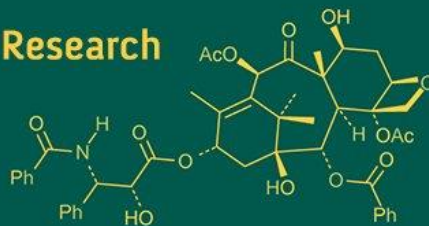


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; 10(6): 1105-1113
www.biochemjournal.com
Received: 04-03-2026
Accepted: 08-04-2026

JS Damadhere
M.Sc. Research Scholar,
Department of Entomology,
Mahatma Phule Krishi
Vidyapeeth, Rahuri,
Maharashtra, India

Dr SA Landge
Assistant Residue Analyst,
AINP on Pesticide Residues
and Contaminants,
Department of Entomology,
Mahatma Phule Krishi
Vidyapeeth, Rahuri,
Maharashtra, India

UK Kadam
Head, Department of
Entomology, Mahatma Phule
Krishi Vidyapeeth, Rahuri,
Maharashtra, India

BY Pawar
AICRP on Betelvine,
Department of Entomology,
Mahatma Phule Krishi
Vidyapeeth, Rahuri,
Maharashtra, India

VS Shinde
Assistant Plant Pathologist,
AICRP on Maize, Mahatma
Phule Krishi Vidyapeeth,
Rahuri, Maharashtra, India

RV Kadu
Junior Entomologist, AICRP
on Fruit Crops, Mahatma
Phule Krishi Vidyapeeth,
Rahuri, Maharashtra, India

Corresponding Author:
JS Damadhere
M.Sc. Research Scholar,
Department of Entomology,
Mahatma Phule Krishi
Vidyapeeth, Rahuri,
Maharashtra, India

Studies of honey bee species in lucerne seed production (*Medicago sativa* L.)

JS Damadhere, SA Landge, UK Kadam, BY Pawar, VS Shinde and RV Kadu

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i6n.8834>

Abstract

Lucerne (*Medicago sativa* L.) is cross-pollinated crop and requires entomophilous pollination for tripping of flowers and subsequent pod and seed set. To find out the best pollinators for lucerne seed production, the present investigation entitled "Studies of honeybee species in Lucerne seed production" was carried at All India Co-ordinated Research Project on Forage Crops & Utilization, Department of Agril. Botany, Mahatma Phule Krishi Vidyapeeth, Rahuri during 2024-2025. Field experiments covered species diversity of flower visitors, foraging ecology of major bee species, impact of managed bee pollination on seed yield. The species diversity of flower visitors of lucerne composed of 905 individuals of 27 different species which belonged to 23 genera, 12 families and 3 orders. The flower visitors predominantly belonged to the order hymenoptera (79.52%, 13 species) followed by lepidoptera (11.09%, 11 species), diptera (9.39%, 3 species). Honey bee species constituted major part of flower visitors 60.74 per cent. Seed yield enhancement through different pollination methods revealed that Open pollination produced the highest seed yield (351.23 kg/ha) with better pod formation, higher test weight (3.76 g), and maximum economic return (BC ratio 4.46), mainly due to efficient pollination by *A. florea* and solitary bees. *A. cerana* recorded the second-highest yield (297.6 kg/ha) with 93.5% germination and 3.15 BC ratio. *T. iridipennis* showed moderate effectiveness (254.82 kg/ha), while *A. mellifera* was less effective (185.44 kg/ha) and economically infeasible. The no-pollination treatment gave the lowest yield (43.55 kg/ha) and poor germination (26%), indicating lucerne needs insect pollinators for optimum seed production.

Keywords: Entomology, insect pest management, crop protection, pesticide residues, biological control, Integrated Pest Management (IPM), agricultural entomology

Introduction

Lucerne (*Medicago sativa* L.) called, 'queen of forage crops', is one of the oldest among cultivated fodder crops also known as alfalfa means best fodder as lucerne is most important cosmopolitan forage crop. Lucerne belongs to family leguminosae and genus *Medicago*. This genus includes 65 species native to Europe, Asia and Africa. Lucerne is propagated or reproduced by seed. Once established, lucerne gives fodder for 3-4 years.

Lucerne is a wonderful winter fodder crop and mainly grown where berseem fails. It is valued for its drought tolerance, high yield, protein content and digestible fibre and as such makes good forage for ruminants. It is used for grazing, hay, silage making, green manure and as cover crop (Burezq, 2021) ^[14]. It is valued for the ability to fix nitrogen during drought when other legumes are not nodulated or not actively fixing nitrogen (Johnson and Rumbaugh, 1981) ^[30]. Lucerne fixes 83 to 594 kg atmospheric nitrogen per hectare (Aganga, 2003) ^[1]. Its presence in rotation maintains the nitrates and organic matter in soil. Lucerne is also recommended for seeding mined soil to reduce erosion and increase forage value (Cogliastro *et al.*, 1990) ^[21].

Lucerne contains approximately 5 times as much protein as sorghum and ample quantity of vitamin 'A'. This forage legume is palatable with 72 per cent dry matter digestibility. It is high in mineral content and contains at least 10 different vitamins such as pantothenic acid, riboflavin, biotin, folic acid, choline, pyridoxine, vitamin B12, K and C. Lucerne contains 20.2 per cent crude protein, 16.2 per cent digestible crude protein, 30.1 per cent crude fibre, 1240 g calcium and 350 g phosphorous per 100 kg lucerne and metabolic energy 2.17 M cal per kg (Banerjee, 1978) ^[5]. In India, area, seed and green forage production of lucerne is nearly 10 lakh hectares, 150 kg ha⁻¹ and 65-130 tones per hectare, respectively. Scarcity of

green fodder is one of the most important limiting factors for achieving "White revolution". The present feed and fodder resources of the country can meet only 48 per cent of the requirement with a vast deficit of 62 and 22 percent of green and dry fodder, respectively. Lucerne being a perennial fodder crop could play a major role in this direction.

Insect pollination is essential for the sustainability of both agricultural and natural ecosystems since 87 per cent of flowering plants and nearly 35 per cent of crops worldwide are dependent on insect and other pollinators. Honey bee pollination enhances crop yield, seed production, and fruit quality, making them an eco-friendly and cost-effective means of improving cross-pollinated crops (Free, 1970)^[23]. Alfalfa is considered an insectary crop because it attracts a large number of insect pollinators (Kulkarni and Kumar, 2020)^[32].

Floral biology of lucerne -The plant bears irregular bisexual flowers, usually purple, blue, violet, yellow, or white, about 1/4" to 1/3" long arranged in axillary racemes in an acropetal pattern. Each raceme contains 5-40 insect-pollinated flowers, and the fruits are spirally twisted pods. The flower has one standard petal, two wing petals, and two fused petals forming the keel. The flower contains ten anthers in diadelphous condition (Brunet *et al.*, 2019)^[12]. Alfalfa flowering extends over a relatively long period, with all flowers in a bloom opening within about one week (McGregor, 1976)^[35]. The unpollinated flower remains open for seven days, but very soon after pollination it wilts and falls off.

Pollination & Fertilization of alfalfa - Standard petal of floret acts as a platform for bees to land on it. The lucerne flower is so constructed that the style and stamens are enclosed in a tense position within the keel. When this tension is released the style and stamens spring forward, causing the pollen (sexual column) to be distributed and the stigma to strike with some force against the standard petal. This process, referred to as "tripping" the flower, allows the pollen to become available for cross and self-pollination. Alfalfa pollen is moist, sticky, heavy and coalescent into large lumps and cannot be transferred by wind, only by pollinating insects. There is self-pollination in alfalfa, however, as stated by Tysdal (1946)^[49], in field conditions less than one percent of flowers pollinated in such a way produce seed.

The available bee forage resources in the lucerne flower are pollen and nectar.

When a honey bee enters its proboscis into a flower and triggers its opening mechanism (tripping), the stamens strike the bee's head, depositing pollen, while the stigma receives pollen from another flower, enabling cross-pollination. Thus the bee both trips and cross-pollinates the flower (Butler *et al.*, 1956)^[15]. opening of the flower is rapid and frequent, stylus strikes the honey bee and throws it away from flower or its proboscis gets stuck between anthers and the honey bee must spend a lot of effort to free its head out of the flower. As this mechanism can be forceful and inconvenient, nectar-collecting honey bees often avoid tripping the flowers and instead extract nectar by squeezing their proboscis between petals without tripping the flowers. As a result, they obtain nectar without pollinating the flowers.

Alfalfa potential for seed production - Although alfalfa produces an enormous number of flowers (about 3.3 billion flowers ha⁻¹) due to its genetics, only a fraction develop into seed-bearing pods (Tysdal, 1946)^[49]. Based on estimates of pod set and seed production, potential seed yields of up to 5,000 kg ha⁻¹ have been suggested (Lesins, 1950)^[34], while pod formation in 46.7% of flowers could result in about 2,000 kg ha⁻¹ of seed yield (Pedersen *et al.*, 1956)^[37]. However, such yields are rarely achieved under field conditions primarily due to poor pollination of alfalfa flowers which is a result of morphological traits of flower, low self fertilization and insufficient presence of adequate pollinators. Practical seed yields are reported to be 20-30 times lower than the theoretical potential (Stjepanović, 1981), with average yields ranging from 250-300 kg ha⁻¹ and maximum yields reaching 800-1000 kg ha⁻¹ under favorable agro-ecological conditions (Erić, 1995).

Pollinators have a vital role in pollinating the lucerne crop. Cross-pollination in lucerne contributes to 89 per cent seed yield (Somerville, 2002)^[45]. Reproductive success in lucerne is influenced significantly by pollinator abundance, their foraging behaviour and pollination characteristics (through the provision of pollen and nectar). Alfalfa, is cross-pollinated because of self-incompatibility and self-sterility. One of the major problems in alfalfa seed production is Insufficient pollination and variation in seed yield, which is heavily affected by environmental conditions. The years with higher rainfall are more favourable to solitary bees, while the dry years are ideal for honey bees. Pollination is mainly dependent on honey bees and other solitary bees. By keeping all this in a view viz. economic and ecological importance of lucerne, problems associated with pollination and its yield potential, investigation was undertaken to study diversity of flowers visitors and their abundance in lucerne seed production plot and impact of managed bee pollination on seed yield obtained by use of honey bee colonies.

2. Materials and methodology

2.1 Materials

- **Seed** - The seeds of lucerne RL-88 variety were used for studies on pollinators in seed production
- **Honey bees**- Honey bee hive of *Apis mellifera*, *Apis cerana indica* and *Trigona irridipenis* in lucerne crop will be placed inside the cage house
- **Pollination cages** - Nylon nets will be used to make pollination cages. The cages of nylon mosquito nets(1 mm) size will be erected over the crop at the commencement of flowering. erect pollination cages
- **Bamboo stick** - Will be used to erect pollination cages
- **Insect collection net** - To collect the different species of inset pollinators visited during flowering period

2.2 Details of experiment

- **Location** : Experimental Farm, AICRP on Forage Crops & Utilization Department of Agril. Botany, MPKV, Rahuri.
- **Crop** : Lucerne
- **Variety** : RL-88
- **Design of experiment** : Randomized Block Design (RBD)
- **Treatment** : 5
- **Replication** : 4
- **Plot size** : 10 x 5 m²

- **Spacing** : 30 cm (line sowing)
- **Season** : Rabi 2024-25
- **Sowing Date** : 4th January, 2024
- **Seed Rate** : 25 kg / ha
- **Fertilizer dose** : 20:150:40 NPK Kg / ha
- **Date of harvesting** : 13th May, 2025

2.3 Treatment details

Table 1: Pollination Treatments and Colony Strength of Different Pollinator Species

Tr. No.	Treatment	Colony strength
T ₁	Controlled pollination only with <i>Apis cerana</i>	5 colonies/ ha
T ₂	Controlled pollination with <i>Apis mellifera</i>	3 colonies/ ha
T ₃	Controlled pollination with <i>Trigona iridipennis</i>	8 colonies/ ha
T ₄	Open pollination	-
T ₅	No insect Pollination	-

2.4 Methodology

2.4.1 Species diversity of flowers visitors

A total of 1000 m² area of lucerne field was selected and the observations pertaining to insect flower visitors and their diversity on lucerne flowers recorded once a week starting from 11th standard meteorological week SMW to 18th standard meteorological week SMW during summer 2025 using transect sampling method. The observations were recorded at three hourly intervals (9.00-12.00 hrs, 12.00-15.00 hrs and 15.00-17.00 hrs) on a selected day of an individual week. The traversing distance was up to 10 m in one direction and total distance of 30 m (3 transect lines) was covered in a span of five min. A total of 24 such transect walks (10 meters each) were made throughout the flowering period and all the flower visiting insects were counted and recorded in a field register. Representative specimens of each insect visitor were caught with a hand net, pinned, labelled and repository of lucerne flower visiting insects was prepared which is later identified with the help of specialist.

$$\text{Per cent forage visit (\%)} = \frac{\text{Total number of individuals of particular species}}{\text{Total number of species recorded}} \times 100$$

2.4.2 To study effect of managed bee pollination seed yield of lucerne

The cages of the size 10 m × 10 m were made by using mosquito net of 288 mesh with four replications for each treatment. After crop maturity and seed setting seed yield, no. of pods per raceme, test weight and germination test are recorded

Seed yield (kg/ha) - Inflorescences from all five replications of each treatment were harvested separately. Seeds were threshed, cleaned, and weighed for each replication. The average seed weight was expressed as kg/plot and later converted to kg/hectare

Number of pods per raceme (P/R)- Five inflorescences were randomly collected from all four replications of each treatment. Pods were separated and counted individually, and the average value was expressed as number of pods per raceme (P/R).

Weight of 1000 seeds (Test weight)

Germination test - Germination tests for each treatment were conducted in four replications of 100 seeds following

ISTA procedures (Anon., 2014) ^[4] using the above paper method. Final germination count was recorded on the fourth day based on normal seedling emergence and growth, and results were expressed as percentage seed germination.

$$\text{Germination percentage (\%)} = \frac{\text{Seeds germinated}}{\text{Total seeds}} \times 100$$

ICBR ratio - The incremental cost-benefit ratio (ICBR) refers to a financial metric that evaluates the additional costs incurred in treatment (Appendix II) compared to the additional benefits gained (Vladica, 2018) ^[52].

B:C ratio

The Benefit-Cost ratio (B:C ratio) is a financial metric used to access the profitability of a treatment. It compares the total present value of benefits generated by treatment to the total present value of cost. The B:C ratio of the respective treatment was calculated by using the following formula, (Akila and Babu, 1994) ^[3]

$$\text{B:C ratio} = \frac{\text{Total present value of benefits}}{\text{Total present value of costs}}$$

3. Results and discussion

3.1 Studies on species diversity of flowers visitors in lucerne seed production

Studies on the species diversity of flower visitors in lucerne fields are essential for improving crop production, supporting biodiversity conservation and promoting sustainable agriculture. The flower visitors of lucerne composed of 905 individuals of 27 different species which belonged to 22 genera, 12 families and 3 orders. These insect visitors visited the lucerne flowers for both pollen and nectar and did not cause any harmful damage to flowers of lucerne plant. The flower visitors predominantly belonged to the order hymenoptera (79.52%) followed by lepidoptera (11.09%), diptera (9.39%). The species were continuously active and spent most of the time of the day in visiting lucerne flowers and found as effective pollinators.

Among 27 flowers visitors, 13 species of 4 families belongs to hymenoptera order. Honey bees included *A. dorsata*, *A. cerana* and *A. florea*, *A. mellifera*. Among honey bee species dominant species was *A. florea* (40.33%) followed by *A. cerana* (11.60%), *A. dorsata* (6.61%), *A. mellifera* (2.20%). Apis species formed 60.74% and 18.78% hymenopteran species were non apis bee species i.e. solitary bee species. This included *T. iridipennis*, *Ceratina (Pithitis) binghami* Cockerell, *Amegilla* spp., *Xylocopa* sp., *Xylocopa latipes*. Other solitary bees includes *Lasioglossum coriaceum* and *Lipotriches* sp. of halictidae family; *Megachile rotundata* of megachilidae family; *Polistes Carolina* of vespidae family.

The next dominating order was lepidoptera with eleven species belonging to five different families. The species included *Eurema hacabe*, *Delias eucharis*, *Calotis etrida* from pieridae family; *Danaus chrysippus* and *Acraea terpsicore*, *Junonia lemonias* from Nymphalidae family; *Cigaritis lohita*, *Leptotes pirithous*, *Rapala manae* from lycaenidae; *Papilio demoleus* from papilionidae and *Colias philodice* from coliadinae family.

The following dominant order in the lucerne plot was diptera. It included three species from two different families viz. *Musca domestica* from muscidae, *Spherophoria* sp. and *Ischiodon scutellaris* from syrphidae.

Table 2: Species diversity of flowers visitors in lucerne seed production

Sr. No.	Pollinator	Scientific Name	Systematic Position
Hymenopteran Insects			
K1	Dwarf honey bee	<i>Apis florea</i>	Apidae
2	Indian honey bee	<i>Apis cerana</i>	Apidae
3	Rock honey bee	<i>Apis dorsata</i>	Apidae
4	European honey bee	<i>Apis mellifera</i>	Apidae
5	Stingless bee	<i>Trigona iridipennis</i>	Apidae
6	Small Carpenter bee	<i>Ceratina (Pithitis) binghami</i> Cockerell	Apidae
7	Leathery Sweat Bee	<i>Lasioglossum coriaceum</i>	Halictidae
8	Alfalfa leaf cutter bee	<i>Megachile rotundata</i>	Megachilidae
9	Blue-banded bee	<i>Amegilla</i> spp.	Apidae
10	Carpenter bee	<i>Xylocopa</i> sp.	Apidae
11	Tropical Carpenter bee	<i>Xylocopa latipes</i>	Apidae
12	Sweat bee	<i>Lipotriches</i> sp.	Halictidae
13	Red paper wasp	<i>Polistes carolina</i>	Vespidae
Lepidopteran Insects			
14	Plain Tiger Butterfly	<i>Danaus chrysippus</i>	Nymphalidae
15	Common Grass Yellow	<i>Eurema hecabe</i>	Pieridae
16	Long-banded Silverline	<i>Cigaritis lohita</i>	Lycaenidae
17	Common Zebra Blue	<i>Leptotes pirithous</i>	Lycaenidae
18	Common Jezebel Butterfly	<i>Delias eucharis</i>	Pieridae
19	Slate Flash	<i>Rapala manea</i>	Lycaenidae
20	Tawny Coster Butterfly	<i>Acraea terpsichore</i>	Nymphalidae
21	Little Orange Tip	<i>Colotis etrida</i>	Pieridae
22	Lemon Butterfly	<i>Papilio demoleus</i>	Papilionidae
23	Lemon Pansy	<i>Junonia lemonias</i>	Nymphalidae
24	Clouded Sulphur Butterfly	<i>Colias philodice</i>	Coliadinae
Dipteran Insects			
25	House fly	<i>Musca domestica</i>	Muscidae
26	Globe-tailed hoverfly	<i>Spherophoria</i> spp.	Syrphidae
27	Yellow-shouldered hoverfly	<i>Ischiodon scutellaris</i>	Syrphidae

The results of present investigations are in line with Chavhan and Shashidhar (2015) ^[19], Kulkarni and Kumar (2020) ^[32], Rauf *et al.* (2021) ^[41], Kumar *et al.* (2024) ^[33] and, who found that the majority of floral visitors belonged to Hymenoptera, followed by Lepidoptera and Diptera. The dominance of honey bees in the present study is consistent with the reports of Vansell and Todd (1946) ^[51], Bieberdorf (1949) ^[7] and Ciurdarescu (1972), Cecen (2008) ^[17, 18] and Jones and Forster (2012) ^[31], who concluded that honey bees were the most frequent visitors to alfalfa flowers. Under Indian conditions, Gachchinamath (1983) ^[24], also recorded *A. cerana* and *A. florea* as predominant floral visitors in lucerne forming more than half of the bee population. Studies conducted in Central

Serbia for ten long year (1997-2007) it was found that honey bees were dominant compared to all other pollinators.

In contrast, Hadfield and Calder (1936) ^[26], Gurr (1955) ^[25] and Wojtowski (1965) ^[53] reported that *Bombus terrestris* was a more effective pollinator of lucerne than the honey bee. Brunet and Stewart (2010) ^[13] found that three species of the genus *Bombus* visited alfalfa flowers significantly more frequent and tripped them much better than the alfalfa leafcutter and honey bees. Conversely, Jevtic and Radovic (2001) ^[29] reported that honey bees formed only 1.1 per cent of pollinators. The presence of syrphid flies (7.82%) as significant floral visitors corroborates the findings of Kulkarni and Kumar (2020) ^[32] and Chavhan and Shashidhar (2015) ^[19], who recorded syrphids as an important component of lucerne pollinator communities in India.

The role of non-*Apis* bees, syrphid flies and butterflies, though numerically less abundant, cannot be overlooked due to their documented efficiency in flower tripping and potential to enhance seed set. Studies by Vansell and Todd

(1946) ^[51] Stephen (1960), Bohart (1962) ^[9], Olmstead and Wooten (1987) ^[36] and Cecen (2008) ^[17, 18] identified leafcutting bees (*Megachile rotundata*) and alkali bees (*Nomia melanderi*) as efficient pollinators. Rauf *et al.* (2021) ^[41] emphasized the high pollination efficiency of solitary bees such as *Megachile cephalotes* and *Amegilla* spp. Bohart and Knewlton (1960) ^[8] showed that these two species can be manipulated, i.e. they can be transported and domesticated in the alfalfa fields. As claimed by Mc Gregor (1976) ^[35] alkali bees and alfalfa leafcutters are more efficient pollinators than honey bees as their primary motive for visiting alfalfa flower is collecting pollen used to feed the brood. Bosch and Kemp (2005) ^[10] determined that alfalfa leafcutter can increase seed yield if brought organized to the seed crop field. Ciurdarescu (1974) ^[20] claimed that honey bee trips from 3.4 to 9.5% of visited flowers, which is much less efficient than some solitary bees which trip 45-100% of visited flowers. Tucker (1956) ^[48] pointed that honey bees which forage nectar trip only 0.3% of visited flowers. He gives great importance to young nectar foragers, citing the fact that they trip 70-85% of visited flowers during their “learning process”. These findings emphasizing that lucerne pollination benefits from a diverse pollinator community rather than reliance on a single bee species.

3.2 Impact of managed bee pollination on seed yield parameters of lucerne

Managed bee pollination, specifically using honeybees, can have a significant positive impact on both the quantity and quality of lucerne seeds. Precise and efficient pollination process by managed honey bees tends to enhance seed uniformity and quality, leading to a higher percentage of

viable and marketable crop seeds, ultimately benefiting the seed production industry. The data pertaining to various qualitative and quantitative parameters viz. lucerne seed

yield (Kg/ha), Number of pods/ racemes (P/R), 1000 seed weight (g), Germination (%) are studied.

Table 3: Impact of managed bee pollination on seed yield parameters of Lucerne

Treatment	Number of pods/ racemes **	Seed yield (kg/ha)	1000 seed weight (g)	Germination (%) *
T ₁ : <i>A. cerena</i>	16.45 (4.11)	297.6	3.58	93.5 (75.72)
T ₂ : <i>A. mellifera</i>	8.75 (3.04)	185.44	2.80	85.25 (68.44)
T ₃ : <i>T. iridipennis</i>	11.2 (3.41)	254.82	3.09	89.75 (71.37)
T ₄ : Open pollination	19.1 (4.42)	351.23	3.76	95 (77.36)
T ₅ : No pollination	2.05 (1.59)	43.55	1.75	26 (30.42)
SE(m)±	0.14	3.74	0.04	1.16
CD at 5%	0.42	11.52	0.12	3.48
CV (%)	8.19	3.98	4.28	7.36

*Figures in the parenthesis are arc sine transformed values

Figures in parenthesis are $\sqrt{x+0.5}$ transformed values

In a column, means followed by same alphabet do not differ significantly ($p=0.5$) by DMRT

The results on the effect of different pollination treatments on seed yield and yield attributes of lucerne revealed significant differences (Table 3). Among the treatments, open pollination (T₄) recorded the highest seed yield (351.23 kg/ha), number of pods per raceme (19.1), 1000-seed weight (3.76 g) and germination (95%). This was followed by *A. cerana* pollination (T₁), which produced 297.6 kg/ha seed yield, 16.45 pods per raceme, 3.58 g test weight and 93.5% germination. *T. iridipennis* pollination (T₃) also showed moderate improvement over the control with 254.82 kg/ha seed yield, 11.2 pods per raceme, 3.09 g

test weight and 89.75% germination. In contrast, *A. mellifera* pollination (T₂) was relatively less effective, recording only 185.44 kg/ha seed yield, 8.75 pods per raceme, 2.80 g weight for 1000 seeds and 85.25% germination. The least performance was observed in the pollination-excluded treatment (T₅), which resulted in only 43.55 kg/ha seed yield, 2.05 pods per raceme, 1.75 g test weight and 26% germination, clearly confirming the indispensability of insect pollination for lucerne seed production.



Apis dorsata



Apis mellifera



Trigona iridipennis



Lasioglossum coriaceum



Ceratina binghami



Amegilla spp



Megachile rotundata



Fig 1: List of comon flowers visitors in lucerne blooms

Cost economics (ICBR and BC)

Cost economics of each treatment was analysed based on net return, ICBR and B:C ratio. Among the different pollination treatments, the open pollinated plot had obtained maximum net return (Rs. 3,26,906) with highest B:C ratio of (4.46), establishing itself as the most effective treatment and economically viable in the lucerne seed production (Table 7). It was followed by the *A. cerana* pollinated plot which recorded net return of Rs. 3,04,860/ha with ICBR (1:16.08) and B:C ratio of (3.15). The next economically moderately beneficial treatment was pollination with *T. iridipennis* which had given net return of Rs. 2,53,524/ha with ICBR (1:14.10) and B:C ratio of (2.72). *A. mellifera* pollination treatment was economically less beneficial with net return of Rs. 1,70,268/ha with ICBR (1:13.14) and B:C ratio of (2.07) which was lower than other treatments of pollination. No pollination was not economically advantageous which had B:C ratio of 0.55. The superiority of open pollination and *A. cerana* pollination treatments corroborates earlier

findings that insect pollinators are crucial for successful pod set and higher seed yields in lucerne. Gregor Similarly, Bieberdorf (1949)^[7] and Rincker *et al.* (1988)^[43] reported that tripping and insect-mediated cross-pollination are prerequisites for seed set in lucerne, which is consistent with the present findings. Jevtic and Radovic (2001)^[29] reported that in the plot without honey bees (*Apis mellifera carnica*) 23.3 kg ha⁻¹ and in cages with bees 158.9-198 kg ha⁻¹ and in open pollination 304 kg ha⁻¹ of seed yield were obtained. Cecen *et al.* (2008)^[17, 18], using isolated cages, found that the bumblebee (*Bombus terrestris* L.) is more successful pollinator of alfalfa compared to the honey bee. In cages with bumblebees, the average seed yield was 56.48 kg ha⁻¹, while in cages with honey bees, 49.2 kg ha⁻¹ and with free pollination 66.19 kg ha⁻¹ was obtained..

The higher seed yield under open pollination and *A. cerana* also confirms the reports of Vansell and todd (1946)^[51] and Olmstead and Wooten (1987)^[36], who documented that seed yields were greatly enhanced with honey bee activity and

drastically reduced in pollination-excluded plots. Comparable observations were also made by Gachchinamath (1983)^[24] and Hosamani *et al.* (2020)^[28], who highlighted the role of *A. cerana* in significantly increasing lucerne and onion seed yield, seed weight and germination. The present study further aligns with Pushpalata (2024), who found that *A. cerana* pollinated plots outperformed *A. mellifera* and *T. iridipennis* in terms of yield and seed quality parameters in onion. Findings of Singh and Dhamwal (1970), Benedek and Gall (1972)^[6] and Pudasaini *et al.* (2014)^[39], demonstrates that insect pollination not only increases seed yield but also improves seed viability and vigor. Likewise, Pashte *et al.* (2015) emphasized that *A. cerana* pollination enhances seed quality traits such as test weight and germination percentage in sesamum and other crops, which parallels the present findings. These findings reinforce earlier reports (Cane, 2002; Divija and Jayanthi, 2022)^[16, 22] that lucerne is a strictly cross-pollinated crop requiring active bee visitation and that bee pollination enhances both quantitative (yield) and qualitative (seed quality, germination) aspects of production.

Previously, Kulkarni and Kumar (2021)^[32] reported that open pollination along with solitary bees produced significantly more seeds as compared to honeybees and pollination-exclusion flowers. Similarly, Breazeale *et al.* (2008)^[11], Brunet *et al.* (2019)^[12] and Pitts-Singer *et al.* (2011)^[38] concluded worldwide that higher abundance of Megachile bees has been linked with enhanced alfalfa seed production due to their superior pollination efficiency.

Many authors claim that the honey bee is not very successful in the pollination of alfalfa (Benedek, 1976; Holm, 1984)^[6], primarily due to the anatomy of the flower. In addition, it was found that many other bee species are much more efficient pollinators of this important forage crops (Richards, 1984; Cane, 2002, Stanisavljević *et al.*, 2003, etc.)^[16, 42, 46]. However, those bee species have their specific drawbacks as they are highly dependent on meteorological conditions and anthropogenic factors. In addition, alfalfa is grown on large areas where there are no natural habitats for spontaneous pollinators. From the above stated, it can be concluded that the pollinating of alfalfa cannot be left to only one species, i.e. spontaneous pollinators, because of their low abundance and frequency and high dependence on various factors.

4. Conclusion

The studies on species diversity indicated that pollinator faunas of lucerne composed of 905 individuals of 27 different species which belonged to 22 genera, 12 families and 3 orders. The flower visitors belonged to the order hymenoptera (79.52%), lepidoptera (11.09%) and diptera (9.39%). Among five pollination treatment, best treatment was open pollination that gave highest seed yield (351.23 kg/ha) with highest germination percentage and seed weight which was at par with *A. cerana* followed by pollination with *T. iridipennis*. *A. mellifera* yielded low seed with less germination percent than other honey bee pollination. However no pollination gave negligible seed yield indicating self-pollination in lucerne is not economically beneficial.

Hence this study revealed Alfalfa, as practically only xenogame species in which pollinators play an important role in increasing the yield of lucerne. The honey bee is not

as effective in pollination of alfalfa as are some solitary bees, but its advantage is in the number of colonies and the ability to manipulate them easily. In the pollination of alfalfa significantly greater importance is of pollen forager bees compared to the nectar foragers. Results of open pollinated field suggests adequate pollination by diversity of flowers visitors viz. honey bees, wild bees, syrphid flies, butterflies is needed for a significant increase in the seed yield with enhanced germination.

References

1. Aganga G. Lucerne, Lablab and *Leucaena leucocephala* Forages: Production and Utilization for Livestock Production. Pak. J. Nutrition. 2003;2(2):46-53.
2. Aguiar WM, Melo GAR, Gaglianone MC. Does Forest phisiognomy affect the structure of orchid bee (*Hymenoptera, Apidae, Euglossini*) communities? A study in the Atlantic forest of Rio de Janeiro state, Brazil. Sociobiology. 2014;61:68-77.
3. Akila S, Babu PCS. Release of different dose of *Trichogramma* and its effect on internode borer, yield and quality of sugarcane. Sugarcane (London). 1994;2:22-23.
4. Anonymous. International rules for seed testing by international seed testing association (ISTA). Seed Science and Technology. 2014;27:215.
5. Banerjee GC. Principles of animal nutrition and feeding practices. Chpt., Animal Feed Stuffs. 1978:1-20.
6. Benedek P, Gall E. The effect of insect pollination on seed onion with observation in the behaviour of honey bees crop. J. Apic. Res. 1972;11:175-180.
7. Bieberdorf GA. Some observations on pollination of alfalfa hay. Academy of science for proceeding of the Oklahoma. 1949:49-51.
8. Bohart GE, Knowlton GF. Managing alkali bees for alfalfa pollination. Logan, Utah State Univ. Extension Services Leaflet. 1960;78:1-8.
9. Bohart GE. Management of wild bees for the pollination of crops. Annu. Rev. Entomol. 1962;17:287-312.
10. Bosch J, Kemp WP. Alfalfa leafcutting bee population dynamics, flower availability and pollination rates in two Oregon alfalfa fields. Journal of Economic Entomology. 2005;98(4):1077-1086.
11. Breazeale D, Fernandez G, Narayanan R. Modeling pollination factors that influence alfalfa seed yield in north-central Nevada. J. Cent. Eur. Agric. 2008;9:107-116.
12. Brunet J, Zhao Y, Clayton MK. Linking the foraging behaviour of three bee species to pollen dispersal and gene flow. Plos One. 2019;14:e0212561.
13. Brunet J, Stewart CM. Impact of Bee Species and Plant Density on Alfalfa Pollination and Potential for Gene Flow. Hindawi Publishing Corporation, Psyche. 2011;Volume 2010(Article ID 201858):7 pages.
14. Burezaq H. Reproductive ecology of forage alfalfa (*Medicago sativa* L.): Recent advances. Plant Reprod. Ecol. 2021;579:100-640.
15. Butler George D, Todd Frank E, Tuttle Donald M. Alfalfa pollination in Arizona. A. Progress Report. Agr. Expt. Sta., University of Arizona (Tucson, AZ). 1956.

16. Cane JH. Pollinating bees (*Hymenoptera: Apiformes*) of U.S. alfalfa compared for rates of pod and seed set. *Journal of Economic Entomology*. 2002;1:22-27.
17. Cecen S, Gurel F, Karaca A. Impact of honeybee and bumblebee pollination on alfalfa seed yield. *Acta Agriculturae Scandinavica, Section B - Soil & Plant Science*. 2008;58(1):77-81.
18. Cecen S. Impact of honeybee and bumblebee pollination on alfalfa seed yield. *Acta Agriculturae Scandinavica, Section B - Soil & Plant Science*. 2008;58(1):77-81.
19. Chavhan VT, Shashidhar V. Pollinator fauna of lucerne and their relative abundance in lucerne at Dharwad, Karnataka. *Karnataka Journal of Agricultural Sciences*. 2015;28(1):112-113.
20. Ciurdarescu G. Pollination as an important factor for the yield alfalfa crops in Romania. Organizing committee XII international grassland congress. Sectional Papers-plant introduction, Breeding and seed production - Moscow. 1974:60-69.
21. Cogliastro Alain, Gagnon Daniel, Coderre Daniel, Bheureur Pierre. Response of seven hardwood tree species to herbicide, rototilling and legume cover at two southern Quebec plantation sites. *Canadian Journal of Forestry*. 1990;20:1172-1182.
22. Divija SD, Jayanthi KPD. Pollination efficiency and foraging behaviour of honey bees and flies to onion *Allium cepa* L. *Journal of Apicultural Research*. 2022;61(5):688-694.
23. Free JB. *Insect Pollination of Crops*. 2nd ed. London Academic Press; 1970:544.
24. Gachchinamath JK. Study on the bee-flora of the Indian bee, *Apis cerana* Fabricius (*Hymenoptera: Apidae*) and its role in pollination of lucerne, *Medicago sativa* Linn [M.Sc. Thesis (Agri)]. Uni. Agri. Sci., Dharwad (India); 1983.
25. Gurr L. A note on the efficiency of *Bombus terrestris* (L.) as a pollinator of lucerne (*Medicago sativa* L.). *N.Z. J. Sci. Tech*. 1955;37A:300.
26. Hadfield JW, Calder RA. Lucerne (*Medicago sativa*) investigations relative to pollination and seed production in New Zealand. *N.Z. J. Sci. Tech*. 1936;17:577-594.
27. Holm SN. Introduction and propagation of the leafcutter bee (*M. rotundata*) in Denmark. *Proc. int. Sym. on Poll.*, 27-30 IX 1983. Versailles, Tasei, Jnied. 1984:455-460.
28. Hosamani V, Venkateshalu, Jagadeesha N, Reddy MS, Gangadarappa PM, Ravikumar B. Effect of Bee Pollination and Attractants on Quantitative and Qualitative Parameters of Onion (*Allium cepa*) Seed Yield and Quality. *Int. J. Curr. Microbiol. App. Sci*. 2020;9(2):1885-1892.
29. Jevtic G, Radovic J. The importance of honey bee (*Apis mellifera carnica*) in pollination and seed yield of alfalfa (*Medicago sativa* L.). Ministry of Education, Science and Technology Development of the Republic of Serbia. 2001:11-24.
30. Johnson DA, Rumbaugh MD. Nodulation and acetylene reduction by certain rangeland legume species under field conditions. *J. Rang. Mgmt*. 1981;34(3):178-181.
31. Jones P, Forster W. Observations on the pollination of lucerne (*Medicago sativa* Linn.) *N. Z.J. Agric. Res*. 2012;8(2):340-349.
32. Kulkarni Narendra S, Kumar Vinod. Studies on Insect Fauna of Lucerne (*Medicago sativa*) and Their Impact on Seed and Forage Yield in Lucerne *Medicago sativa*. IGC Proceedings (1989-2023). 2020. Available from: <https://uknowledge.uky.edu/igc/23/2-2-1/>
33. Kumar S, Keerthi MC, Mahesha HS, Arunkumara CG, Shivakumara KT, Saini RP, Yadav VK. Foraging behaviour and pollinator dynamics in lucerne crop of the Bundelkhand region: Implications for crop management in the subtropics. *Animal Biology*. 2024;74(4):401-413.
34. Lesins K. Investigations into seed setting of lucerne at ultuna Sweden. *Ann. Roy. Agr. Col. (Uppsala: Sweden)*. 1950;17:441-483.
35. Mc-Gregor SE. Alfalfa in: *Insect pollination of cultivated crop plans*. Agricultural Handbook, US Department of Agriculture No. 496. 1976:64-75.
36. Olmstead AL, Wooten DB. Bee pollination and productivity growth: The case of alfalfa. *American Journal of Agricultural Economics*. 1987;69:56-63.
37. Pedersen MW, Petersen HL, Bohart GE, Levin MD. A comparison of the effect of complete and partial cross-pollination of alfalfa on pod set, seeds per pod and seed weight. *Agron. Jour*. 1956;48:177-180.
38. Pitts-Singer TL, Cane JH. Bee pollination of lucerne: problems and solutions. 2011.
39. Pudasaini RB, Thapa VR, Poudel PR. Effect of Pollination on qualitative characteristics of rapeseed in Chitwan, Nepal. *International Journal of Biological, Biomolecular, Agriculnursl. Food and Biotechnological Engineering*. 2014;8(12):65-68.
40. Pushpalatha M. Studies on species diversity of pollinators and foraging ecology of major pollinator species associated with onion flowers (*Allium cepa* L.) [Ph.D. Thesis]. Univ. Agric. Sci., Rahuri (India); 2024.
41. Rauf A, Saeed S, Ali M, Nadeem Tahir MH. Comparative efficiency of native insect pollinators in reproductive performance of *Medicago sativa* L. in Pakistan. *Insects*. 2021;12(11):1029.
42. Richards KW. Alfalfa leafcutter bee management in Western Canada. Publication 1495/E Agricultural Canada: Ottawa, Canada. 1984:53.
43. Rincker CM. Alfalfa seed production in the Pacific Northwest. In: *Proceedings Annual Farm Seed Conference*. Am. Seed Trade Association, Kansas City, Mo. 25 November. 1988:13-19.
44. Singh JP, Dharamwal SS. The role of honey bees in seed setting on onion at Pant Nagar, Dist. Nainital, Uttar Pradesh, India. *Indian Bee Journal*. 1970;32(1/2):23-27.
45. Somerville D. Lucerne pollination (Agfact DAI-134). New South Wales Department of Primary Industries. 2002.
46. Stanisavljević Lj, Krunić M, Brajković M, Jevtić G. Značaj medonosne i drugih vrsta pčela za oprašivanje gajenih biljaka. XI Naučno savetovanje sa međunarodnim učešćem, 6-9. februar, Poljoprivredni fakultet, Zemun, Zbornik radova. 2003:82-88.
47. Stjepanović M. Utjecaj ekoloških faktora na osjemenjavanje nekih sorata lucerne na području Istočne Slavonije [Doktorska disertacija]. Poljoprivredni fakultet Osijek; 1981.

48. Tucker KW. Honey bee pollinators of alfalfa in Minnesota. 15th Alfalfa Imp. Conf. Rpt. St. Paul, Minn. 1956:32-33.
49. Tysdal HM. Is Tripping Necessary For Seed Setting In Alfalfa? Amer. Soc. Agron. Jour. 38:515-535.
50. Umaerus M, Akerberg E. Pollination and seed setting in red clover and lucerne under Scandinavian conditions. Herb. Abstr. 1959;29:157-164.
51. Vansell GH, Todd FE. Alfalfa tripping by insects. Amer. Soc Agron. Jour. 1946;38:470-478.
52. Vladica V. How to calculate incremental cost benefit ratio? ResearchGate. 2018. Available from: <https://www.researchgate.net/post/How-to-calculate-incremental-costbenefit-ratio/5bb2b2bfeb038993023a07db/citation/download>
53. Wojtowski. Zastosowanie blonkowek pszolowatych rodzaju *Bombus* latr. (*Hymenoptera: Apoidea*) do zapylania planteji nasiennych rostin motylkowych. Roozn. Wyz. Szk. Roln. Poznen. 1965;24:223-274.