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Investigating the impact of supplementary pollination methods on seed production and yield in Lucerne (*Medicago sativa* L.)

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Abstract

A study was carried out at the Indian Grassland and Fodder Research Institute, Southern Regional Research Station and at UAS Campus, Dharwad, over two consecutive rabi-summer seasons. The aim was to investigate the impact of supplementary pollination techniques on the seed setting and yield of lucerne (*Medicago sativa* L.) Cv. RL-88. The experiment was set up using a randomized block design with three replications, comprising seven different treatments. Analysis of data collected over the two years indicated that T₆ (which involved the application of insect attractants during various flowering periods) resulted in a significantly higher total number of pods per raceme (14.62), number of filled pods per raceme (9.47), total number of pods per plant (291.34), number of filled pods per plant (172.67), number of seeds per pod (7.34), 1000 seed weight (3.29 g), pod set (62.10 %), seed set (63.45 %), seed yield per plant (3.77 g), seed yield per plot (358.27 g), seed yield per hectare (299.51 kg). The study reveals that effective pollination management significantly influences seed setting and seed quality in crop production. Notably, spraying insect attractants during flowering leads to higher seed setting and yield compared to other methods. This underscores the vital role of insect pollinators in ensuring successful fertilization and seed development. By attracting more pollinators to the crops, these attractants improve pollination efficiency, resulting in increased seed setting rates and ultimately greater seed yield. The findings emphasize the importance of employing efficient pollination strategies, like insect attractants, to enhance seed setting and overall productivity in agricultural systems.

Keywords: Lucerne, supplementary pollination, seed setting, seed yield, insect attractants

Introduction

Lucerne, also known as alfalfa and popularly regarded as the "queen of forage crops" in India, covers the third-largest area (11.35%) after sorghum and berseem. Despite its high palatability and nutritional value, with a protein content of 15-20%, digestibility of 60-70%, and a dry matter intake of 4.28 kg per 100 kg body weight (Pathak and Jakhmola, 1983) ^[13], its cultivation area has not seen significant growth. This lack of expansion is likely attributed to the insufficient availability of high-quality seeds.

There is a significant demand for lucerne seed among government departments, resource-rich farmers, and private dairy owners in peninsular states. This demand underscores the need to focus research efforts on factors influencing seed yields. Genetic and environmental factors, along with the presence of pollinators, play crucial roles in determining lucerne seed yields.

Pollination in *Medicago* species varies greatly, spanning from complete self-sterility to very high self-fertility. For commercial cultivars, cross-pollination occurs in more than 80% of cases (Free, 1993) ^[5], with tripping being essential to facilitate this process and subsequent seed production in lucerne (McGregor, 1976; Rincker *et al.*, 1988) ^[10, 15]. Bees, primarily foraging for nectar or pollen, are the main agents responsible for floret tripping.

Pollinators, such as bees and other insects, play a crucial role in boosting the seed yield of lucerne. Lucerne plants offer both pollen and nectar to these pollinators, attracting them to the flowers. In return, these pollinators facilitate the pollination process, transferring pollen between flowers. This is particularly important for lucerne because it is a cross-pollinated crop, meaning that pollen from one plant must reach the stigma of another plant for successful fertilization and

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seed production. Adequate pollination is essential for achieving a substantial increase in the seed yield of lucerne. Without effective pollination, the formation of seeds would be limited, leading to reduced yields. Therefore, the presence and activity of pollinators are vital for maximizing the productivity of lucerne crops.

The findings of the present study are intriguing considering that the lucerne flower requires tripping prior to pollination. Thus, it was anticipated that non-Apis bees would be the primary pollinators. Similarly, Cecen (2008) [2] and Jones and Forster (2012) [4] identified honey bees as the dominant and efficient pollinators of lucerne. In contrast, Jevtic and Radovic (2001) [8] reported that honey bees constituted only 1.1 percent of the pollinator population. These discrepancies suggest that the composition of lucerne pollinator fauna is largely influenced by the density and distribution of different pollinator species in a given area.

Fruit boost demonstrates greater efficacy in attracting various pollinators compared to other bee attractants. However, it remains unused at the field level due to its status as a test attractant. Instead, sugar solution is preferred over fruit boost. For application on a one-hectare area, the recommended dosage is 5 kg of sugar dissolved in 50 liters of water.

In lucerne cultivation, the production of seeds relies heavily on the efficiency of pollination. However, there is a notable scarcity of research focusing on this crucial aspect. Therefore, the current study was initiated to address this gap and to explore the most effective pollination mechanisms that could lead to enhanced seed setting and ultimately increase the seed yield of lucerne.

Materials and Methods

The experiment was conducted at Indian Grassland and fodder research institute, Regional research station, UAS campus, Dharwad during two consecutive rabi seasons to investigate the effect of supplementary pollination techniques on seed setting and seed yield of lucerne (*Medicago sativa* L.) Cv. RL-88. The field experiment was laid out in randomized block design with three replications consisting of seven treatments. The treatments were imposed when the crop begins the flowering period at different intervals i.e flowering at 25%, 50% and 75% stages respectively.

Treatment consisted of T₁ - Control (No supplementary pollination), T₂ - General beating with sticks, T₃ - Manual tripping of flower, T₄ - Rope pulling, T₅ - Blowing wind with empty dusters, T₆ - Spraying of insects attractants and T₇ - Water jet spraying

Five random competitive plants were selected for recording the observations and the means of five plant observations were used for further statistical analysis. Seed setting and seed yield parameters viz., number of pods per raceme, number of pods per plant, number of filled pods per raceme, number of filled pods per plant, number of seeds per pod, number of seeds per plant, pod set percentage, seed set percentage, 1000 seed weight, seed yield per plant, seed yield per plot and seed yield per hectare were recorded in all the treatments.

- 1. Number of Pods per Raceme and Number of Pods per Plant:** The total number of pods present on randomly selected racemes and individual plants within each treatment plot was counted. Standardized counting methods were employed to ensure accurate data collection.
- 2. Number of Filled Pods per Raceme and Number of Filled Pods per Plant:** The selected racemes and plants were examined, and the number of fully developed pods containing seeds was recorded. Careful counting was

conducted to distinguish filled pods from empty ones.

- 3. Number of Seeds per Pod and Number of Seeds per Plant:** A representative sample of pods from the selected plants was opened, and the number of seeds in each pod was counted. The average number of seeds per pod was calculated, and then multiplied by the total number of pods per plant to determine the number of seeds per plant.
- 4. Calculating Pod Set Percentage and Seed Set Percentage:** The percentage of pods and seeds that successfully developed relative to the total number of flowers or pods produced was determined. The formula (Number of filled pods/seeds ÷ Total number of pods/seeds) x 100 was used for calculation.
- 5. 1000 Seed Weight:** Random samples of seeds from each treatment were weighed, and the number of seeds was counted. The weight of 1000 seeds was calculated by dividing the total weight by the number of seeds and multiplying by 1000.
- 6. Recording Seed Yield per Plant, Seed Yield per Plot, and Seed Yield per Hectare:** All plants within each treatment plot were harvested, and the seeds were threshed and weighed to determine the seed yield per plant and per plot. The seed yield per plot was multiplied by the appropriate conversion factor to calculate the seed yield per hectare.

Statistical analysis

The data obtained from the experiment underwent statistical analysis using a Randomized Complete Block Design (RCBD), following the methods outlined by Sundarajan *et al.* (1972) [16]. Analysis of Variance (ANOVA) was conducted to assess the significance of the results. Critical differences were computed at a significance level of five percent. In the tables, critical difference values were provided for observations that exhibited significance at this level. This rigorous statistical approach ensured robust evaluation of the experimental findings, allowing for reliable interpretation of the results and identification of significant differences among treatments.

Results and Discussion

The study evaluated various seed setting and seed yield parameters across different treatments aimed at enhancing pollination. The treatments included T₁ (Control), T₂ (General beating with sticks), T₃ (Manual tripping of flower), T₄ (Rope pulling), T₅ (Blowing wind with empty dusters), T₆ (Spraying of insect attractants), and T₇ (Water jet spraying). The results demonstrated significant differences in seed setting and yield parameters among these treatments. This suggests that different pollination techniques had varying impacts on the reproductive success of the crop. Parameters such as number of pods per raceme, number of pods per plant, number of filled pods per raceme, number of filled pods per plant, number of seeds per pod, number of seeds per plant, pod set percentage, seed set percentage, 1000 seed weight, seed yield per plant, seed yield per plot, and seed yield per hectare were assessed across all treatments, and the findings indicate that treatment methods significantly influenced these parameters.

Yield parameters were also significantly influenced by supplementary pollination techniques. Spraying of insect attractants i.e. 10 percent jaggery solution (T₆) recorded significantly higher total number of pods per raceme (14.62), number of filled pods per raceme (9.47), total number of pods per plant (291.34), number of filled pods per plant (172.67), number of seeds per pod (7.34), 1000 seed weight (3.29 g), pod

set (62.10 %), seed set (63.45 %), seed yield per plant (3.77 g), seed yield per plot (358.27 g), seed yield per hectare (299.51 kg) (Table 1, 2, 3, 4 and 5). The significant improvement in yield parameters observed with the application of supplementary pollination techniques, specifically the spraying of insect attractants such as 10 percent jaggery solution (T_6), can be attributed to enhanced pollination efficiency. Insect attractants, like jaggery solution, are known to attract a diverse range of pollinators, including bees and other beneficial insects, to the lucerne flowers. This increased pollinator activity leads to more effective cross-pollination, resulting in improved fertilization rates and subsequent seed production. The higher number of pods per raceme and per plant, increased seed set percentage, and greater seed yield per plant, plot, and hectare observed with T_6 treatment indicate the positive impact of enhanced pollination on overall reproductive success and yield of lucerne crops.

While, control-no supplementary pollination treatment (T_1) recorded lowest total number of pods per raceme (12.72), number of filled pods per raceme (5.87), total number of pods per plant (229.34), number of filled pods per plant (114.84), number of seeds per pod (6.52), 1000 seed weight (3.07 g), pod set (53.31 %), seed set (53.42 %), seed yield per plant (2.41 g), seed yield per plot (243.41 g), seed yield per hectare (203.78 kg) (Table 1 to 5). The observed differences in plant reproductive parameters between the control group (no supplementary pollination treatment, T_1) and other treatment groups can likely be attributed to the absence of additional pollination inputs. Without supplementary pollination, the natural pollination process may not be efficient enough to ensure optimal fertilization of flowers, leading to reduced pod formation, seed set, and ultimately lower seed yield per plant and per hectare. This inefficiency may stem from limitations in pollinator activity, incomplete pollen transfer, genetic compatibility issues, unfavorable environmental conditions, and altered resource allocation within the plant. Consequently, the control group exhibited lower total number of pods per raceme, number of filled pods per raceme, total number of pods per plant, number of filled pods per plant, number of seeds per pod, pod set, seed set, seed yield per plant, seed yield per plot, seed yield per hectare, and 1000 seed weight compared to treatment groups receiving supplementary pollination.

The higher number of pods per raceme and increased seeds per pod observed in the study can be largely attributed to the combined effects of cross-pollination and reduced ovule abortion, as elucidated by Cooper and Brink in their 1940 research. Cross-pollination facilitates the transfer of pollen from one plant to another, promoting genetic diversity and potentially enhancing fertilization rates. Moreover, reduced ovule abortion, a process where unfertilized ovules fail to develop into seeds, contributes to higher pod numbers per raceme. Additionally, the greater number of seeds per pod can be linked to improved pollen fertility and higher rates of fertilization, particularly under elevated temperatures, as highlighted by Brinks and Cooper in 1936. Warmer temperatures can enhance the viability of pollen grains and facilitate more successful fertilization events, leading to a higher number of seeds per pod. Overall, the combined influence of cross-pollination, reduced ovule abortion, and improved pollen fertility and fertilization success under favorable temperature conditions explains the observed increase in both pods per raceme and seeds per pod in the study.

The 1000 seed weight found higher in T_6 (3.29 g) and was on par with T_3 and lower seed weight was recorded in T_1 (3.07 g). The observed difference in 1000 seed weight, with higher values in T_6 and lower values in T_1 , can be attributed to variations in

endosperm development among the treatments. Endosperm, a tissue within seeds that provides nutrients to the developing embryo, plays a crucial role in determining seed weight. As suggested by Partap and Partap in 2000, the higher 1000 seed weight in T_6 may be linked to an increased rate of endosperm development in this treatment group. Adequate nutrient availability and favourable environmental conditions in T_6 might have facilitated optimal endosperm formation, resulting in larger and heavier seeds. Conversely, the lower seed weight in T_1 could be due to suboptimal conditions for endosperm development, potentially caused by factors such as nutrient deficiencies or environmental stressors. Overall, differences in endosperm development among the treatments likely contributed to the observed variations in 1000 seed weight.

It was estimated that with the spraying of jaggery solution the increase in the seed set (63.45 %) and seed yield (299.51 kg/ha.) was accounted for 10.03 percentage and 95.73 kg / ha over untreated control (T_1 53.42 % and 203.78 kg/ha respectively). The increase in seed set and seed yield attributed to the spraying of jaggery solution can be explained by its potential effects on enhancing pollination and subsequent reproductive processes in the plants. Jaggery solution, being rich in sugars and other nutrients, can serve as a supplemental food source for pollinators, attracting them to the crop plants and increasing their activity. This increased pollinator activity likely leads to more efficient pollen transfer and higher rates of fertilization, resulting in a higher seed set percentage. Additionally, the sugars present in the jaggery solution may also directly contribute to enhanced seed development and maturation, leading to increased seed yield per hectare. The improvement in both seed set and seed yield over the untreated control (T_1) suggests that the application of jaggery solution positively influences various stages of the reproductive process in the plants, ultimately leading to higher seed production.

The beneficial effect of applying a bee attractant in improving the number of pods per plant can be elucidated by its ability to enhance pollination efficiency. Bee attractants, by attracting more pollinators to the crop plants, increase the frequency of flower visitation and subsequent pollen transfer between flowers. This increased pollinator activity results in more effective pollination, leading to the retention of a higher proportion of flowers and consequently, the production of more pods per plant. The retention of flowers due to optimum pollination ensures that a greater number of flowers successfully develop into pods, thereby increasing the overall pod count. The reported enhancement of pod number by 61.38% due to pollination in other leguminous crops, as mentioned improved pollination can significantly augment pod production in plant populations. Overall, the application of bee attractant facilitates optimal pollination, which in turn increases the number of pods per plant through enhanced flower retention and successful pod development.

The observed beneficial effects of using bee attractants on both the number of seeds per pod and seed weight have been documented across various crops, as reported by several studies. Guruprasad and Viraktamath (2003) ^[6] observed similar improvements in niger, while reported positive outcomes in sunflower, and Jayaramappa *et al.* (2011) ^[7] documented these effects in the ridge gourd crop. These enhancements in seed-related parameters can be attributed to the increased efficiency of pollination facilitated by bee attractants. By attracting more pollinators to the flowering plants, bee attractants enhance the frequency and effectiveness of pollen transfer between flowers, leading to a higher rate of successful fertilization and subsequent seed development. Additionally, the application of indigenous bee attractants has also been linked to improvements in seed

weight in other oilseed crops like sunflower, as noted by Manjunath (2003) ^[9] and as well as in mustard, as reported by Murasing and Viraktmath (2002) ^[11]. This enhancement in seed weight could be a result of improved pollination leading to better seed development and maturation, ultimately resulting in larger and heavier seeds. Overall, the consistent positive outcomes observed across various crops highlight the significant role of bee attractants in enhancing pollination efficiency and subsequently improving seed-related parameters in diverse agricultural settings. The increase in yield observed in the crop treated with insect attractants can be attributed to the synergistic impact of several factors. Firstly, the higher number of pods per plant, facilitated by improved pollination efficiency due to increased insect activity, contributes to a greater potential for seed production. Secondly, the enhanced number of seeds per pod, likely a result of more effective fertilization processes, further amplifies seed yield potential. Additionally, the heavier seeds produced in the treated crop indicate improved seed

quality, which can translate to higher overall yield. These findings align with those of Jevtic and Radovic (2001) ^[8], who noted increased seed yield in lucerne following the application of sugar syrup, suggesting that supplemental nutrient sources can enhance pollination and subsequent seed production.

The endorsement of these results by previous studies further reinforces the significance of insect pollination in enhancing crop yield. This collective body of evidence underscores the pivotal role of insect pollinators, such as honey bees, in optimizing reproductive processes and ultimately enhancing crop productivity. By facilitating efficient pollen transfer and promoting favourable conditions for seed development, insect attractants contribute to increased yield potential, highlighting the importance of considering pollination management strategies in agricultural practices aimed at maximizing crop output (Pedersen *et al.* 1956) ^[14].

Table 1: Effect of supplementary pollination techniques on number of pods per raceme and number of filled pods per raceme of lucerne Cv.RL-88

Treatments	Total number of pods per raceme			No. of filled pods per raceme		
	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
T ₁	12.93	12.50	12.72	5.97	5.77	5.87
T ₂	14.00	13.50	13.75	7.54	7.20	7.37
T ₃	14.60	13.90	14.25	8.57	8.07	8.32
T ₄	14.46	13.90	14.18	7.07	6.84	6.96
T ₅	12.83	12.66	12.75	6.07	5.84	5.96
T ₆	14.83	14.40	14.62	9.70	9.24	9.47
T ₇	13.06	12.80	12.93	6.04	5.87	5.96
Mean	13.82	13.38	13.60	7.28	6.98	7.13
SEm±	0.32	0.28	0.30	0.54	0.50	0.52
CD@5%	1.01	0.86	0.93	1.68	1.54	1.61

Treatments: T₁ - Control (No supplementary pollination), T₂ - Gentle beating with sticks, T₃ - Manual tripping of flower, T₄ - Rope pulling, T₅ - Blowing wind with empty dusters, T₆ - Spraying of insects attractants (10 % Jaggery solution) and T₇ - Water jet spraying

Table 2: Effect of supplementary pollination techniques on total number of pods per plant and number of filled pods per plant of lucerne Cv.RL-88

Treatments	Total Number of pods per plant			Number of filled pods per plant		
	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
T ₁	226.67	232.00	229.34	119.34	110.34	114.84
T ₂	253.34	258.67	256.01	133.00	122.01	127.51
T ₃	276.34	281.67	279.01	155.34	142.01	148.68
T ₄	239.67	245.00	242.34	127.34	114.01	120.68
T ₅	231.00	236.33	233.67	125.00	111.67	118.34
T ₆	288.67	294.00	291.34	180.00	165.34	172.67
T ₇	246.67	252.00	249.34	128.67	117.01	122.84
Mean	251.77	257.10	254.43	138.38	126.06	132.22
SEm±	8.71	8.73	8.72	8.17	7.71	7.94
CD@5%	26.97	27.07	27.05	25.34	23.89	24.60

Treatments: T₁ - Control (No supplementary pollination), T₂ - Gentle beating with sticks, T₃ - Manual tripping of flower, T₄ - Rope pulling, T₅ - Blowing wind with empty dusters, T₆ - Spraying of insects attractants (10 % Jaggery solution) and T₇ - Water jet spraying

Table 3: Effect of supplementary pollination techniques on number of seeds per pod and 1000 seed weight (g) of lucerne Cv.RL-88

Treatments	Number of seeds per pod			1000 seed weight (g)		
	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
T ₁	6.50	6.54	6.52	3.04	3.09	3.07
T ₂	6.73	6.64	6.69	3.19	3.24	3.22
T ₃	7.10	7.18	7.14	3.23	3.28	3.26
T ₄	6.53	6.60	6.57	3.18	3.23	3.21
T ₅	6.50	6.57	6.54	3.04	3.09	3.07
T ₆	7.43	7.24	7.34	3.26	3.31	3.29
T ₇	6.60	6.67	6.64	3.12	3.17	3.15
Mean	6.77	6.82	6.79	3.15	3.20	3.18
SEm±	0.14	0.11	0.12	0.03	0.04	0.03
CD@5%	0.42	0.33	0.37	0.09	0.11	0.10

Treatments: T₁ - Control (No supplementary pollination), T₂ - Gentle beating with sticks, T₃ - Manual tripping of flower, T₄ - Rope pulling, T₅ - Blowing wind with empty dusters, T₆ - Spraying of insect's attractants (10 % Jaggery solution) and T₇ - Water jet spraying

Table 4: Effect of supplementary pollination techniques on pod set percentage and seed set percentage of lucerne Cv.RL-88

Treatments	Pod set (%)			Seed set (%)		
	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
T ₁	52.40	54.21	53.31	54.88	51.96	53.42
T ₂	56.00	54.81	55.41	54.71	53.80	54.26
T ₃	60.46	58.73	59.60	58.41	56.33	57.37
T ₄	56.20	55.11	55.66	55.36	53.40	54.38
T ₅	55.40	55.08	55.24	56.41	53.52	54.97
T ₆	62.42	61.78	62.10	64.60	62.29	63.45
T ₇	56.26	55.90	56.08	54.34	52.27	53.31
Mean	57.02	56.52	56.77	56.96	54.80	55.88
SEm±	1.27	1.04	1.14	1.38	1.36	1.36
CD@5%	3.92	3.22	3.53	4.27	4.21	4.22

Treatments: T₁ - Control (No supplementary pollination), T₂ - Gentle beating with sticks, T₃ - Manual tripping of flower, T₄ - Rope pulling, T₅ - Blowing wind with empty dusters, T₆ - Spraying of insect's attractants (10 % Jaggery solution) and T₇ - Water jet spraying

Table 5: Effect of supplementary pollination techniques on seed yield per plant (g), seed yield per plot (g) and seed yield per hectare (kg) of lucerne Cv.RL-88

Treatments	Seed yield (g/plant)			Seed yield (g/plot)			Seed yield (kg/ha)		
	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
T ₁	2.36	2.46	2.41	249.23	237.58	243.41	208.95	198.61	203.78
T ₂	2.56	2.68	2.62	264.38	251.28	257.83	221.58	210.03	215.81
T ₃	3.18	3.29	3.24	327.09	311.95	319.52	273.84	260.59	267.22
T ₄	2.46	2.58	2.52	254.26	246.62	250.44	213.15	206.14	209.65
T ₅	2.37	2.48	2.43	251.48	238.88	245.18	210.83	199.70	205.27
T ₆	3.78	3.75	3.77	371.91	344.63	358.27	311.19	287.82	299.51
T ₇	2.47	2.58	2.53	263.18	245.30	254.24	220.58	205.04	212.81
Mean	2.74	2.83	2.79	283.08	268.03	275.56	237.16	223.99	230.58
SEm±	0.20	0.19	0.20	17.96	16.06	16.99	14.97	13.38	14.16
CD@5%	0.63	0.58	0.60	55.68	49.78	52.68	46.40	41.48	43.90

Treatments: T₁ - Control (No supplementary pollination), T₂ - Gentle beating with sticks, T₃ - Manual tripping of flower, T₄ - Rope pulling, T₅ - Blowing wind with empty dusters, T₆ - Spraying of insect's attractants (10 % Jaggery solution) and T₇ - Water jet spraying

Conclusion

The significant results regarding seed setting and seed quality components under the influence of supplementary pollination techniques highlight the importance of pollination management in crop production. Specifically, the spraying of insect attractants during the flowering period resulted in notably higher seed setting and seed yield compared to alternative treatments. This outcome underscores the pivotal role of insect pollinators in ensuring effective fertilization and subsequent seed development. By attracting more pollinators to the crop plants, insect attractants enhance pollination efficiency, leading to increased seed setting rates. Consequently, the higher seed setting translates into greater seed yield, reflecting the direct impact of pollination management practices on crop productivity. These findings underscore the significance of implementing effective pollination strategies, such as the application of insect attractants, to optimize seed setting and ultimately enhance seed yield in agricultural systems.

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