International Journal of Agriculture Sciences
ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 15, Issue 3, 2023, pp.-12229-12235.
Available online at https://bioinfopublication.org/pages/jouarchive.php?id=BPJ0000217

Research Article

USING HONEY BEE (*APIS CERANA* FAB.)- AN ALTERNATIVE TO HAND POLLINATION IN SUNFLOWER HYBRID (KBSH-44) SEED PRODUCTION

S.M. SAJJANAR*
Senior Farm Superintendent, Main Agricultural Research Station, University of Agricultural Sciences, Raichur, 584104, Karnataka, India
*Corresponding Author: Email - sajjan9@rediffmail.com

Received: March 04, 2023; Revised: March 26, 2023; Accepted: March 28, 2023; Published: March 30, 2023

Abstract: The study was conducted at Main Agricultural Research Station, UAS, Raichur during *rabi*-2020-21 to utilize honey bees in sunflower hybrid seed production. Totally, thirty species of floral visitors were recorded on parental lines of sunflower hybrid (KBSH-44), among them 15 were hymenopterans, 7 lepidopterans, 5 coleopterans and 3 dipterans. *Apis dorsata* was predominant pollinator (20.08 ±10.45 bees/ 5 capitula/ 5 min.) on CMS parental line (CMS 17A), followed by *A. cerana* and least was *A. florea*. The peak foraging activity of *A. dorsata* was recorded at 1100-1200 hr with 33 bees, *A. cerana* recorded two peak foraging activity, one at 0900-1100 hr (23 bees) and another at 1500-1600 hr (19 bees) and *A. florea* recorded at 1000-1200 hr (8 bees). The maximum Shannon-Wiener index of diversity (H=1.28) of floral visitors on CMS line under open condition was recorded at 0800-0900 hr and 1000-1100 hr of the day and least (H=0.56) was at 1700-1800 hr. More number of honey bees were recorded in fertility restorer line (RHA 95C-1) compared to the CMS line. *A. cerana* under caged condition (three colonies per acre) without pollen load on CMS line were recorded highest mean number of nectar foragers (80.25±22.97 bees), followed by *A. cerana*, *A. dorsata* and *A. florea* foragers from natural colonies in open pollination condition. Whereas, highest mean number of nectar foragers (35.08±19.31) of *A. cerana* under caged condition (three colonies per acre) with pollen load were recorded, followed by *A. cerana*, *A. dorsata* and *A. florea*. Nectar foragers with pollen load on CMS line play an important role in transfer of pollen grains from restorer line to CMS line and carryout effective pollination. *A. florea* with pollen load spent more foraging duration (12.11 sec/capitulum) on CMS line, followed by *A. cerana* and least in case of *A. dorsata* in open pollination. Bees without pollen load spent significantly greater nectar foraging duration as compared with bees with pollen load, both in caged and open pollination situations. Significantly highest seed yield per hectare (887.74 kg), per cent filled seeds (94.44 %), test weight (5.04 g), volume weight (39.18 g/100 ml), kernel percentage (75.34 %), highest kernel to husk ratio (3.06) and highest seed quality & associated parameters were recorded in hand + open pollinated plots, followed by the *A. cerana* @ 3 colonies per acre treated plots, hand pollination, open pollination and least seed quality parameters were recorded in control treatment.

Keywords: *Honey bees, Foraging activity, Sunflower pollination*

Citation: S.M. Sajjanar, (2023) Using Honey Bee (*Apis cerana* Fab.)- An Alternative to Hand Pollination in Sunflower Hybrid (KBSH-44) Seed Production. International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 15, Issue 3, pp.- 12229-12235.

Academic Editor / Reviewer: S. M. Chavan, Dr A. H. Barad

Introduction

Sunflower hybrid seed production through hand pollination is very difficult task due to shortage of agricultural labour and it is very expensive when there is availability of labour. Thus, using honey bees as pollinators for good quality and quantity of sunflower hybrid seed production as an alternative to hand pollination is need of the hour.

Honey bees are the most important insect pollinators of cultivated crops worldwide. While some insects visit the flowers of only a small number of plant species. Honey bees will visit all flower from which they can harvest reward. Honey bees are one of the few pollinating insects that can be managed. They can be delivered to a crop when required and various management options available to influence the honey bees' flower visiting behavior [1]. Honey bees are essential for transfer of pollen grains from fertility restorer line to cytoplasmic male sterile line [11]. Many experiments have consistently confined that commercial yield levels could be increased to an extent of 45 to 50 per cent in sunflower, sesamum and niger [13].

The species richness and foraging behavior of honey bees on sunflower is genotype specific and is influenced by morphometric variations of the plant, which includes flower shape, flower structure, head size, floret length, corolla length, stigma pigmentation and many other factors. Whereas, nectar and pollen are the source of food for honey bees, which attract or restrict bee visitation to the host plant flower [19]. Sunflower is an allogamic plant which needs insects during flowering for pollination especially honey bees for seed production.

Pollen grains of sunflower are heavy and sticky in nature due to this wind plays a very minor role in transfer of pollen from one flower head to other, which can be done by using honey bees [7]. Productivity variables are depending upon the number of honey bee colonies and their visitation rate to the flowers. However, effects were nonlinear for visitation rates, there is anoptimum (mean of 8-10 visits per flower), beyond which more honey bees are not beneficial (and can even be detrimental) for crop productivity [20].

One acre of sunflower hybrid seed production required around 42 skilled labourers for transfer of pollen from male capitulum to cytoplamsic male sterile capitulum. The present study aims to address this important issue in sunflower hybrid seed production by utilizing Indian honey bee, *Apis cerana* Fab. and calculation of benefit:cost ratio of honey bee pollination v/s hand pollination.

Material and Methods

The present investigation on was carried out during 2020-21 at Main Agricultural Research Station, UAS, Raichur (Latitude16 °12'N and Longitude 77 °20'E) which is located in the II zone of Karnataka state with following five treatments in six replications,T1: Crop caged with three colonies of *Apis cerana* (six frame strength) per acre; T2:Hand pollination; T3:Open pollination; T4: Hand + open pollination; T5:Control (crop covered with mosquito nylon net). The parental lines CMS 17A (3 lines) and RHA 95C-1(1 line) of sunflower hybrid KBSH-44 were used for hybrid seed production.

Pollinator fauna and foraging activity on parental lines of sunflower hybrid
In order to record the species abundance and diversity of flower visitor, ad-libitum sampling of five capitula was done for 5 minutes duration hourly intervals, commencing from 0600 hr to 1800 hr.

Diversity of flower visitors on parental lines of sunflower hybrid
Representative samples of flower visitors were collected by various methods of collection *viz*., visual scanning, sweep net sampling and bee bowls [3].
The frequency of visits by each species was recorded in order to identify the most abundant species visiting sunflower capitulum. Pollinator population count was used to compute Shannon-Weiner index of diversity (H) by using the following formula:-
H=-∑pi x1npi Where, pi is the proportion of the i^{th} species.
The dominant species on any given sampling day was determined by the Berger-Parker dominance index 'd' which gives the proportion of the total numbers of individuals ina sample that is due to the dominant species and was calculated by d=ni/NT Where, ni is the number of individuals of the ith species on sampling date and NT is the total number of individuals in the sample [23].

Foraging activity of honey bee
The pollen and nectar foraging activity of different honey bee species in open plot and foraging activity of *Apis cerana* Fab. in caged pollinated plots were determined on the capitulum during flowering period for 5 min. The forager bees with pollen pellets in their corbicula (even small loads) were classified as pollen foragers. The honey bees without pollen loan in their corbicula treated as nectar foragers. Both pollen and nectar foragers were recorded during flowering period from 0600 to 1800 hr of the day at hourly interval in three replications and were expressed as number of pollen or nectar foragers per 5 capitula per 5 minutes.

Time spent by bees on the flower heads
The time when forager bee landed on the capitulum till leaving was recorded by using a digital stop watch and considered as time spent by the bee/capitulum in open pollination plots for *Apis* species. Similarly, separate observations were made on nectar and pollen collection in CMS and R line from 0600 up to 1800 hr at an hourly interval during the flowering period. The mean time spent by bee per capitulum is expressed as time (sec) spent/capitulum.

Nectar yield and total soluble solids (TSS)
Quantity of nectar and TSS in the nectar produced by the disc florets (n=25) of CMS and R line was estimated. The randomly selected capitula were enclosed with butter paper cover to avoid the visit of pollinators. Next day the covers were removed from the capitula and the quantity of nectar produced per floret was measured from 0600 to 1800 hr at two hourly intervals by using calibrated capillaries/micro syringe. The quantity of nectar collected was expressed in micro litre (µl)/floret [3]. The quality of nectar in terms of sugar (TSS) content was assessed by using hand refractometer and expressed in percentage.

Effect of different pollination conditions on seed quality, seed yield and yield attributing characters
In the field experiment, from each treatment thirty sunflower heads were harvested separately to record following yield attributes.

Percent seed filling
In each head, total numbers of filled and unfilled seeds were counted. The ratio of number of filled seeds to the total number of seeds per head was expressed in percentage.

Test weight
Hundred filled seeds were counted from each treatment in all replications and its mean weight was expressed in grams.

Volume weight
Hundred ml of filled seeds were collected from each treatment in all replications and its mean weight was expressed in g/100ml.

Kernel to Husk (K/H) Ratio, Husk and Kernel percent: Collected hundred seeds from each treatments and were manually dehusked. Both weight of kernel and husk were determined to calculate the husk percentage, kernel percentage and kernel to husk ratio.

Germination percent
Hundred seeds were placed on moist blotting paper and kept for observation. The germination counts were taken five days later and percent seed germination was computed.

Seed vigour Index
Ten seedlings from each replication from all treatments were randomly taken to record root and shoot length. Seed vigour index(VI) was calculated by using the following formula –
VI= RL+SL x GP
Where in, RL=Root length; SL=Shoot length; GP=Germination percentage

Percent oil content
Oil content of seeds of hybrid sunflower (KBSH-44) produced from different treatments were analysed by using NMR facility at AICRP (Sunflower), Main Agricultural Research Station, UAS, Raichur.

Table-1 List of floral visitors on parental lines of sunflower hybrid (KBSH-44)

Order	Family	Sl.No.	Scientific name
Hymenoptera	Apidae	1	*Apis dorsata* Fabricius
		2	*Apis cerana indica* Fabricius
		3	*Apis florea* Fabricus
		4	*Xylocopa aestuans* (Linnaeus)
		5	*Xylocopa fenestrate* (Fabricius)
		6	*Amegilla* sp.
		7	Unidentified sp.
	Halictidae	8	*Lassooglossum* sp.
		9	Unidentified sp.
	Vespidae	10	*Vespa tropica* (Linnaeus)
		11	*Ropalidia marginata* (Lepeletier)
		12	*Poslistes* sp.
	Sphecidae	13	Unidentified sp.
	Megachilidae	14	*Megachile disjuncta* (Fabricius)
		15	*Megachile lanata* (Fabricius)
Lepidoptera	Sphingidae	16	Unidentified sp.
	Nymphalidae	17	*Danaus chrysippus* Linnaeus
		18	*Junonia lemonias* (Linnaeus)
		19	*Tirumala limniace* (Cramer)
	Pieridae	20	*Catopsilia* sp.
	Erebidae	21	*Amata passalis* (Fabricius)
		22	*Amata cyssea* (Stoll)
Coleoptera	Scarabaeidae	23	*Gametis versicolor* (Fabricius)
	Coccinellidae	24	*Coccinella transversalis* Fabricius
		25	*Chilomenes sexmaculata* Fabricius
	Chrysomelidae	26	*Monolepta* sp.
		27	*Leptisma* sp.
Diptera	Syrphidae	28	*Eristalinus* sp.
	Sarcophagidae	29	Unidentified sp.
	Muscidae	30	Unidentified sp.

Statistical analysis
The data from the field experiment was analyzed statistically for comparing treatments following ANOVA for Randomized Block Design and results were interpreted at 5 per cent level of significance in order to compare the dependency of sunflower hybrid on honey bee pollination for per cent seed filling, test weight, germination percentage and oil content over other treatments.

Calculation of cost benefit ratio of honey bee pollination v/s hand pollination
The benefit cost ratio of the different modes of pollination was calculated for hybrid seed production by considering the current cost of inputs.

Table-2 Abundance, diversity and dominance of floral visitors on CMS parental line (CMS 17 A) of sunflower hybrid (KBSH-44) under open condition

Time(hrs)	Number of floral visitors/5 capitula/5 min.							Total	"H"Value	"d"value
	Apis species			Non-*Apis* species						
	Apis cerana	*Apis dorsata*	*Apis florea*	Hymenoptera	Diptera	Lepidoptera	Coleoptera			
0600-0700	10	7	1	0	0	0	1	19	1.02	0.52
0700-0800	20	18	2	2	0	0	1	43	1.09	0.46
0800-0900	22	25	3	4	1	1	1	57	1.28	0.43
0900-1000	23	27	6	5	0	0	0	61	1.16	0.44
1000-1100	23	31	8	8	0	1	0	71	1.28	0.43
1100-1200	15	33	8	7	0	0	0	63	1.19	0.52
1200-1300	11	30	4	7	0	0	0	52	1.11	0.57
1300-1400	7	26	2	6	0	0	0	41	1.02	0.63
1400-1500	10	21	2	4	0	1	0	38	1.17	0.55
1500-1600	19	14	1	3	1	0	0	38	1.11	0.50
1600-1700	11	7	0	1	0	1	0	20	0.99	0.55
1700-1800	6	2	0	0	0	0	0	8	0.56	0.75
Total	177	241	37	47	2	4	3	511		
Mean±SD	14.75±6.35	20.08±10.45	3.08±2.84	3.91±2.77	0.16±0.38	0.33±0.49	0.25±0.45			
"d"value	0.346	0.471	0.072	0.091	0.003	0.007	0.005			

Table-3 Abundance, diversity and dominance of floral visitors on fertility restorer parental line (RHA 95 C-1) of sunflower hybrid (KBSH-44) under open condition

Time(hrs)	Number of floral visitors/5 capitulum/5 min.							Total	"H"Value	"d"vale
	Apis species			Non-*Apis* species						
	Apis cerana	*Apis dorsata*	*Apis florea*	Hymenoptera	Diptera	Lepidoptera	Coleoptera			
0600-0700	14	16	3	1	0	1	1	36	1.23	0.44
0700-0800	26	26	6	1	1	1	1	62	1.22	0.41
0800-0900	30	40	10	1	0	0	0	81	1.03	0.49
0900-1000	40	44	18	2	0	0	0	104	1.11	0.42
1000-1100	40	49	21	1	1	0	0	112	1.13	0.43
1100-1200	50	49	25	3	0	1	1	129	1.22	0.38
1200-1300	44	46	25	0	0	0	0	115	1.07	0.40
1300-1400	38	38	22	1	1	0	0	100	1.16	0.38
1400-1500	32	34	18	2	0	0	0	86	1.15	0.39
1500-1600	28	20	17	0	0	1	0	66	1.14	0.42
1600-1700	20	8	12	1	0	0	1	42	1.21	0.47
1700-1800	8	4	6	3	0	0	0	21	1.32	0.38
Total	370	374	183	16	3	4	4	954		
Mean ±SD	30.83±12.48	31.16±15.98	15.25±7.66	1.33±0.98	0.25±0.45	0.33±0.49	0.33±0.49			
"d"value	0.387	0.392	0.191	0.016	0.003	0.004	0.004			

Table-4 Nectar foragers of Apis species on CMS parental line (CMS 17 A) of sunflower hybrid (KBSH-44) under caged (3 colonies/acre) pollination as compared with open condition

Time (hrs)	Number of floral visitors/5 capitula/5 min.							
	Foragers of *A. cerana* under caged condition (3 colonies/acre)		Foragers of *Apis* species from natural colonies					
			Apis cerana		*Apis dorsata*		*Apis florea*	
	Bees with Pollen load	Bees without pollen load	Bees with Pollen load	Bees without pollen load	Bees with Pollen load	Bees without pollen load	Bees with Pollen load	Bees without pollen load
0600-0700	29	62	3	7	0	7	0	1
0700-0800	48	104	8	12	3	15	1	1
0800-0900	60	107	7	15	6	19	1	2
0900-1000	63	106	6	17	5	22	2	4
1000-1100	57	91	3	20	5	26	3	5
1100-1200	44	66	2	13	5	28	2	6
1200-1300	30	62	2	9	4	26	1	3
1300-1400	10	93	1	6	2	24	1	1
1400-1500	8	104	2	8	0	21	1	1
1500-1600	23	65	4	15	0	14	0	1
1600-1700	34	63	1	10	0	7	0	0
1700-1800	15	40	0	6	0	2	0	0
Total	421	963	39	138	30	211	12	25
Mean±SD	35.08± 19.31	80.25± 22.97	3.25± 2.52	11.50± 4.58	2.50± 2.43	17.58± 8.58	1.00± 0.95	2.08± 1.97
t-test@5%	*		*		*		NS	

Results and Discussion

Floral visitor on parental lines

Thirty species of floral visitors were recorded on parental lines of the sunflower hybrid (KBSH-44), of which15 species were from five families of Hymenoptera (Apidae, Vespidae, Halictidae, Megachilidae and Sphecidae), seven species from four families of Lepidoptera (Nymphalidae, Erebidae, Pieridae and Spingidae), five species from three families of Coleoptera (Chrysomelidae, Coccinellidae and Scarabaeidae) and one each species from three families of Diptera (Syrphidae, Sacrophagidae and Muscidae) [Table-1]. Similarly, [12] from Thirupathi, who recorded two families of Hymenoptera, four families of Lepidoptera and three families of Coleoptera and one family of Diptera visiting sunflower capitulum. Recorded 12 species of insect visitors, of which nine belonged to Hymenoptera, one to Diptera, one to Lepidoptera and one was a Coleopteran species at Pantanagar [8].

Abundance of floral visitors on parental lines KBSH-44

On CMS 17 A line, *A. dorsata* was predominant pollinator (20.08 ±10.45 bees/ 5 capitula/ 5 min.), followed by the *A. cerana* and least was *A. florea*. The peak foraging activity of *A. dorsata* was recorded at 1100-1200 hr with 33 bees/ 5 capitula/ 5 min, *A. cerana* recorded two peak foraging activity pattern, one at

Table-5 Pollen and nectar foragers of Apis species on fertility restorer parental line (RHA 95 C-1) under caged (3 colonies/acre) and open condition

Time (hrs)	Number of bees/5 capitula/5 min.							
	Foragers of *A. cerana* under caged condition (3 colonies/acre)		Foragers of *Apis* species from natural colonies					
			Apis cerana		*Apis dorsata*		*Apis florea*	
	Pollen	Nectar	Pollen	Nectar	Pollen	Nectar	Pollen	Nectar
0600-0700	93	46	8	6	10	6	2	1
0700-0800	145	60	16	10	18	8	4	2
0800-0900	151	65	18	12	28	12	6	4
0900-1000	148	66	24	16	30	14	12	6
1000-1100	140	63	26	14	34	15	14	7
1100-1200	102	55	34	16	32	17	16	9
1200-1300	61	34	30	14	30	16	17	8
1300-1400	20	22	26	12	26	12	16	6
1400-1500	18	19	20	12	24	10	14	4
1500-1600	69	21	18	10	16	4	15	2
1600-1700	102	35	16	4	6	2	12	0
1700-1800	62	13	6	2	4	0	6	0
Total	1111	499	242	128	258	116	134	49
Mean±SD	92.58± 47.65	41.58± 19.94	20.16± 8.33	10.66± 4.53	21.50± 10.44	9.66± 5.66	11.16± 5.23	4.08± 3.11
t-test@5%	*		*		*		*	

Table-6 Nectar foraging duration of Apis species on CMS parental line (CMS 17 A) under caged pollination as compared with open pollination

Time (hrs)	Mean time(sec/capitulum) spent for nectar collection							
	Foragers of *A. cerana* under caged condition (3 colonies/acre)		Foragers of *Apis* species from natural colonies					
			Apis cerana		*Apis dorsata*		*Apis florea*	
	Bees with Pollen load	Bees without Pollen load	Bees with Pollen load	Bees without Pollen load	Bees with Pollen load	Bees without Pollen load	Bees with Pollen load	Bees without Pollen load
0600-0700	3.60^{k}	3.34^{k}	1.14^{g}	4.57^{g}	0.00	2.06^{h}	0.00	5.59^{h}
0700-0800	6.33^{i}	9.60^{j}	3.88^{f}	10.93^{f}	1.55^{e}	6.77^{g}	5.18^{f}	12.29^{g}
0800-0900	13.26^{g}	25.60^{i}	6.90^{e}	16.31^{e}	2.87^{d}	11.18^{f}	10.52^{d}	20.16^{f}
0900-1000	29.60^{a}	53.94^{a}	11.27^{bc}	26.32^{c}	5.67^{c}	21.38^{d}	14.81^{bc}	30.57^{c}
1000-1100	29.54^{a}	50.06^{b}	13.76^{a}	37.89^{a}	7.68^{b}	31.38^{a}	17.68^{a}	39.92^{a}
1100-1200	28.00^{b}	47.80^{c}	12.26^{ab}	35.54^{a}	9.57^{a}	29.75^{a}	16.14^{b}	37.54^{b}
1200-1300	24.40^{c}	45.66^{d}	11.55^{b}	34.54^{a}	10.18^{a}	27.07^{b}	13.53^{c}	35.58^{b}
1300-1400	18.86^{d}	44.00^{e}	9.89^{cd}	30.88^{b}	8.46^{b}	25.00^{c}	10.23^{de}	32.22^{c}
1400-1500	15.54^{e}	40.20^{f}	9.17^{d}	29.52^{bc}	0	20.13^{d}	8.80^{e}	28.08^{d}
1500-1600	14.46^{f}	38.80^{g}	8.57^{d}	22.83^{d}	0	18.06^{e}	0	23.92^{e}
1600-1700	10.06^{h}	39.00^{g}	6.56^{e}	18.85^{e}	0	9.71^{f}	0	0
1700-1800	5.34^{i}	29.33^{h}	0.0	11.56^{f}	0	7.38^{g}	0	0
Mean	16.58	35.61	8.63	23.31	6.56	17.48	12.11	26.58
Sem±	0.13	0.34	0.52	1.14	0.31	0.60	0.50	0.69
CV(%)	1.42	1.67	11.37	8.46	14.08	5.99	10.78	5.42
t-test@5%	*		*		*		*	

Table-7 Foraging duration of Apis species on fertility restorer parental line (RHA 95 C-1) under caged and open pollination

Time (hrs)	Mean time (sec/capitulum) spent for pollen and nectar collection							
	Foragers of *A. cerana* under caged condition (3 colonies/acre)		Foragers of *Apis* species from natural colonies					
			Apis cerana		*Apis dorsata*		*Apis florea*	
	Pollen	Nectar	Pollen	Nectar	Pollen	Nectar	Pollen	Nectar
0600-0700	22.60^{f}	2.80^{j}	1.69^{f}	2.56^{j}	1.81^{g}	1.43^{i}	4.20^{g}	4.49^{g}
0700-0800	25.14^{e}	7.00^{i}	5.20^{e}	8.52^{i}	3.32^{fg}	5.58^{h}	9.60^{f}	10.42^{f}
0800-0900	26.33^{d}	20.73^{h}	9.95^{d}	14.13^{g}	5.43^{ef}	10.50^{g}	14.54^{d}	17.20^{d}
0900-1000	28.06^{c}	47.60^{a}	15.28^{bc}	25.19^{d}	8.71^{cd}	18.22^{e}	18.28^{bc}	26.15^{c}
1000-1100	30.40^{b}	45.20^{b}	20.53^{a}	36.11^{a}	10.36^{cd}	31.44^{a}	20.66^{a}	38.10^{a}
1100-1200	32.80^{a}	43.54^{c}	19.57^{a}	34.22^{b}	12.94^{ab}	28.18^{b}	18.88^{b}	37.61^{a}
1200-1300	27.26^{cd}	41.40^{d}	15.88^{b}	33.20^{b}	14.56^{a}	28.47^{b}	18.60^{bc}	34.54^{b}
1300-1400	25.07^{e}	39.00^{e}	14.19^{bc}	29.85^{c}	10.64^{cd}	23.36^{c}	16.87^{c}	32.90^{b}
1400-1500	22.94^{f}	39.40^{e}	13.12^{c}	25.81^{d}	10.89^{bc}	20.73^{d}	14.44^{d}	27.86^{c}
1500-1600	20.27^{g}	36.73^{f}	10.12^{d}	21.20^{e}	8.43^{d}	15.02^{f}	12.03^{e}	14.95^{e}
1600-1700	14.73^{h}	31.74^{g}	7.42^{e}	16.80^{f}	5.85^{e}	7.84^{h}	7.81^{f}	0
1700-1800	9.46^{i}	21.26^{h}	6.52^{e}	11.13^{h}	3.73^{efg}	0.00	5.46^{g}	0
Mean	23.75	31.36	11.53	21.56	8.05	17.33	13.44	24.42
Sem±	0.32	0.22	0.76	0.59	0.75	0.80	0.63	0.73
CV(%)	2.36	1.22	11.41	4.74	16.26	8.81	8.12	6.28
t-test@5%	NS		*		*		NS	

0900-1100 hr and another at 1500-1600 hr and *A. florea* recorded peak foraging activity at1000-1200 hr [Table-2]. Among non-*Apis* species, hymenopterans were the predominant (3.91±2.77 bees/ 5 capitula/ 5min.) floral visitors and their peak foraging activity was at 1000-1100 hr, followed by lepidopterans, coleopterans and lowest was recorded in case of dipterans. In case of fertility restorer line (RHA 95 C-1) *A. dorsata* was the predominant pollinator (31.16±15.98 bees/ 5 capitula/ 5 min.), followed by the *A. cerana* and least was *A. florea*. The peak foraging activity of *A. dorsata* was recorded at 1000-1200hr with 49 bees/5 capitula/5 min, *A. cerana* recorded peak foraging activity at 1100-1200 hr and *A. florea* recorded peak foraging activity at 1100-1300 hr [Table-3]. Among non-*Apis* species, hymenopterans were predominant (1.33±0.98 bees/ 5 capitula/ 5 min.) and peak foraging activity was observed at 1100-1200 hr, followed by lepidopterans,

Table-8 Nectar secretion and its total soluble solids (TSS) content in the flowers of parental lines of sunflower hybrid (n=25 florets)

Time (hrs)	Sunflower hybrid (KBSH-44)			
	Cytoplasmic male sterile line (CMS 17A)		Restorer line (RHA 95 C-1)	
	Nectar (µl/floret)	TSS (%)	Nectar (µl/floret)	TSS (%)
0600	1.00^{f}(0.00)	1.00^{f}(0.00)	1.00^{g}(0.00)	1.00^{g}(0.00)
0800	1.08^{e}(0.17)	5.51^{e}(29.37)	1.01^{f}(0.01)	5.31^{f}(27.22)
1000	1.26^{d}(0.59)	5.87^{d}(33.46)	1.06^{e}(0.13)	6.12^{e}(36.48)
1200	1.36^{c}(0.86)	6.41^{c}(40.21)	1.08^{d}(0.17)	6.50^{d}(41.32)
1400	1.52^{a}(1.32)	7.11^{b}(49.58)	1.16^{a}(0.34)	6.92^{c}(47.00)
1600	1.52^{a}(1.31)	7.27^{a}(51.93)	1.10^{c}(0.21)	7.27^{a}(51.98)
1800	1.41^{b}(1.01)	7.15^{b}(50.11)	1.15^{b}(0.33)	7.04^{b}(48.68)
Mean	1.30	5.76	1.08	5.74
Sem±	0.003	0.027	0.001	0.028
CV (%)	0.333	0.824	0.154	0.853

Table-9 Effect of different modes of pollination on hybrid seed yield and yield attributing characters of sunflower hybrid (KBSH-44)

Treatments	Seed yield (kg/ha)	Percent filled seeds	Test weight (g)	Volume weight (g)	Kernel (%)	Husk (%)	K:H Ratio
T1:*A. cerana* 3colonies/acre	869.69^{a}	93.76^{a}	4.94^{a}	38.67^{a}	74.14^{a}	25.86^{d}	2.88^{a}
T2:Hand pollination	805.25^{b}	92.04^{b}	4.52^{b}	37.53^{b}	68.75^{b}	31.25^{c}	2.19^{b}
T3:Open pollination	448.01^{c}	76.12^{c}	2.63^{c}	29.05^{c}	63.23^{c}	36.77^{b}	1.74^{c}
T4:Hand +Open pollination	887.74^{a}	94.44^{a}	5.04^{a}	39.18^{a}	75.34^{a}	24.66^{e}	3.06^{a}
T5:Control	116.11^{d}	0.00^{d}	0.94^{d}	7.95^{d}	0.00^{d}	100.00^{a}	0.00^{d}
Mean	625.36	71.27	3.61	30.47	56.29	43.70	1.97
Sem±	6.55	0.50	0.04	0.20	0.43	0.43	0.06
CV(%)	2.34	1.57	2.75	1.52	1.72	2.21	7.47

Table-10 Effect of different modes of pollination on seed quality, associated parameters and B:C ratio of sunflower hybrid (KBSH-44)

Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	Seed vigour Index	Oil content (%)	B:C Ratio
T1:*A. cerana* 3colonies/acre	93.76^{a}	12.10^{b}	20.10^{b}	1154.89^{b}	38.36^{b}	1.55:1
T2:Hand pollination	92.04^{b}	11.43^{c}	19.33^{c}	1071.51^{c}	36.06^{c}	1.34:1
T3:Open pollination	76.12^{c}	11.00^{d}	16.75^{d}	853.52^{d}	25.46^{d}	1.08:1
T4:Hand +Open pollination	94.44^{a}	12.53^{a}	20.76^{a}	1204.19^{a}	38.90^{a}	1.48:1
T5:Control	0.00^{d}	0.00^{e}	0.00^{e}	0.00^{e}	10.05^{e}	0.27:1
Mean	71.27	9.41	15.38	856.82	29.76	1.14
Sem±	0.50	0.10	0.12	9.38	0.12	
CV(%)	1.57	2.57	1.76	2.44	0.92	

coleopterans and lowest was dipterans. The corolla length of disc florets of CMS 17A line was 4.46 mm and RHA 95C-1 line was 4.45 mm. The variation in abundance, diversity and dominance of pollinators on parental lines could be due to the variation in availability of rewards (pollen and nectar) and variation in corolla length of disc florets. The present findings on abundance and foraging hours are in agreement with the findings of [[18] who showed that, *A. cerana* and *A. florea* were the most frequent visitors of sunflower capitula and maximum visitation was made during 0600-1100 hr, whereas comparatively lowest activity was observed during 1200-1430 hr of the day. The activity of the pollinators was more frequent in the forenoon (0900-1100 hr) and in the later afternoon (1600-1700 hr) [5].

Diversity and dominance of floral visitors on parental lines of KBSH-44

The maximum Shannon-Wiener index of diversity (H) of floral visitors (H=1.28) under open condition war recorded at 0800-0900 hr and 1000-1100 hr of the day and least (H=0.56) was recorded at 1700-1800 hr. The highest (d=0.471) Berger Parker dominance index between the floral visitors were recorded in case of *A. dorsata* and lowest was in case of dipterans (d=0.003), whereas between the hours of the day, highest dominance was recorded at 1700-1800 hr (0.75) and lowest was recorded at 0800-0900hr and 1000-1100hr (0.43) on CMS parental line (CMS 17A)[Table-2]. In case of fertility of restorer parental line (RHA 95C-1), maximum Shannon-Wiener index of diversity (H) of floral visitors (H=1.32) on fertility restorer parental line in open condition was recorded at 1700-1800hr of the day and least (H=1.03) was recorded at 0800-0900 hr. The highest (d=0.392) Berger Parker dominance index between the floral visitors of RHA 95C-1 was recorded with *A. dorsata* and lowest with dipterans (d=0.003), whereas highest dominance between the hours of the day was recorded at 0800-0900hr (0.49)[Table-3]. The findings of the present study on diversity indices are similar to the findings of [4] who reported the Shannon-Wiener diversity index H was calculated and found to be 1.49 for order Hymenoptera, 1.4 for the species of order Diptera and 1 for the species under order Lepidoptera in West Bengal.

Foraging activity of *Apis* species on CMS parental line (CMS 17 A)

The highest mean number of nectar foragers (80.25±22.97 bees/5 capitula/5 min.) of *A. cerana* under caged condition (three colonies per acre) without pollen load on cytoplasmic male sterile parental line (CMS 17A) of sunflower hybrid (KBSH-44) were recorded, followed by *A. dorsata*, *A. cerana* and *A. florea* foragers from natural colonies in open pollination condition, whereas, highest mean number of nectar foragers (35.08±19.31 bees/ 5 capitula/ 5 min.) of *A. cerana* under caged condition (three colonies per acre) with pollen load were recorded, followed by *A. cerana* (3.25±2.52 bees/ 5 capitula/ 5min.), *A. dorsata* (2.50±2.43 bees/5 capitula/5 min.) and *A. florea* foragers from natural colonies in open pollination condition [Table-4]. The two peak nectar foragers of *A. cerana* with pollen load in caged condition (three colonies per acre) was recorded between 0900-1000 hr and 1600-1700hr (63 and 34 bees/5 capitula /5 min. respectively) and without pollen load recorded at 0800-0900 hr and 1400-1500hr (107 and 104 bees/5 capitula/ 5 min) of the day.

There was significant difference in number of nectar foragers with and without pollen load in caged condition. Foragers from natural colonies of *A. cerana* with pollen load recorded two peaks of nectar foraging activity at 0700-0800 hr (8 bees/5 capitula/5 min.) and1500-1600 hr (4 bees/5 capitula/ 5 min.). Whereas, *A. cerana* without pollen load recorded two peaks at 1000-1100 hr and 1500-1600 hr of the day. Nectar foragers with pollen load on CMS lines are plays important role in transferring of pollen grains from restorer line to cytoplasmic male sterile line and carried out effective pollination. There are two peak nectar foraging activity of honey bees during 0800-1100 hr in the morning and 1400-1600 hr in the evening. There is significant difference in number of nectar foragers with and without pollen load in caged condition and open pollination by *Apis cerana* and *Apis* dorsata. But there is no significant difference in number of nectar foragers of *A. florea* with and without pollen load in open pollination. That leads to effective transfer of pollen from restorer line to the cytoplasmic male sterile lines.

Foraging activity on fertility restorer parental line (RHA95C-1)

Pollen and nectar foragers (no. of bees/ 5 capitula/ 5 min.) on restorer line under caged (3 colony/ acre) and open condition was recorded. *A. cerana* recorded highest mean number of pollen foragers (92.58±47.65 bees/5 capitula/5 min.) compared with nectar foragers (41.58±19.94 bees/5 capitula/5min.) under caged condition (three colonies per acre), whereas in open condition *A. dorsata* was the more abundant pollenforager and *A. cerana* was the more abundant nectar forager compared with other *Apis* species on restorer line RHA 95C-1 and least pollen and nectar foragers were recorded with *A. florea* [Table-5]. *A. cerana* recorded two peaks of pollen foraging activity in caged at 0800-0900 hr and 1600-1700 hr (151 bees and 102 bees / 5 capitula/ 5 min. respectively) and open condition only one peak pollen foraging activity at1100-1200 hr (34bees/5capitula/5min.), whereas, two nectar foraging activity of *A. cerana* in caged condition at 0900-1000 hr and 1600-1700 hr 66 bees and 35 bees/5 capitula /5 min. respectively) and in open condition only one peak in nectar foraging activity was recorded at 1100-1200 hr with 16 bees/5 capitula/ 5 min. *A. dorsata* and *A. florea* recorded only one peak in pollen and nectar foraging activity between 1100-1300 hr of the day. There were statistically significant differences in the number of nectar and pollen foragers of *A. cerana* on RHA 95C-1 parental line in caged condition and natural colonies in open pollination. The variation in the abundance of insect pollinators, nectar foragers and pollen foragers on restorer lines of selected sunflower hybrids was mainly due to variation in corolla length of disc florets, variation in the availability of nectar quantity and sugar concentration, availability of pollen in disc florets. Similar findings were also reported by [16] where in female parental (CMS lines) of sunflower hybrids were visited mainly by major nectar collectors due to greater availability of nectar. Pollen foragers of A. mellifera were more abundant on male fertile flowers and the nectar foragers were more abundant on male sterile flowers [10]. The higher activity of the bees in the morning may be attributed to the abundant availability of pollen and also nectar in the sunflower heads. Pollen availability gradually decreased due to pollen foraging by honey bees, there by resulting in decreased bee activity. In the evening probably the stigma would have pushed remaining pollen out of anther tube resulting in increased bee visits to sunflower heads [21].

Foraging duration of *Apis* species on CMS parental line (CMS 17 A)

The mean nectar foraging duration with pollen load of *A. florea* spent more foraging duration (12.11 sec/flower) on the flowers of CMS parental line, followed by *A. cerana* and least duration was recorded in case of *A. dorsata* in open pollination. Whereas, maximum nectar foraging duration (16.58 sec/capitulum) was recorded by *A. cerana* in caged pollination (3 colonies per acre) [Table-6]. *A. florea* without pollen load spent more mean nectar foraging duration (26.58 sec/capitulum) in open pollination compared with *A. cerana* (23.31 sec/capitulum) and *A. dorsata* (17.48 sec/capitulum). But in caged condition (3 colonies of *A. cerana*/ acre) it recorded maximum nectar foraging duration of 35.61 sec/capitulum. Bees without pollen load spent significantly greater nectar foraging duration as compared with bees with pollen load, both in caged and open pollination situations.

Foraging duration on fertility restorer parental line (RHA 95 C-1)

The maximum mean pollen and nectar foraging duration (13.44and 24.42 sec/capitulum respectively) was recorded by the *A. florea* in open pollination, followed by the *A. cerana* and least was recorded in case of *A. dorsata* [Table-7]. The pollen (23.75sec/capitulum) and nectar (31.36 sec/capitulum) foraging duration by the *A. cerana* in caged pollination (3 colonies/acre) registered highest mean foraging duration as compared with the which spent time for collection of pollen and nectar in open pollination. The time spent by *A. cerana* in caged condition and *A. florea* in open pollination for nectar foraging was on par with pollen foraging. *A. dorsata* and *A. cerana* recorded significantly higher nectar foraging duration as compared with pollen duration in open pollination.

The time spent by *A. cerana* in caged condition and *A. florea* in open pollination for nectar foraging was on par with pollen foraging. *A. dorsata* and *A. cerana* recorded significantly greater nectar foraging duration compared with pollen foraging duration in open pollination. The present findings were in agreement with findings of [14] reported that, *Apis cerana* was the most dominant pollinator with maximal foraging activity at 1100 hr and 1400 hr while *A. dorsata* and *A. florea* were present at lower density. *A. florea* spent highest time (44.1 sec), followed by *A. cerana* (34.54 sec) and *A. dorsata* (31.9 sec). [17] reported that *A. dorsata* spent only 1.5 minutes as these bees swiftly collected floral rewards. They could also visit several flowers in a shorter time, making them efficient pollen vectors. The floral handling time was more for nectar collection (90 sec) than pollen collection (30sec).

Nectar secretion and its total soluble solids (TSS) content in the nectar of parental lines

Nectar secretion/production by both the parental lines of sunflower hybrid was recorded. 1.30 and 1.08 µl/floret was produced by CMS 17A and RHA 95C-1 respectively. Cytoplasmic male sterile parental lines produce more quantity of nectar as compared with fertility restorer line. CMS parental line attract more number of nectar foragers compared with fertility restorer parental line [Table-8]. The mean total soluble solids (TSS) of nectar produced by CMS 17A and RHA 95C-1 lines was recorded 5.76 and 5.74 % respectively. [9] reported that the mean nectar yield per floret of various sunflower genotypes showed a significant differences. The mean nectar yield per floret ranged from 0.21 mg (265R) to 0.59 mg (586R). CMS lines were found to have more mean nectar content as compared with R lines of sunflower (0.4 and 0.37 mg/floret, respectively). Bees have differential preference for one of the two parental lines. This difference can be explained by differences between lines for nectar production and for concentration and quality of sugars [2].

Effect of different modes of pollination on hybrid seed yield, yield attributing characters, seed quality and its associated parameters

The significantly highest seed yield per hectare (887.74kg), percent filled seeds (94.44%), test weight (5.04 g), volume weight (39.18 g/100 ml), kernel percentage (75.34 %) and highest kernel to husk ratio (3.06) was recorded with hand+open pollination treatment, this was statistically on par with by *A. cerana* @ 3 colonies per acre (869.69kg, 93.76, 4.94g, 38.67g/100ml,74.14% and 2.88 K:H ratio, respectively), followed by hand pollination, open pollination and least was recorded with control [Table-9]. The highest germination percentage (94.44 %), shoot length (12.53 cm), root length (20.76 cm), seed vigour index (1204.19) and oil content (38.90 %) were recorded in hand + open pollination plots, followed by *A. cerana* @ 3 colonies per acre treated plots (93.76 %, 12.10 cm, 20.10 cm, 1154.89 and 38.36 %), hand pollination, open and pollination and least seed quality and its associated parameters were recorded in control treatment [Table-10]. Similar results were reported by [22] who conducted an experiment on effect of bee pollination in sunflower with three treatment *i.e*. pollination without insects, open pollination and bee (*A. cerana* himalaya) pollination. Crop pollinated by honey bees enhanced seed set (23.43 %) compared to pollination without insects and 18.31% compared to open pollination. The increase in seed yield in bee pollinated treatment was 80.49% greater than pollinated without insects and 11.75% than open pollination. The germination of seeds from bee pollinated plants was 30.41% higher than seeds from plants without insects and 5.35% higher than open pollinated plants. In case of the female parent of sunflower hybrid, the filled seed weight, seed filling percentage and seed oil content were highest in the plot pollinated only by honey bees [15].

Benefit:cost ratios of different modes of pollination

The numerically highest benefit:cost ratio was recorded in *A. cerana* @ 3 colonies per acre (1.55:1), followed by hand + open pollination treatment (1.48:1), hand pollination (1.34:1), open pollination (1.08:1) and control (0.27:1) treatments [Table-10]. Similar finding were recorded by [6] who reported that, the benefit cost from the beekeeping was 1.81 which indicates higher yield and less cost of production of beekeeping contributed higher gross return and benefit cost ratio.

Conclusion

Sunflower hybrid seed production through hand pollination is very difficult task due to shortage of agricultural labour and it is also very expensive when there is

availability of labour. To overcome this problem, we have to use honey bee colonies required for higher quantity and good quality sunflower hybrid seed production as an alternative to hand pollination.

Application of research: Study of three colonies of *Apis cerana* per acre was assessed to be effective for the production of good quality sunflower hybrid seeds.

Research Category: Pollination

Acknowledgement / Funding: Authors are thankful to Senior Farm Superintendent, Main Agricultural Research Station, University of Agricultural Sciences, Raichur, 584104, Karnataka, India

****Principal Investigator or Chairperson of research: Dr Sanganna M. Sajjanar**
University: University of Agricultural Sciences, Raichur, 584104, Karnataka, India
Research project name or number: Research station study

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: Main Agricultural Research Station, Raichur, 584104, Karnataka, India

Cultivar / Variety / Breed name: Sunflower Hybrid (KBSH-44)

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

References

[1] Anonymous (2012) Focus, (7), 6-7.
[2] Basualdo M., Rodriguez E. and Bedascarrasburre E., (1999) *Ann. encuentro de investigadores en temas relacionados a la apicultura, Azul, Argentina*.
[3] Belavadi V. V. and Ganeshaiah K. N. (2013) *Insect pollination manual. NICRA project on effects of climate change on pollinator populations. Department of Agricultural Entomology, UAS, Bangalore*.
[4] Biswanath B. and Kakali B. (2015) *Int. J.Curr.Sci*., 18, 76-87.
[5] Delaude A., Tasel J. N. and Roller M. (1978) *In Proc. IV Int. Sym.On Pollination, Maryland*, 29-40.
[6] Devkota K., Dhakal S. C. and Thapa R. B. (2016) *Agric.& Food Secur*., 5, 1-6.
[7] Free J. B., (1963) *J. Appl. Ecol*., 1(1), 19-27.
[8] Goswami V., Khan M. S. and Usha (2013) *Journal of Applied and Natural Science*, 5(2), 294-296.
[9] Gowda J., Bhat N. S. and Kusuma B.K. (2003) Indian *Bee J*., 65(1&2), 36-43.
[10] Greenleaf S. S. and Kremen C. (2006) *Proceedings of the National Academy of Science, USA*, 13890-13895.
[11] Hoffman D. G. and Chambers M. (2006) *Environ. Entomol*., 35(4), 1103-1108.
[12] Jadhav A. J., Sreedevi K. and Rajendraprasad P. (2011) *Curr. Biotica*., 5(3), 344-350.
[13] Melnichenko A. N. and Khalifman I. A. (1960) *Pollination of agricultural crops, Vol-III, Amerind Publication Co. Pvt. Ltd, New Delhi*, 406.
[14] Panda P., Nanda U. K., Mohapatra H. and Padhi J. (1988) *Indian Bee J.,* 51(3), 97-98.
[15] Rajagopal D., Veeresh G.K., Chikkadevaiah, Nagaraja N. and Kencharaddi R. N. (1999) *Indian J. Agric. Sci*., 68(1), 40-43.
[16] Rajasri M., Kanakadurga K., Durgarani V. and Anuradha C. H. (2012) *Int. J. of Applied Biology and Pharmaceutical Technol*., 3(2), 216-221.
[17] Ramya M., Muthuraman M. and Saravanan P. A. (2014) *Madras Agric. J.,* 101(10-12), 379-381.
[18] Rangarajan S., Mahadevan N. R. and Iyemperumal S. (1974) *Indian J. Agric. Sci*., 44, 66-67.
[19] Rinku, Chaudhary O. P. and Kaushik H. D. (2017) *Indian J. Eco*., 44(5), 536-542.
[20] Rollin O. and Garibaldi L. A. (2019) *J. Appl. Eco*., 56, 1152-1163.
[21] Singh B. (1977) *Thesis Abstract Haryana Agric. Uni. Hisar, India,* 3, 76-77.
[22] Singh M. P., Singh K. I. and Devi C. S. (1998) *In, Asian Bees and Beekeeping, Progress of Research and Development (Eds.M.Mustaka,L.R.Verma,S. Wongsiri, K. K. Shrestha and U. Pratap). Oxford and IBH Publishing Company, NewDelhi,* 186-189.
[23] Southwood T. R. E. (1988) *Ecological methods. Third edition. Methuen, London, UK.*