Floral Biology and Foraging Behavior of Dominant Flower Visitors of Lucerne (*Medicago sativa* L.)

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Received : December 2023 *Accepted* : February 2024 Abstract

A total of 36 parameters with regard to floral biology of lucerne was recorded. The number of days taken for complete anthesis of florets in the inflorescence ranged from 15-17 days. Anther dehiscence was observed after 1 to 72 hr of the anthesis and the pollen grains were viable from 1 to 120 hr after anthesis. The stigma receptivity was observed 54 hr before anthesis and continued upto 96 hr after anthesis. The scientific facts of floral biology helped to determine the type and mechanism of pollination required. Among the dominant floral visitors, *Amegilla zonata* (38 florets/4 bees) visited maximum number of florets and *Apis florea* (46.75 seconds/floret) spent maximum forging duration for collection of floral rewards.

Keywords : Floral biology, Floral visitors, Foraging behaviour

UCERNE (Medicago sativa L.) is a perennial I flowering plant which belongs to the family Fabaceae and originated from south-central Asia. It is mainly cultivated as forage crop for livestock fodder all over the world (Roopa et al., 2022) and It is also called as Alfalfa or 'Queen of Forage Crops' and used for grazing, hay, silage making, green manure and as cover crop (Burezq, 2021 and Jahangirr Ahmad Magray and Sharma, 2022). The United States is the world's largest alfalfa producer with an area of 9 million hectares followed by Argentina with an area of 6.9 million hectares. Globally, China ranks fifth with an area of 4.7 million hectares (Yuegao and Cash, 2009). In India, lucerne occupies about one million ha and provides 60-130 tonnes of green forage per ha. It is grown as a farm crop in Gujarat, Maharashtra, Punjab, Western districts of U.P and West Bengal. After sorghum and berseem, lucerne is the third most important forage crop in India (Shekara et al., 2021).

The lucerne flower consists of a standard petal and two smaller wing petals on either side, opposite to which there are two fused petals called the keel. The female sexual column is held under considerable pressure by the keel petals. The flower also contains ten anthers in diadelphous condition (Brunet *et al.*, 2019).

Pollination in lucerne flowers does not occur without the sexual column being released to fertilize the pollen grains, which later germinate on the column and proceed down to the ovule to form viable seed. The release of the sexual column is called 'tripping' of the flower. The action of many insects foraging on the lucerne flowers for honey and pollen, will often cause tripping to occur and thus facilitate the process of pollination. The floral biological aspects helped to determine the type and mechanism of pollination required. The foraging behaviour of dominant floral visitors enables to know the peak activity in addition their length of the foraging period for effective pollination of crop.

MATERIAL AND METHODS

The study was carried out in the Research Farm of ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Attur, Yelahanka campus, Bengaluru (13.096°N, 77.5666°E, 874 m. above MSL), Karnataka during 2022-23. The popular lucerne variety 'RL-88' suitable for cultivation in all agro-climatic zones of the country under irrigated condition was chosen and the crop was sown on 22nd November, 2022 in 200m² area with a spacing of 10cm x 45cm, as per the recommended package of practices.

Floral Biology of Lucerne

Detailed aspects of floral biology of lucerne were studied both in the field and laboratory (Belavadi and Ganeshaiah, 2013). The floral parameters such as flower length, number of stamens per flower, length of stamens and carpel were recorded in the collected flowers at laboratory. The flower bud development, time of anthesis, anther dehiscence, pollen viability, stigma receptivity and longevity of flowers (n=10) was studied in the field.

Duration of Flower Bud initiation to Flower Opening

Twenty-five unopened mature flower buds were selected one day prior to the flower opening and tagged. The flower buds were observed for the sequence of opening. The number of days taken for the opening of the flowers was recorded. The average number of days between the date on which the flower buds initiated and their sequence of opening was computed.

Anther Dehiscence

The anther dehiscence of lucerne in which before and after opening of the flower was recorded. Twentyfive fully matured floral buds on the previous day of opening were plucked from the plant and the stamens were observed for anther dehiscence using a hand lens from 6:00 to 18:00 hrs (Belavadi and Ganeshaiah, 2013).

Pollen Viability

Immediately after anther dehiscence, the required number of flowers were plucked from the labelled plant and brought to the laboratory. The pollen grains were dusted on a glass slide and few drops of acetocarmine solution (2%) was added, then the cover slip was placed over it. The slide was left for 4-5 minutes for proper staining, the slides were then examined under microscope for viability. The deeply stained pollen grains were considered as viable and non-stained pollen grains were considered as nonviable (Derin and Eti, 2001).

Stigma Receptivity

Totally 25 randomly selected flower buds before anthesis were plucked from the plant and brought to the laboratory. The petals, sepals and anthers were removed from the floral buds. Later a few drops of Hydrogen peroxide (6%) were placed on the surface of stigma and observed under microscope for bubble formation. The appearance of bubbles on the stigmatic surface indicated that the stigma was receptive. The same procedure was repeated after anthesis till the flower closed completely (Belavadi and Ganeshaiah, 2013).

Number of Florets visited by Floral visitors

The individual insects of major floral visitor species were tracked for their landing on different florets for the collection of floral rewards until leaving, for the period of one minute and the number of florets visited during this period was counted and expressed in terms of number of florets visited by the bees/insects per minute. The same observation was replicated four times from 0600 to 1800 hrs at two-hour interval (Belavadi and Ganeshaiah, 2013).

Total Foraging duration of Major Floral visitors for Collection of Floral rewards from the Florets

The major floral visitors were individually tracked from 0600 to 1800 hrs at two-hour interval for alighting on the florets for floral reward collection until leaving the florets and this time gap was considered as time spent per floret per insect visitor. The same observations were replicated four times and recorded the total time spent by major floral visitors (Belavadi and Ganeshaiah, 2013).

RESULTS AND DISCUSSION

Floral Architecture of Lucerne

Lucerne (Medicago sativa L.) belongs to the family Fabaceae. Flowering started in the month of February, 2023 after 28 days of first cutting and 58 days after sowing the seeds. The plant had raceme inflorescence in an acropetal manner with trifoliate leaves. The average number of raceme inflorescence per plant and number of florets per inflorescence was 16.6±4.72 and 31.2 ± 7.70 , respectively. The flower of each inflorescence was called as floret, which was pea shaped, having a length of 8.5±1.43mm, diameter $(2.8\pm0.79\text{mm})$, along with the longevity of 5.5 ± 1.08 days. The floret was purple in colour bearing five petals of which one was standard petal having length and breadth of 17±1.03 and 4.1±0.74mm, respectively, besides two wing petals (6.5 ± 0.97 and 3.4 ± 0.52 mm) and two fused keel petals (6.1 ± 0.74 and 3.6 ± 0.84 mm) enclosing the sexual column. There were five green sepals with varied length (upper lobe-3.1±0.74mm and lower lobe- 2.4 ± 0.52 mm) and breadth (5.8 ± 0.79 mm). Stamens were in diadelphous (9+1) condition enclosed within keel petal. The length and breadth of stamen was 5.2±0.79mm and 1.5±0.53 mm, respectively. The type of attachment of filament to anther was dorsified, the mode of anther dehiscence was longitudinal and the time of anther dehiscence was 1 to 76 hr after anthesis. The pollens were subprolate in shape with length and breadth of 19.57 µm and 19.23 µm and it was viable from one hour after anthesis, for upto five days. The stigma enclosed within keel petal with length and breadth of 6.3 ± 0.48 mm and 2.3 ± 0.48 mm, respectively and it became receptive before two days of anthesis. The ovary was hypogynous in position with monocarpellary, unilocular and containing many ovules. The nectaries were present at the base of the staminal column and primarily on the receptacle. The

and inflorescence was 3.4±0.77 and 14.2±1.62 days, respectively. The peak time of anthesis was between 1000 am to 1200pm. The total flowering duration was 62 days with a pod initiation at late bloom phase for about 2-3 weeks for pod setting.

The available bee forage resources in the flower were pollen and nectar. The flowers pollinated by means of entomophily through tripping mechanism leading to the cross pollination. Self-pollination was also observed in lucerne but it is less compared to entomophily (Table 1). Pedersen (2002) reported that alfalfa is a typical flowering plant which had either purple, yellow or white coloured irregular bisexual flowers. Inflorescence is racemose, with a standard petal, which acts as a platform for bees to land on it, with two smaller wing petals on each side. The flower of alfalfa is not pollinated unless the keel petal is forced to open to release the sexual column. The discharge of the sexual column is referred to as 'tripping' of the flower. Further, Brunet et al. (2019) stated that temperature also affects the self-tripping mechanism in the flowers of alfalfa.

TABLE 1 Floral biology and floral architecture of lucerne (Medicago sativa L.) during 2023

Floral parameters	Observation/ (Mean value \pm S.D.)
Type of inflorescence	Racemose
Number of raceme inflorescences per plan	16.6±4.72
Number of florets per inflorescence	31.2±7.70
Shape of flower	Pea/ Butterfly like shape (pentamerous flowers)
Length of floret (mm)	8.5±1.43
Diameter of floret(mm) 2.8±0.79
Longevity of floret	5.5±1.08
No. of petals per flowe	er Five
Colour of petal	Purple
Length of the petals(m	 m) a. Standard petal-17±1.03 b. Wing petal-6.5±0.97 c. Keel petal-6.1±0.74

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Floral parameters Observ	ration/ (Mean value \pm S.D.)
Breadth of the petals (mm)	a. Standard petal-4.1±0.74
	b. Wing petal-3.4±0.52
	c. Keel petal-3.6±0.84
lo. of sepals per flower	Five
Colour of the sepal	Green
length of the sepals (mm)	Upper lobe-3.1±0.74 and lower lobe-2.4±0.52
Breadth of the sepals (mm)	5.8±0.79
lo. of stamen per flower	Diadelphous condition- 9+1
ength of the stamen(mm)	5.2±0.79
Breadth of the stamen(mm)	1.5±0.53
nther attachment to filament	Dorsified
lode of anther dehiscence	Longitudinal
ime of anther dehiscence	1 to 76 hr after anthesis
hape of the pollen	Subprolate
ength of pollen	19.57 μm
readth of pollen	19.23 μm
ime at which the pollen ecome viable	1 hr after anthesis and viable upto 5 days
ength of the pistil(mm)	6.3±0.48
readth of the pistil(mm)	2.3±0.48
eceptivity of stigma	Before 2 days of anthesis
vary	Monocarpellary and unilocular with many vules
osition of the ovary	Hypogynous
ocation of the nectaries	Base of the staminal
	column and primarily on
Lunden af Jame (1 – C	ine receptacie
umber of days taken for omplete anthesis of floret	3.4±0.//
umber of days taken for omplete anthesis of	14.2±1.62
nflorescence	
eak time of anthesis	1000 to 1200 hr
otal flowering duration	62 days
vailable bee forage resource	Nectar and pollen
ollination type	Cross pollination
Aode and mechanism of ollination	Entomophily through tripping
Self-pollination	Present

Number of Days taken from Bud initiation to Flower Opening

Totally 25 randomly selected florets were tagged at different days in January, 2023. The observation on flower bud initiation was taken from 21^{st} January to 9^{th} February, 2023. The duration taken for floral bud initiation to complete flower opening was ranged from 10 (25-01-23 to 04-02-23) to 17 (25-01-23 to 11-02-23) days with a mean of 13.72 ± 1.84 days (Table 2).

TABLE 2

Number of days taken from flower bud initiation to flower opening in lucerne during 2022-23

Plant No.	Date of bud initiation	Date of flowering	Days taken for flower opening (days)
1.	21-Jan-23	03-Feb-23	13
2.	21-Jan-23	05-Feb-23	15
3.	21-Jan-23	06-Feb-23	16
4.	21-Jan-23	02-Feb-23	12
5.	21-Jan-23	05-Feb-23	15
6.	23-Jan-23	08-Feb-23	16
7.	23-Jan-23	06-Feb-23	14
8.	23-Jan-23	07-Feb-23	15
9.	23-Jan-23	05-Feb-23	13
10.	23-Jan-23	04-Feb-23	12
11.	24-Jan-23	07-Feb-23	14
12.	24-Jan-23	08-Feb-23	15
13.	24-Jan-23	05-Feb-23	12
14.	24-Jan-23	09-Feb-23	16
15.	24-Jan-23	04-Feb-23	11
16.	25-Jan-23	11-Feb-23	17
17.	25-Jan-23	07-Feb-23	13
18.	25-Jan-23	10-Feb-23	16
19.	25-Jan-23	05-Feb-23	11
20.	25-Jan-23	04-Feb-23	10
21.	26-Jan-23	09-Feb-23	14
22.	26-Jan-23	08-Feb-23	13
23.	26-Jan-23	07-Feb-23	12
24.	26-Jan-23	09-Feb-23	14
25.	26-Jan-23	09-Feb-23	14
	Range	10 to 17	
	Mea	13.72±1.84	

Note : n= 25 florets (unopened inflorescence containing immature floral buds)

Days required for Complete Anthesis of Florets in the Inflorescence

The buds developed from the bottom to top, the lower flowers opened first and then it progressed to top region. The number of days taken for complete anthesis of florets in the inflorescence ranged from 15-17 days, with a mean of 15.8 ± 0.84 . The sequence of opening of the florets for the period of 15.8 ± 0.84 days attracted many floral visitors by offering floral rewards leading to pollination of lucerne flowers (Table 3).

TABLE 3

Days required for complete anthesis (flower opening) of florets in inflorescence during maximum bloom period, 2022-23

Date of Date of inflorescence tagged Date of complete anthesis of florets in inflorescence		
6 January, 2023	16	
6 January, 2023	15	
9 January, 2023	17	
8 January, 2023	15	
22 February, 2023 10 January, 2023		
Mean ± SD		
Range		
	Date of complete anthesis of florets in inflorescence 6 January, 2023 6 January, 2023 9 January, 2023 8 January, 2023 10 January, 2023 5 SD	

Note : n=5 inflorescence with mature floral buds

Anther Dehiscence

On the previous day of flower bud opening, twentyfive matured florets at bud stage were randomly selected and tagged. Next day, all the opened flowers were observed for anther dehiscence from to 1 to 72 hr after anthesis, with an interval of six hours. The number of anthers dehisced ranged from 4 (1, 60, 66 and 72 hours after anthesis) to 16 per cent (30 hours after anthesis). The maximum number of anthers dehisced in the flowers were recorded at 30 hr (16%) after anthesis, followed by 36 hr after anthesis (12%) compared to the rest of the hours in which anther dehiscence was tested (Table 4). However, Bohart and

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Time of anther dehiscence in the flowers of lucerne
during maximum bloom phase during 2023

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Time after anthesis (hr)	No. of anthers which dehisced	Anther dehiscence (%)		
1	1	4		
6	3	12		
12	2	8		
18	1	4		
24	2	8		
30	4	16		
36	3	12		
42	2	8		
48	2	8		
54	2	8		
60	1	4		
66	1	4		
72	1	4		
Range	1-4	4-16		
$Mean \pm SD$	$1.78{\pm}1.05$	7-0.04		
	<i>Note</i> : $(n=25 \text{ florets})$			

George (1957) stated that, the pollen in alfalfa flowers shed in the bud stage and covers the stigma before the flower was open. Further, Shama Rao and Solomon (1957) reported that dehiscence takes place longitudinally on either side of the central axis. It commences when the 'standard' begins to separate from the keel in the bud and is completed when the flowers are in the half-open stage (0900-1000 am) which are ready for tripping.

Pollen Viability

Pollen grains collected from randomly selected flowers immediately after anthesis were examined for pollen viability by staining pollen with acetocarmine solution. It was tested from 1 hr up to 120 hr (5 days) after anthesis viable (Plate 1). Whereas, the pollen grains from 126 to 144 hr (6 days) after anthesis were non-viable. Pankiw and Bolton (1965) stated that, pollen remains viable for 8 days in alfalfa. Further, Zhang *et al.* (2005) reported that, the pollen viability was 51.87 ± 2.08 per cent and it dropped to 25.39per cent after 7 days of anthesis.

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Plate 1: Viable pollen showing change in colour

Stigma Receptivity

The stigma receptivity of randomly selected 25 floral buds before anthesis and after anthesis (from 1 to 144 hr) were tested separately by placing a drop of hydrogen peroxide on the stigmatic surface. The stigma receptivity in tested flowers started from 1 to 54 hr (2 days) before anthesis (Plate 2) and nonreceptivity was observed from 60 to 144 hr (6 days) before anthesis. The stigma receptivity in tested flowers after anthesis started from 1 to 96 hr (4 days) and non-receptivity was observed from 102 to 144 hr (6 days), respectively. According to Pankiw and Bolton (1965), the ovules of florets with exposed stigmas remained fully functional for 6 hours, but then



Plate 2: Bubbling indicating stigma receptivity

declined rapidly to almost 0 at 48 hours. Further, Zhang *et al.* (2008) reported that the stigma vitality can last for 4-6 days, but the optimum pollination time was the first day of flowering in *M. varia* martin.cv. Xinmu. No. 1.

Number of Florets visited by Dominant Floral visitors

The number of florets visited by *A. cerana* Fab., *A. florea* Fab., *A. dorsata* Fab., *A. zonata* L., *M. lanata* Fab., *C. Binghami* Cockerell and *H. Westwoodi* (Gribodo) varied among different time intervals (Table 5). *Apis cerana* Fab. visited lowest number of florets (7 florets/4 bees/min.) during 0600-

Number of florets visited by major bee floral visitors (No. of florets/4 bees/min.) on lucerne inflorescence during maximum bloom phase, 2023

Species Time (hr)	A. cerana	A. florea	A. dorsata	A. zonata	C. binghami	M. lanata	H. westwoodi
0600-0800	7	0	0	0	0	0	0
0800-1000	20	14	12	30	7	18	7
1000-1200	20	17	9	38	19	25	13
1200-1400	17	15	11	37	14	27	15
1400-1600	18	15	8	33	12	18	10
1600-1800	18	8	12	32	8	13	6
1800-2000	0	5	5	0	0	0	0
Mean±SD	14.29±7.72	10.57±6.35	8.14±4.37	24.29±16.82	8.57±7.07	14.43±10.91	7.29±5.88
SE (m)±	0.87	0.56	0.82	1.59	0.77	1.19	0.71
Florets visited/bee	3.57	2.64	2.03	6.07	2.14	3.61	1.82

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0800 hr, whereas, it visited maximum number of florets from 0800-1200 hr (20 florets/4 bees/min.). Apis florea Fab. visited maximum number of florets during 1000-1200 hr (17 florets/4 bees/min.) followed by 1200-1600 (15 florets/4 bees/min.) and the lowest visits were recorded at 1800-2000 hr (5 florets/4 bees/ min.). A. dorsata Fab. visited lowest number of florets during 1800-2000 hr (5 florets/4 bees/min.) and it visited maximum number of florets during 0800-1000 hr and 1600-1800 hr (12 florets/4 bees/ min.). Among Apis species, A. cerana visited maximum number of florets (3.57/bee/min.), followed by A. florea (2.64/ bee/min.) and A. dorsata (2.03/bee/min.). These findings are supported by Taha et al. (2016) reported highest number of Carniolan bees (4.00 and 4.22 bees/ $m^2/min.$) and dwarf bees (5.50 and 5.87 bees/ $m^2/min.$) at 1700-1800 hr and the lowest (2.54 and 2.77bees/ m²/min.; 4.35 and 4.73bees/m²/min.) at 0800-0900 hr during 2013 and 2014, respectively on the flowers of lucerne in Saudi Arabia.

The number of florets visited by *A. zonata* L. (38 florets/4 bees/min.) and *C. Binghami* Cockerell (19 florets/4 bees/min.) was maximum during 1000-1200 hr and the lowest (30 and7 florets/4 bees/min.)

during 0600-0800 hr, respectively. Similarly, the number of florets visited by *M. lanata* Fab. (27 florets/4 bees/min.) and *H. Westwoodi*_(15 florets/4 bees/min.) was maximum during 1200-1400 hr and the lowest (13 and 6 florets/4 bees/min.) was during 1600-1800 hr, respectively. Among non-*Apis* bees, *A. Zonata*_L. visited maximum number of florets (6.07/bee/min.), followed by_*Megachile lanata* Fab. (3.61/bee/min.), *C. Binghami* Cockerell (2.14/bee/min.) and the lowest (1.82/bee/min.) was recorded in case of *H. Westwoodi* (Gribodo).

Foraging duration of Major Floral visitors for Collection of Floral Rewards

The time spent per floret by *A. cerana* Fab., *A. florea* Fab., *A. dorsata* Fab., *M. lanata* Fab., *C. Binghami* Cockerell and *H. Westwoodi* (Gribodo) varied significantly among different time intervals (Table 6). *A. cerana* (27.5 sec./floret) and *A. florea* (46.75 sec./ floret) spent significantly maximum time per floret during 1000-1200 hr and lowest time was spent between 1600-1800 hr (7.25 sec./floret) and 1800-2000 hr (15.25 sec./floret), respectively. Similarly, *A. dorsata* (17.5 sec./floret) and *M. lanata* (6.25 sec./

Foraging duration (seconds/floret) of major bee floral visitors for collection of floral rewards from florets of
lucerne during maximum bloom phase (2023)

TABLE 6

SpeciesTime (hr)	A. ceran	a A. florea	A. dorsat	a A. zonata	C. bingham	i M. lanata	H. westwoodi
0600-0800	12.25 ^d	0.00	0.00	0.00	0.00	0.00	0.00
0800-1000	15.5 °	26.25 °	9.75 ^d	2.25 ª	7.25°	5.75 ^{ab}	4.5 ^b
1000-1200	27.5 ª	46.75 ª	12.75°	2.75 ª	11.75 ª	4.75 ^{bc}	7.5 ª
1200-1400	21.25 ^b	31.75 ^ь	17.5ª	3.25 ª	8.75 ^b	6.25 ª	4.75 ^b
1400-1600	12.5 ^d	23.5 ^d	8.5 °	2.75 ª	5.25 ^d	5.5 ^{ab}	2.75 °
1600-1800	7.25 °	17.75 °	15.25 ^b	2.5 ª	3.25 °	3.75°	2.25 °
1800-2000	0.00	$15.25^{\text{ f}}$	9.75 ^d	0.00	0.00	0.00	0.00
Mean±SD	13.75 ± 8.98	23.03±14.54	10.5±5.66	1.93±1.35	5.18±4.43	3.71±2.66	3.11±2.71
F test	*	*	*	NS	*	*	*
SE (m)±	0.50	0.39	0.50	-	0.39	0.50	0.32
CD @5%	1.51	1.18	1.50	-	1.17	1.48	0.97

Note:*-Significant at p=0.05, NS= Non-significant

floret) spent significantly maximum time per floret during 1200-1400 hr and lowest time spent between 1400-1600 hr (8.5 sec./floret) and 1600-1800 hr (3.75 sec./floret), respectively.

The forager bees of *C. binghami* (11.75sec./floret), *H. Westwoodi* (7.5 sec./floret) has taken significantly maximum foraging duration during 1000-1200 hr and the lowest (3.25 and 2.25 sec./floret) during 1600-1800 hr, respectively. There was no significant variation in the foraging duration of *A. zonata* (2.5-3.25 sec./floret) among different time of intervals. The mean foraging duration of *A. florea* (23.03 \pm 14.54) was maximum among *Apis* species and it was higher in *C. Binghami* (5.18 \pm 4.43) among non-*Apis* bees.

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