**Do Alterations in Irrigation Frequency and the Foliar Applications of Plant Bio-Regulators on Berseem Clover (*Trifolium alexandrinum* L.) affect the Abundance and Activities of Pollinators?**

**Ram Prasad Ghimire1 and Rita Amgain2**

*1National Pasture and Fodder Research Program, Nepal Agricultural Research Council, Lalitpur, Nepal*

*2National Soil Science Research Center, Nepal Agricultural Research Council, Lalitpur, Nepal*

**Abstract**

The implementation of research findings to improve the seed yield and quality of Berseem clover can potentially disturb the abundance and behaviors of pollinators, leading to reduced pollination and seed setting. To evaluate the impact of irrigation frequencies combined with the number of plant bio-regulator sprays on pollinators’ visitation and activities, a study was conducted at the research site of Directorate of Agricultural Research, Khajura, Banke. The study employed a Randomized Complete Block Design, with 12 treatments and three replications. The experimental treatments involved combinations of irrigation frequencies of one, two, three, and four times @ 666.67 kiloliters of water ha-1 applied at 15-day intervals after one-cut at 60 days after sowing and one, two, and three foliar sprays of plant bio-regulators (P2O5 @ 2 kg ha-1 + KNO3 @ 4 kg ha-1) administered at 7-day intervals during the pre-flowering stage. The results of the study indicated that the population abundance of pollinators, foraging duration on Berseem clover flowers, and pollinator diversity did not show significant differences (P>0.05) among the treatments. The findings support the elevation of irrigation frequency and plant bio-regulator sprays for Berseem clover seed plants, considering the perspectives of pollinator populations and activities.

**Keywords:** *Diversity, Duration, Foraging, Honey bee, Pollinators’ population.*

**Introduction**

Berseem clover (*Trifolium alexandrinum* L.) plays a significant role as the popular winter fodder legume, serving as a vital component in the winter feeding strategies for the ruminants in the Terai regions of Nepal. The plant demonstrates excellent fodder yield, generating five to six cuttings with a quality harvest comprising about 20% crude protein, alongside superior digestibility (approximately 65%) and increased palatability, solidifying its position as a top choice for winter fodder. Despite its many advantages, including long-duration availability from November to May and excellent persistency, Berseem clover faces a significant challenge in Nepal- the issue of lower seed yield and inferior seed quality. This limitation poses a hurdle to the effective seed production of Berseem clover in the field, despite its otherwise favorable characteristics for livestock feeding (Tiwari and Yadav, 2014).

Implementing suitable irrigation management and technological interventions can significantly enhance the seed yield and quality of Berseem clover. Recent researches in Nepal, based on a series of experiments, has conclusively demonstrated that employing irrigations after a cut at 60 DAS for fodder, along with foliar sprays of plant bio-regulators (2 kg P2O5 + 4 kg KNO3 ha-1) at pre-flowering stage, markedly improves both the seed yield and seed quality of Berseem clover in the western Terai of Nepal (Ghimire, 2022). The incorporation of these technologies, emphasizing the optimization of irrigation management and the implementation of strategies to improve seed setting under high-temperature stress using plant bio-regulators as suggested by Singh and Kang (2004), Patil, et al., (2005) and Bakheit, et al., (2012), has the potential to enhance seed productivity and elevating seed quality in Berseem clover within the Terai region of Nepal.

However, seed production of Berseem clover requires interventions for maximum pollination, fertilization and seed setting. Being an entomophilous plant type, pollinator insets’ visit is vital during the flowering and fruit setting time in order to achieve good seed yield (Martiniello, et al., 2003; El-Naby, et al., 2012; Jat, et al., 2014). So higher number of visits of pollinators and their massive grazing are beneficial to the seed yield and quality of Berseem clover. Several authors reported that sufficient visits and foraging of pollinator insects, especially honey bees, for tripping and other activities on flowers of Berseem clover is a critical factor for seed production (Dixit, et al., 1989; Martiniello, et al., 2003; El-Naby, et al., 2012; Jat, et al., 2014). Conversely, any treatment aimed at enhancing seed yield and quality may disrupt the abundance and activities of pollinators, introducing complexities to such technologies for Berseem clover seed production. Consequently, it becomes imperative to conduct a study of pollinators. Hence, a study was conducted to evaluate the impact of recommended technologies, including irrigation management and plant bio-regulator sprays, on the population and activities of pollinators.

**Materials and Methods**

The study was conducted at the Regional Agricultural Research Station (RARS) located in Khajura, Banke, Nepal at an elevation of 156 masl. The soil composition at the experimental site was sandy loam. The region undergoes extremely hot summers and mild winters. The meteorological station at RARS, Khajura, diligently monitored and documented meteorological data throughout the entire experimental duration. In March 2019, the daily average of maximum temperature, minimum temperature, relative humidity (RH) at 08:45 hours, and RH at 17:45 hours NST were recorded as 31.48°C, 13.66°C, 78.02%, and 47.46%, and for April 2019, the corresponding figures were 36.21°C, 19.34°C, 63.05%, and 42.88%, respectively.

**Experimental details and crop management**

The experiment was conducted in a Randomized Complete Block Design (RCBD) with 12 treatments. The combination of one-, two-, three-, and four-times irrigations @ 666.67 kiloliters of water ha-1 at 15-day intervals after one cut at 60 days after sowing (DAS) and one- two-, and three-times foliar sprays of the plant bio-regulators (P2O5 @ 2 kg ha-1 + KNO3 @ 4 kg ha-1) at 7-day intervals during the pre-flowering stage were used as the treatments. Each treatment was replicated three times, with the plot size of 4×3 m² as an experimental unit. The 'Greengold' cultivar was used, and the procedures outlined by Vijay, et al., (2017) for crop management were followed. Field preparation included thorough tillage and the dry sowing method, with fertilizers applied at a basal dose of 20:60:40 kg ha-1 N:P2O5:K2O during land preparation. Sowing was carried out using the line sowing method @ 20 kg seed ha-1, with a row-to-row distance of 40 cm. Before sowing, seed was inoculated with *Rhizobium leguminosarum* bv. *Trifolii*. Initial irrigations were applied at sowing and at 12 days after sowing (DAS) to facilitate germination and seedling establishment. Continuous monitoring of diseases and insect pests was performed, and hand weedings were conducted at 15 and 30 DAS for effective weed management. Green fodder was harvested once at 60 DAS, and the crop was subsequently left for seed production.

**Observations on pollinators’ abundance and activity**

Observations on the diversity and abundance of pollinators’ population, as well as their foraging activities, were conducted from March 16, 2019 to April 15, 2019 commencing from the 25% flowering stage of Berseem clover.

The Sight Count Method was employed to determine the populations of pollinators during the experimentation. The observations were conducted in randomly selected one m² areas in each experimental plot. The pollinators’ population data were collected by recording their visits over 11 observation days, spanning from March 16, 2019, to April 15, 2019, with observations made on the 1st, 4th, 7th, 10th, 13th, 16th, 19th, 22nd, 25th, 28th, and 31st days of the observation period. On each observation day, data were collected three times at 09:00 AM, 12:00 PM, and 03:00 PM, respectively. The number of pollinators visiting the flowers within the selected one m² area during a one-minute duration was observed, counted, and recorded from each plot at 09:00 AM, 12:00 PM, and 03:00 PM on every observation day by using stop watch.

Apart from counting the pollinators, the duration spent by the pollinators engaged in foraging on Berseem clover flowers was observed and documented on observation days using the Sight Count Method (SCM). During each observation day, specifically in the morning (09:00 AM), at noon (12:00 PM), and in the afternoon (03:00 PM), the active foraging time on flowers by the visiting pollinators was recorded through three observations of 1-minute intervals (during the first, third, and fifth minutes of the five-minute observation duration), using a stopwatch. The average foraging time was computed from these three observations, and the time spent by the pollinators minute-1 was calculated.

Diversity among pollinators was investigated using the Pan Trap Method (PTM). Each experimental plot was equipped with one fluorescent yellow pan trap. These traps, constructed from plastic bowls with dimensions of 15 cm in diameter and 10 cm in depth, were partially filled with water mixed with a small amount of detergent powder. The traps were positioned before 09:00 AM, and during the installation period and different observation times (09:00 AM, 12:00 PM, and 03:00 PM), they were carefully examined. Specimens of pollinators captured in the yellow pan traps were collected and counted at 24-hour intervals during the mornings. The collected pollinator specimens from the experimental fields were transported to the insect taxonomy laboratory, where the identification of the pollinators was conducted.

**Statistical analysis**

The data underwent square root transformation for analysis, and the statistical analysis was performed using Statistical Tool for Agricultural Research 2.0.1 (STAR, 2014) software.

**Results**

**Population abundance of pollinators**

The non-significant difference (p>0.05) on the total count of pollinators was observed in the experimental plots across various treatments during 11 observation days, with total numbers ranging from 568.75 to 755.55 in different plots (Table 1). Diverse results were observed regarding the number of pollinators visiting the experimental plots of Berseem clover on a specific observation day throughout the experimental duration (Figure 1).

**Table 1:** Effect of different treatments on pollinators’ population abundance

|  |  |
| --- | --- |
| **Treatments** | **Total number of pollinators** |
| One irrigation, one spray | 578.50 |
| One irrigation, two sprays | 701.25 |
| One irrigation, three sprays | 603.50 |
| Two irrigations, one spray | 599.75 |
| Two irrigations, two sprays | 568.75 |
| Two irrigations, three sprays | 644.25 |
| Three irrigations, one spray | 623.75 |
| Three irrigations, two sprays | 700.25 |
| Three irrigations, three sprays | 755.55 |
| Four irrigations, one sprays | 665.00 |
| Four irrigations, two sprays | 608.50 |
| Four irrigations, three sprays | 627.25 |
| SEM | 28.04 |
| F-Probability | NS |
| CV% | 13.58 |

SEM= Standard error of mean, CV= Coefficient of variation, NS= Non-significant

**Figure 1:** Number of pollinators visited on the experimental plots under different treatments at RARS, Khajura, Banke in different observation dates

**Diversity of Pollinators**

The diversity of the pollinators grazed in the experimental plots under different treatments are presented in the Figure (2). Honey bees were observed as the predominant pollinators across all treatments in the experimental plots. The primary honey bee species observed in the study were *Apis Cerana* and *Apis dorsata*. In addition to honey bees, various insects such as butterflies and syrphid flies were also identified as pollinators in the field. The percentage of honey bees among the total pollinators ranged from 79.20% to 91.21%, and was significantly higher (p<0.001) than other pollinators.

**Figure 2:** Number of honey bees and other pollinators visited in the experimental plots under various treatments during the different hours of a day

**Foraging durations of pollinators on flowers**

The Figure (3) illustrates the total foraging duration (sec min-1) of honey bees on Berseem clover flowers in different hours of the day. The average time spent by honey bees for various treatment combinations showed non-significant differences (p>0.05).

**Figure 3:** Mean time spent by honey bees for foraging on the flowers of Berseem clover under various treatments during different hours of the day.

**Discussion**

The assistance of pollinator insects, particularly in tripping, is essential for the pollination and fertilization processes during the flowering and fruit-setting period in Berseem clover. Consequently, the foraging or grazing behaviors of pollinators during this flowering period significantly influence its seed yield and quality, aligning with observations in other legumes (Jat, et al*.*, 2014). Therefore, any technological interventions aimed at enhancing the seed yield and quality of Berseem clover must carefully consider the maintaining of pollinator populations and their foraging activities. Number of studies in Nepal, including Ghimire (2022), and other countries (Singh and Kang, 2004, Bakheit, et al*.*, 2012) recommended elevating the irrigation frequencies to the seed plants after taking a last cut for fodder, which is a one-irrigation in farmers’ practice (Tiwari and Yadav, 2014). Additionally, simultaneous applications of plant bio-regulators was also recommended, especially under high temperature conditions (Bakheit, 1989, Patil, et al., 2005, Ghimire, 2022). This brings forth the significant concern regarding the potential impact of these technological interventions on the populations and activities of pollinators, determining whether such effects exist or not. However, in the study, neither the number of pollinators’ visits, nor their diversity, nor their time spent in foraging on Berseem clover flowers was affected by the different treatments. Similar findings were reported in tomatoes by Barbosa, et al., (2019), in which the population of pollinators was not influenced by irrigation methods. In contrast, Park, et al., (2015) found that the use of agrichemicals led to a reduction in pollinator populations. But, in our study the population abundance was not altered by the foliar spray of plant-bio-regulators containing P2O5 and KNO3. The observed results in the study might be due to the potential intoxicating effects of these chemicals and sprays applied before flowering.

The results of the pollinators’ diversity study are in line with other studies. Other authors reported that the honey bees are most dominating and more important species in case of Berseem clover pollination among the pollinator insects (Bakheit, 1989; Dixit, et al*.*, 1989; Jat, et al*.*, 2014). The *Apis* spp., especially *Apis mellifera*, *Apis dorsata*, *Apis florea*, *Apis indica* etc. species of honey bees are common pollinators of Berseem clover crop, among them *A. dorsata* is the most dominant species in the North Indian conditions (Taha and Bayoumi, 2009). The *A. mellifera* is the most effective species of honey bees for pollinating the Berseem clover (Vijay, et al*.*, 2017). In addition to honey bees, there are number of additional pollinators for pollinations to Berseem clover; such as, other bee species, butterflies, flies, wasps, birds, beetles, moths, ants, bats, other vertebrates etc. (FAO, 2018).

There are no distinct differences in the mean time spent by pollinators on the flowers of Berseem clover under different treatments. The grazing hours of honey bees in the Berseem clover field exhibited significant variations based on the time of day. The population dominance of honey bees was further heavier in 12:00 PM and 03:00 PM than in 09:00 AM for all treatments. According to a study by Jat, et al., (2014), the highest pollinators visits occurred at 12:00 PM, 02:00 PM, and 04:00 PM, with a notable reduction during early mornings. Vijay, et al., (2017) also reported a peak visiting time of 11:00 AM, followed by a gradual decline in pollinators’ populations, coinciding with the opening of flowers and pollinators’ activities. In the afternoon, honey bees' activities increased again, primarily focused on nectar collection rather than pollen gathering.

**Conclusion**

The results revealed that the varying the application of one to four irrigations at the rate of 666.67 kiloliters of water ha-1 in 15-day intervalsafter taking a cut for fodder at 60 DAS and one to three sprays of plant bio-regulators (P2O5 @ 2 kg ha-1 + KNO3 @ 4 kg ha-1) in 7-day interval at pre-flowering stage of Berseem clover do not alter the population, diversity and foraging duration of the pollinators. It suggests that the elevated irrigation frequencies and the use of plant bio-regulators are safe for pollinators, allowing the adoption of these technologies in farmers' fields without negatively impacting pollinator abundance and activities.

It's worth noting that in this study, the plant bio-regulators were sprayed during the pre-flowering stage, which could be a contributing factor to the non-significant difference in the impact of plant bio-regulator spray on pollinator abundance. Increasing the number of plant bio-regulators applications beyond three sprays could be the areas of the further research.

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