 (2.84)	4.63 (2.36)	2.77 (1.94)	4.83 (2.38)
AM	7.70 (2.95)	5.57 (2.56)	2.03 (1.73)	5.10 (2.41)	7.23 (2.87)	5.97 (2.64)	3.17 (2.04)	5.46 (2.52)
V	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	7.97 (2.99)	5.17 (2.48)	2.57 (1.89)	5.23 (2.45)
BH	0.67 (1.28)	0.17 (1.08)	0.00 (1.00)	0.28 (1.12)	3.53 (2.13)	0.00 (1.73)	1.47 (1.57)	1.67 (1.81)
A	11.60 (3.55)	4.73 (2.40)	2.60 (1.88)	6.31 (2.61)	12.13 (3.62)	7.43 (2.90)	4.03 (2.24)	7.87 (2.92)
L	2.00 (1.72)	5.00 (2.44)	1.00 (1.38)	2.67 (1.85)	0.99 (1.14)	0.67 (1.24)	0.33 (1.14)	0.66 (1.17)
O	3.67 (2.16)	1.33 (1.52)	3.00 (1.98)	2.67 (1.89)	2.67 (1.88)	1.67 (1.63)	2.00 (1.72)	2.11 (1.74)
Mean	4.60 (2.21)	3.00 (1.91)	1.40 (1.49)	3.00 (1.86)	5.95 (2.51)	3.65 (2.14)	2.33 (1.79)	3.98 (2.14)

CD_(0.05) (2021): Insects (0.19), Day hours (0.12), Insect× Day hours (0.33)

CD_(0.05) (2022): Insects (0.19), Day hours (0.13), Insect× Day hours (0.34)

AC- *Apis cerana*; AM- *Apis mellifera*, V- *Vespa* sp. 1, A-ants, BH- *Bombus haemorrhoidalis*, L- Lepidopterans, O- other insects

Figures in the parentheses are $\sqrt{x+1}$ transformed values

(34, 35). However, from India large sized bumble bees and sunbirds have also been reported as pollinators of *G. superba* (36). At Eastern Cape, South Africa butterflies (*Eronia cleodora* subspecies *cleodora* (Pieridae) and *Papilio demodocus* subspecies *demodocus*) were reported as the most common visitors to *G. superba* (34). In the present study, apart from insects of Pieridae and Papilionidae families, other insects from families Apidae, Vespidae and Nymphalidae were also reported as floral visitors to glory lily under Nauni (Solan) conditions during 2021 and 2022. Among the various pollinators, irrespective of day hours, *B. haemorrhoidalis* visited significantly maximum number of flowers followed by *A. cerana*, *A. mellifera* and *P. bianor* (Fig. 4) while during different day hours, irrespective of species foraging rate of all insects was significantly maximum during 1600-1800 hours (4.91 number of flowers visited per minute). In contrary, the foraging speed of all insects was significantly maximum during 800-1000 hours such as 14.79 seconds spent on one flower and irrespective of day hours, foraging speed of *A. mellifera* was significantly maximum followed by *A. cerana*, *P. bianor*, *B. haemorrhoidalis* and *Vespa* spp. (Fig. 4). Hence,

three kinds of pollination in glory lily including melittophilus (honey bee), sphenophilus (wasps) and psychophilus (butterfly) was observed and these three insects may play an efficient role in glory lily pollination.

The effect of different pollination systems on the yield parameters of glory lily focusing on seed colchicine content along with fruit set, pod length, test weight and percentage of germination was analyzed. Maximum fruit set, pod length and test weight were obtained in hand pollination system and these yield parameters were statistically like the pollination system assisted with blower pollination (Fig. 5). However, the yield parameters, test weight and percent germination in pollination system assisted with honey bee pollination such as, $2.04 \pm 0.01\text{g}$ and $79.02 \pm 0.26\%$, respectively were also statistically similar to hand and blower pollination treatments which represents that although bee pollination system not resulted in maximum fruit set and pod length but other crucial yield parameters like test weight and percentage germination in bee pollination were found to be significantly similar to blower and hand pollination system.

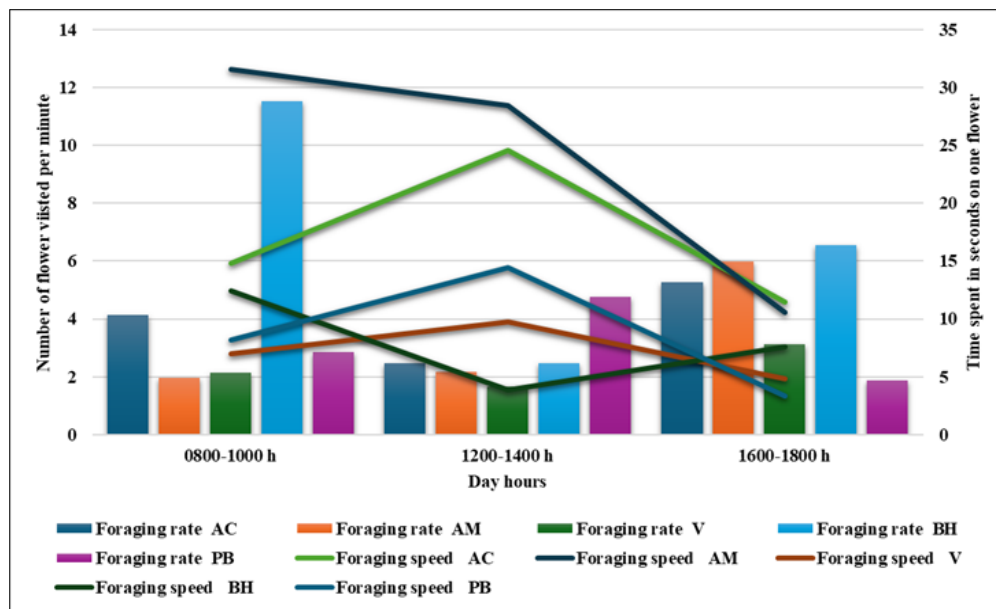


Fig. 4. Foraging behaviour viz., Foraging rate, Foraging speed and Loose pollen grains of important insect visitors on *G. superba* (*AC- *A. cerana*; AM- *A. mellifera*, V- *Vespa* sp. 1, BH- *B. haemorrhoidalis*, PB- *Papilio bianor*).

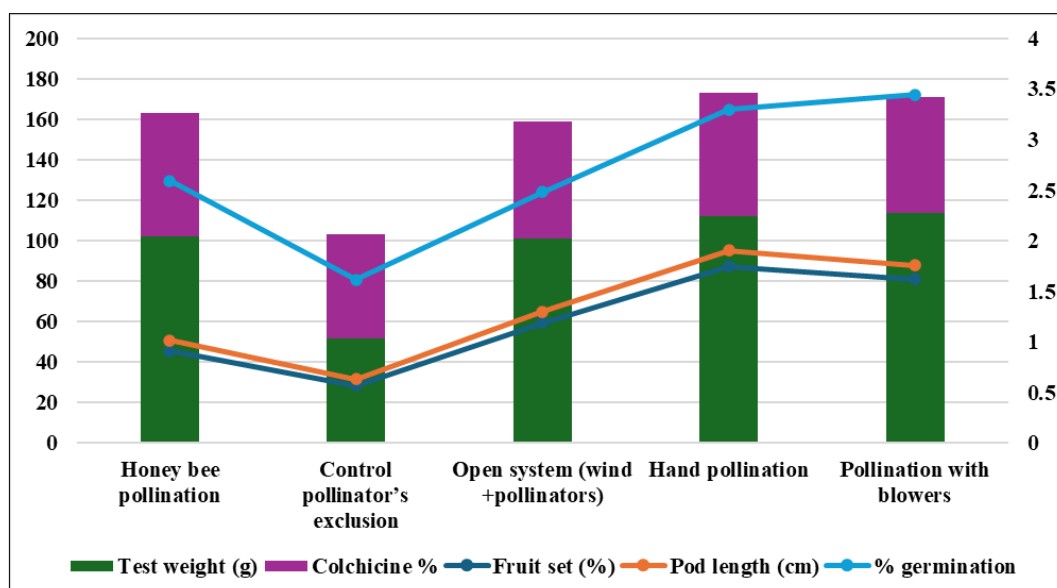


Fig. 5. Effect of different pollination systems on yield parameters of *G. superba*.

About 96 % pod set, 8.4 cm pod length, 74 number of seeds per pod, 54 % germination were observed at Tamil Nadu, while 30 to 40 % seed set in self and hand-pollination treatments (21, 36). 80 80 % seed set in assisted pollination treatments and 66.66 % seed set in open pollination treatments (23). About 97 %, germination percentage after removal of sarcotesta along with other chemical treatments had been observed (37). In present study germination percentage after removal of sarcotesta without any other chemical treatments under laboratory ranged from 60 to 85 % which is like previous findings. The similar peaks of colchicoside and colchicine in HPLC chromatogram (Fig. 6) highlighted that the alkaloid including colchicoside and colchicine content present in whole seed of *G. superba* obtained from honey bee pollination and hand pollination system was similar, which showed the effectiveness of honey bee pollination for glory lily. The colchicine content in seed from assisted pollination treatments and open pollination treatment of present study are in line with results of previous research reported 1.16 % seed colchicine content in open pollination treatment (23). In present study, the effect of honey bee pollination on seed germination percentage,

number of seeds per pod and test weight of were significantly similar with hand and blower pollination treatments while the effect of honey bee pollination treatment on fruit set percentage and pod length was found to be statistically lower than hand and blower pollination treatments.

Conclusion

This study highlights the role of insect pollinators especially honey bees in enhancing the pollination, seed yield and colchicine content of *Gloriosa superba*. Honey bee pollination treatment was observed as best for seed germination percentage, number of seeds per pod and test weight which proved to be an effective and sustainable alternative to traditional pollination methods. Hence, it can be utilized for conservation, sustainable production and crop utilization. Integrating sustainable harvesting methods with biotechnological advancements like micropropagation and genetic improvement will help in long-term sustainability and use of this priceless medicinal plant.

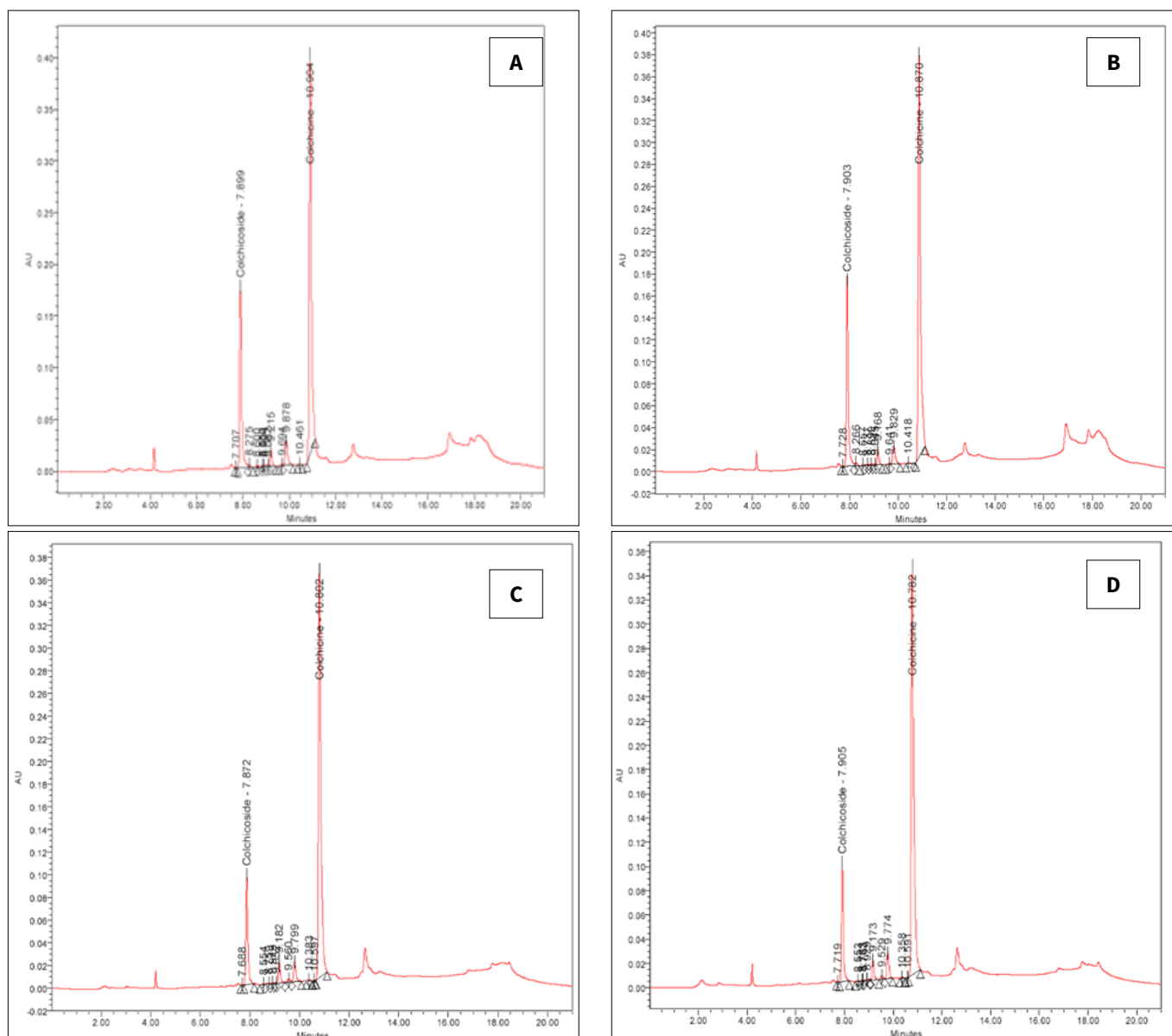


Fig. 6. HPLC chromatogram of alkaloid percentage (colchicoside and colchicine) in glory lily seeds obtained from A) Bee pollination system B) Hand pollination system C) Pollination with blower D) Open pollination.

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Authors' contributions

PR, RKT and YPS conceptualized and designed the research, PR conducted experiments, PR, RKT and MT analysed the data. PR wrote the manuscript and RKT, MT, MAW reviewed and corrected it. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest: None

Ethical issues: None

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