

Impact of Different Pollinator on Seed Production of Alfalfa (*Medicago Sativa* L.) CV. RL-88

ARVIND KUMAR P.R.

avya1054@gmail.com

CHANNAKESHA, B. C.,

BELAVADI, V.

Tharini K.B.

SIDDARAJU, R.

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Abstract

An experiment was conducted and designed with two objectives firstly to find out the efficiency of insects visiting to flowers of alfalfa and secondly how it's affecting the rate of seed production. The experimental design used for the study was Factorial Randomized Block Design with three replication with gross plot size: (2.1 m x 2.0 m=4.20 m²) during kharif and rabi season. It has been seen that the alfalfa flower was visited by approximately 10 species so, the frequency of pollinator visiting flower was tested after 1 hr interval from 6.00 am till 5.00 pm. Further the impact of different pollinator on time of anthesis, anther dehiscence, pollen viability and stigma receptivity was tested after 2hr of interval from 6.00 am to 6.00 pm. The results found from the data that the *A. dorsata* (Time spent per flower by *A. dorsata* (10.5 ± 7.10 in *kharif* and 19.00 ± 14.15 in *summer*), *A. florea* (Time spent per flower by *A. florea* (8.00 ± 4.37 in *kharif* and 13.08 ± 8.42 in *rabi / summer*) and *Xylocopa* sp. (Time spent per flower by *Xylocopa* sp. 8.41 ± 5.01 in *kharif* and 11.16 ± 5.56 in *summer*). These are the most frequent visitor and efficient pollinator of alfalfa flowers both in *kharif* and *rabi / summer* compared to other Lepidopteran pollinators Five treatments were designed as control plot, hand pollination, pollination by *Aphis dorsata*, *Aphis florea* and *Xylocopa* Spp. The effect of pollination on seed production of alfa-alfa were investigated using Number of pod set, % pod set, Total No. of seeds/ pod, Filled seeds/ pod, % filled seed. The data were collected from two different seasons analysis was carried out by considering major aspects using *spss* software. The results of seed yield indicate that interaction between treatment groups and seasons was significant at 5%. The highest seed yield was observed in flowers visited with *Apis dorsata* (92%), followed by *Xylocopa* sp. and other treatment. The results of this experiment show that *Apis dorsata* is recommended as an alternative pollinator to honeybee and other pollinator for alfalfa in commercial seed production.

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is widely known as lucerne and rightly called as “Queen of Forage”. In India, it occupies an area of one million hectares and provides 60 to 130 t ha⁻¹ of green forage, and seed yield. In Karnataka, it occupies an area of 3121.23 ha which accounts for 0.03% of the net cropped area (2023). It's having nutritional importance as rich in fiber content, good source of Vit-K, other vitamins in traces and minerals. It also has important bioactive components' like saponins, coumarins, flavonoids, phytosterols, phytoestrogen and some amount of alkaloids. The saponin content helpful to reduce the cholesterol content in the body. By considering the nutritional value there is need to focus on alfa-alfa crops improvement.

Botanically, alfalfa belongs to the family Fabaceae. Inflorescence is a peduncle raceme, peduncle much longer than petiole, calyx teeth as long as the tube corolla 6–12 mm long, violet to pale lavender. Flowers are complete, axillary and borne at the top of the plant. Each flower consists of tubular calyx, 5-sepals, which are united, 5-petals and 10-stamens (9 + 1) Pistil is simple; stigma is covered with thin waxy membrane. Fruit falcate or in a loose spiral of 1–4 turns, glabrous to appressed pilose, containing around 8–12 seed, It normally lives four to eight years under better agronomic cultivation practices

depending on variety and climate. The plant has approximately height of up to 1 meter and possesses deep root system (Asaadi et al., 2014).

According to physiology of plant the flower opening is around 6 am and remains up to 10 am. The Anther dehiscence was observed immediately after opening of the flower. The flower visitors belonging to 7 families of 2 orders. Of these, 5 were Hymenopterans (50 per cent) and five species of Lepidoptera (50 per cent). The order Hymenoptera was represented by 2 families including 4 species of Apidae (80 per cent) and one species of Scolidae (10 per cent). Peak foraging activity of all the flower visitors was recorded between 10.00 am and 14.00 pm which decrease by noon. The peak activity of pollinators (*A. dorsata* and *A. florea*) coincides with pollen viability and stigma receptivity was found maximum from 10.00 am to 14.00 pm (Cecen et al., 2007).

It is cross-pollinated crop because of self-incompatibility and self-sterility mechanism. It superficially resembles clover with many clusters. Each cluster contains ten to twenty purple flowers and these flowers are fertilized by means of insects that process popularly called by “tripping mechanism”. Tripping is a phenomenon described by the Hens law (1867), it mean the release of sexual column from the keel petal when the bees are lands on flower and probe for nectar. Upon release, the column strikes the standard petal, sometimes striking the underside of the head of the bees first, at times with such a force that the bee can extricate its head. When the flower is tripped the pollens were dusted upon the bee and is then carried to another flower. At the same time, pollen brought from another flower is accidentally rubbed upon the stigma and thus cross pollination occurs. Cross-pollination increases the percentage of flowers forming seed pods, the number of seeds per pod and the size of seeds (Delephane & Mayer, 2000).

Seed production in alfalfa requires high expertise, timeliness and conducive climate conditions. A commercial variety of alfalfa is self-sterile and requires insect pollination to facilitate tripping mechanism. Seed production requires the presence of various insect pollinators when the fields of alfalfa are in full bloom. Due to this, seed yield of alfalfa varies from 2.2 to 4.0 q ha⁻¹. The high plant density of flowers influence the activity of wild pollinators, particularly bumble bees and large solitary bees that are physically capable of tripping the flower. Among the different pollinators recommended in forage crops, alfalfa leaf cutter bee is world's most effectively used and intensely managed solitary bee because of their gregarious nature, presence of trichomes at lateral surface of the abdomen which facilitate the pollen dispersal in turn results in effective pollination (Theresa *et al.*, 2011). Rapid industrialization and urbanization, intensification of agricultural land, environmental pollution and intensive chemical usage have been destroying beneficial pollinating insects in the environment. This has made it necessary to sustain natural pollinators and utilize commercial bee colonies as pollinators for seed, vegetable and fruit production in many field and horticultural crops. One of the major constraints encountered in seed production is the lack of technology to carry over the seeds until the next planting season of the several factors which affect the yield & seed quality. In commercial forage seed production, human intervention may be necessary to innovative crop management technique as well the proper synchrony on pollinator's activity when crop is at full bloom (Diriba et al., 2014).

The present investigation is focused on finding the frequency of different types of pollinator visiting to alfalfa and their impact on seed production. In the present article we compare the potential impact of different pollinators on seed set in alfalfa in two different seasons, i.e. rabi and kharif by considering the yield related trait.

MATERIALS AND METHODS

Materials and site

An experiment was conducted under irrigated condition during the *kharif* and *rabi*, at E-6 block of the Department of Seed Science and Technology, University of Agricultural Sciences, GKVK, Bengaluru. The experimental plot soil is of red sandy clay loam with slightly acidic (pH 6.26) and the electrical conductivity was normal (0.12 m mhos/cm at 25 °C). The nitrogen (240 kg ha⁻¹) was low, whereas the phosphorus was high (62.83 kg ha⁻¹) and the potash was medium (190.83 kg ha⁻¹). During the previous season of *kharif*, the site was grown with red gram crop for seed purposes with the recommended package of practices and, in the previous season of *rabi*, sunflower was grown for seed purposes with normal agronomic practices. The climate in the research region is typically Mediterranean, with dry and hot summers, and mild and rainy winters. The experimental material; used in the study was alfalfa (*Medicago sativa* L.) cv. RL-88 procured from Indian Grass land And Fodder Research Institute (IGFRI), Southern Regional Research Station, Dharwad (Karnataka)

Experimental design and Treatment

The Field experiment is laid out in Factorial Randomized Block Design with three replication with gross plot size: 2.1 m x 2.0 m=4.20 m². The seeds of alfalfa were sown @ 5 kg per hectare, with the line spaced 30 cm apart and 10 cm between the plant-to-plant seeds were sown at a shallow depth of 2-3 cm. After sowing light irrigation was given to maintain the optimum moisture for uniform seed germination, and Common agronomic practices were followed for maintaining the crops. The five treatments were considered as control plot (T₁) (Caged plots), hand pollination (T₂), pollination by *Aphis dorsata* (T₃), *Aphis florea* (T₄) and *Xylocopa Spp* (T₅).

The following observations were made

Visual counting was employed to catch per trap the insects visiting flowers of alfalfa. The collected per trapped insects were then identified for proper record. Number of flowers visited per unit time was calculated by following individual marked bee (Most frequent visitors, n=30 for each species) from the time it entered the field till the time it escaped from vision or left the field and the number of flowers visited per trip was recorded by following a particular marked bee (Most frequent visitors, n=30 for each species) from the time it entered the field till the time it left the field. Detailed observations were recorded on the floral biology of Alfalfa viz., days taken for time of anthesis, time of anther dehiscence, time of pollen viability, time of stigma receptivity using standard procedure. After that the time of activity

and relative abundance of *Apis dorsata*, *Apis florea* and *Xylocopa sp.* was estimated using standard procedure. Following observations of five treatments were recorded and further analyzed statically.

Number of pod set: The total numbers of pods (both filled and chaffy) present in each plant of 25 randomly tagged plants were counted. The average was calculated and expressed as total number of pods per plant.

% pod set: Twenty racemes were chosen from each plot in each flowering period and flowers were counted on them. At the end of the podding period, pod numbers were counted on these chosen racemes and divided by flower numbers and then multiplied by 100 for podding rate.

Total No. of seeds/ pod: Twenty pods were randomly selected from each plot, then total number of seed number was counted in each pod and finally average seed numbers were determined.

Filled seeds/ pod: Number of filled seeds per panicle was counted manually from 10 randomly selected panicles and mean number of filled seeds per panicle was determined.

% filled seed. The proportion of filled seeds to the total seeds per panicle was recorded and expressed as per cent, by using the formula

$$\text{Seed setting (\%)} = \frac{\text{Number of filled seeds}}{\text{Total number of seeds (Both filled and unfilled)}} \times 100$$

Statistical analysis: Data were statistically analyzed by ANOVA using the *spss* software analysis.

RESULTS AND DISCUSSION

Pollinators are essential in many plant species which are cross pollinated, in which pollen from another plant is necessary for seed set and they may increase seed yield or quality even in self-compatible forms (Free and Williams, 1976). Alfalfa plants started flowering 30 to 35 days after sowing and blooming period varied from 55 to 60 days. Purseglove (1968) observed that flowers of pigeonpea opened between 1 am and 3 pm and that the flowers remained open for pollinator visitation for about six hours. In present study, it was found that alfa-alfa plant starts anthesis commenced around 06.00 am and continued till 10.00 am. The Anther dehiscence commenced immediately after opening of the flower mentioned in Table 3 & Table 4. The difference in time of flower opening and longevity of flowers could be due to the variation in the climatic conditions where the two studies were conducted. The flowers opened fully when a larger or heavier flower visitor sat on the petals.

Flowers of alfalfa visited by total of 10 species of flower visitors belonging to 7 families of 2 orders of these, 5 were Hymenopterans (50 per cent) and five species of Lepidoptera (50 per cent). The order Hymenoptera was represented by 2 families including 4 species of Apidae (80 per cent) and one species of Scolidae (10 per cent). In the present study the peak foraging activity of all the flower visitors was

seen between 10.00 am and 14.00 pm which reduce by noon. The different flower visitors noticed during the crop growth period. The peak activity of pollinators *A. dorsata* and *A. florea* coincides with pollen viability and stigma receptivity was found maximum from 10.00 am to 14.00 pm. Similar observations were made by Ahmad and Aslam (2002) and Dobromilska (1997) and they stated that, for all vegetable crops the peak foraging activity was observed early in the morning from 9-11 am which largely decreased their after probably due to the hot weather in May-June. Other researchers also found that the peak foraging time of pollinators is in morning during hot weather.

According to the observations *Apis dorsata* (Time spent per flower by *Apis dorsata* (10.5 ± 7.10 in *kharif* and 19.00 ± 14.15 in *summer*), *Apis florea* (Time spent per flower by *Apis florea* (8.00 ± 4.37 in *kharif* and 13.08 ± 8.42 in *rabi / summer*) and *Xylocopa* sp. (Time spent per flower by *Xylocopa* sp. 8.41 ± 5.01 in *kharif* and 11.16 ± 5.56 in *summer*) was found to be most frequent visitor and efficient pollinator of alfalfa flowers in both *Kharif* and *summer* compared to other Lepidopteran pollinators (table 1 & 2). Similar observations were made by Gemmil-Herren and Ochieng (2008) and they reported that *Xylocopa* sp. as effective pollinators of vegetable crops on the basis of floral visits and fruit set. It has been also suggested that the presence of wild habitat near the cultivated land increases the pollination chances of crop by wild bees.

When we analyzed the result of five treatment it was found that the different pollination services studied the highest pod set per cent was recorded in T₅: *Xylocopa* sp. (53.33 and 66.67), which closely on par with T₃: *Apis dorsata* (36.67 and 50.00). Similarly, the seed set per cent also differed significantly among the pollination service viz., the highest seed set per cent recorded in T₃: *Apis dorsata* (92.56), followed by T₅: *Xylocopa* sp. (87.77), the lowest recorded in T₄: *Apis florea* (71.43 and control plots (T₁) (72.00) during the *kharif* season (table 5). The *rabi / summer* season sown crop the seed set percentage found considerably higher in compared to *kharif* viz., T₅: *Xylocopa* sp. (81.25), followed by in T₃: *Apis dorsata* (74.57), T₄: *Apis florea* (70.21). Whereas, the lowest recorded in control plots (66.00) depicted in table 6. The results find support from the findings of Vipin Krishna (2002) in alfalfa, Sharma and Singh (2003) in clover, Sridhar *et al.* (2004), Sreedhara *et al.* (2012) and Bekele Mechalu (2013) in alfalfa. Knapp and Teuber (1994) observed that flowers were easier to trip when the humidity was low coupled with high soil moisture. This might have increased the pollen tube length and “auto-tripping of flowers” consequently led to increase in all the yield attributes. Hence, the temperature, bright sunshine in the absence of pollinating insects also set seed well without insect visitation and later sowing might be optimum for better fertilization and seed setting diversification of more plant energy to the development of reproductive organ.

The impact of flower abundance and pollinator movement on seed yield and quality is of economic importance. The hypothesis says that seed set is lower when standing crop of open flowers is high than when standing crop is low. This could occur if pollinators move more among flowers on the same plant, causing self-pollination, when flowers are abundant (Stanisavljevic *et al.*, 2012). By the rule self-seeds are of low seed quality compared to crossed once which are resulted from the delayed sown crop

(August) at that the pollinators activity is maximum and achieve almost 100 % crossed seed. So, the present study revealed that the *A. dorsata* and *Xylocopa sp.* proved to be an efficient pollinator in alfalfa in the Bengaluru region since they visit maximum flower, higher pod and seed set percentage among the 10 species of flower visitors.

Conclusion

Pollinators are major contributor for seed production in cross pollinated crop like alfa-alfa. As having lots of importance in nutritional aspect, alfa-alfa is important crop pollinated by many insects among all the pollinator frequently visits the plant is *A. dorsata* and *Xylocopa sp.* The findings of this study suggest that even though all insects but *A. dorsata* is a majorly used a valuable alternative pollinator for alfa-alfa for higher seed production.

Declarations

Author Contribution

A: worked as research worker, data collection, compilation of manuscript. B: Manuscript correction
C: Manuscript correction and Table analysis
D: Field data collection, analysis
E: Manuscript correction

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References

1. ASAADI, M., BAHAREH D. AND BAHMAN P., 2014, Determination the best time to harvest and seed rates in order to increase the yield production of alfalfa (*Medicago sativa* L.) seed. *International Journal of Biosciences.*, **5** (5): 100-106.
2. AHMAD, M. AND ASLAM, M., 2002, Pollinators visiting carrot (*Daucus carota*) seed crop. *J. Res. Sci.*, **13**(1): 31–35.
3. CECEN, SEMIHA, GUREL, FEHMI AND KARACA, AYSE (2007) 'Impact of honeybee and bumblebee pollination on alfalfa seed yield', *Acta Agriculturae Scandinavica, Section B - Plant Soil Science*, 58:1, 77 – 81
4. DIRIBA G., MEKONNEN H., ASHENAFI M. AND ADUGNA TOLERA., 2014, Biomass yield potential and nutritive value of selected alfalfa (*Medicago sativa* L.) cultivars grown under tepid to cool sub-moist agro-ecology of Ethiopia. *Journal of Agricultural Research and Development.*, **4**(1): 7-14.
5. DOBROMILSKA, R., 1997, Plonowanie odmian papryki słodkiej w zależności od sposobu zapylania. *Mat. VII Ogólnopol. Zjazdu Hod. Roslin Ogrod.*, 11&13: 399-402.

6. ELUMALAI KANNAN. 2012, Economics of production, processing and marketing of fodder crops in Karnataka. *Research Report.*, **11** :48.
7. FREE, J. B. AND WILLIAMS, I. H., 1976, Pollination as a factor limiting the yield of field beans (*Vicia faba* L.). *J. Agric. Sci.*, (Cambridge), **87**: 395-399.
8. GEMMILL-HERREN, B., OCHIENG, A.O., 2008, Role of native bees and natural habitats in eggplant (*Solanum melongena*) pollination in Kenya. *Agric. Ecosyst. Environ.*, **127**: 31–36.
9. HENSLOW, C., 1867, Note on the structure of *Medicago sativa*, as apparently affording facilities for intercrossing of distinct flowers. *J. Linn. Soc.*, **9**:327-329.
10. KNAPP, E.E. AND TEUBER L.R., 1994, Selection progress for ease of floret tripping in alfalfa. *Crop Sci.*,**34**:323–326.
11. PURSEGLOVE, J. W., 1968, Tropical crops: Dicotyledons 1, Dicotyledons 2. John Wiley and Sons, New York, USA. pp: 719.
12. SHARMA, D. AND GREEN, J. M., 1980, Pigeon pea. In hybridization of crop plants. Madison, WI, USA: *American society of Agronomy and crop science society of America*. pp: 471-481.
13. STANISAVLJEVIC, R., BEKOVIC, D., DJUKIC, D., STEVOVIC, V., TERZIC, D., MILENKOVIC, J. AND DJOKIC, D., 2012, Influence of plant density on yield components, yield and quality of seed and forage yields of alfalfa varieties. *Romanian Agril. Res.*,**29**: 245-253.
14. THERESA, L., PITTS-SINGER. AND JAMES, H. C., 2011, The alfalfa leaf cutting bee (*Megachile rotundata*). The World's most intensively managed solitary bee. *Ann. Rev. Entomol.*, 56: 221–37.

Tables

Tables 1 to 6 are available in the Supplementary Files section.

Supplementary Files

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