Plant Archives Vol. 25, Supplement 1, 2025 pp. 1863-1868 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.supplement-1.255

OPTIMIZING PLANTING TIME AND BIOINOCULANT USE FOR ENHANCED DEHYDROGENASE ACTIVITY IN RHIZOSPHERIC SOIL OF GLADIOLUS CV WHITE PROSPERITY

Divya[1], Arvind Malik[2*], Neha[2] and Raveena[1]
[1]Maharana Pratap Horticultural University, Karnal, Haryana, 132001, India
[2]Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, 125004, India
*Corresponding author E- Mail: asmalik007@gmail.com
(Date of Receiving : 24-09-2024; Date of Acceptance : 25-11-2024)

ABSTRACT

The present experiment was conducted at Agri-tourism Centre, CCSHAU, Hisar (Haryana) during 2020-21 for Optimizing planting time and bioinoculant use for enhanced Dehydrogenase activity in Gladiolus cv White prosperity rhizosphere soil. This experiment was done to explore the best planting time and best bio inoculant treatment. The experiment consists of growing white prosperity cultivar of gladiolus at four different planting times (1st fortnight of October, 2nd fortnight of October, 1st fortnight of November and 2nd fortnight of November) and with eight different treatments of bio inoculants T_1: Recommended dose of fertilizers (RDF), T_2: RDF + *Azotobacter*, T_3: RDF + Phosphate solubilizing bacteria, T_4: RDF + Mycorrhiza, T_5: RDF + *Azotobacter* + PSB, T_6: RDF + PSB + Mycorrhiza, T_7: RDF + *Azotobacter* + Mycorrhiza and T_8: RDF + *Azotobacter* + PSB + Mycorrhiza. The data recorded revealed that 1st fortnight of October with T_8 treatment for gladiolus performed better results in terms of dehydrogenase enzyme activity.
***Keywords*:** bioinoculants, rhizosphere, gladiolus, mycorrhiza, azotobacter

Introduction

Gladiolus (*Gladiolus spp.*) commonly referred to as the "sword lily," is a prominent ornamental plant known for its vibrant flower spikes and wide variety of colors. Native to the subtropical regions of South Africa, gladiolus is a perennial bulb plant known as the "queen of bulb plants" for its beautiful and graceful blooms (Sultana *et al.,* 2013), (Elmer and Kamo 2018). The growth and quality of gladiolus are significantly influenced by essential nutrient elements, particularly nitrogen and phosphorus. These nutrients play a critical role in promoting robust growth, enhancing flower development, and ensuring the longevity of the blooms. (Dhakal *et al.,* 2017) However, the extensive use of chemical fertilizers to supply these nutrients has led to challenges such as soil nutrient imbalances and deterioration of soil health (Cárceles *et al.,* 2022). The excessive reliance on synthetic fertilizers has raised concerns about environmental sustainability and soil quality, necessitating a shift towards more integrated nutrient management practices (Krasilnikov *et al.,* 2022).

To address these issues and improve the quality of gladiolus cultivation, there is a growing focus on integrating bioinoculants into nutrient management strategies. Bioinoculants are beneficial microorganisms that include various bacteria, fungi, and other microbes, which can enhance soil health and plant growth. These microorganisms promote nutrient availability, improve soil structure, and support plant resilience to stress (Kamath *et al.,* 2023). Notable examples include plant growth-promoting rhizobacteria (PGPR) such as *Azotobacter* and *Phosphorus Solubilizing Bacteria* (PSB), as well as arbuscular mycorrhizal fungi (AMF) (Nagrale *et al.,* 2023). The application of bioinoculants is recognized for its potential to reduce dependency on chemical fertilizers by enhancing the natural nutrient cycling

processes and improving nutrient uptake by plants (Negi *et al.,* 2024 (Bittencourt *et al.,* 2023).

In conclusion, integrating bioinoculants into the management of gladiolus cultivation offers a promising approach to enhancing soil health, improving nutrient use efficiency, and achieving high-quality flower production while minimizing environmental impacts. This approach aligns with contemporary agricultural practices aimed at sustainability and reduced ecological footprints.

Dehydrogenase enzymes play a crucial role in the soil ecosystem by catalyzing the oxidation-reduction reactions necessary for the decomposition of organic matter. These enzymes, primarily found in soil microbes, are vital in converting complex organic substrates into simpler compounds, a process essential for nutrient cycling and maintaining soil fertility (Wolińska and Stępniewska, 2023). The presence and activity of dehydrogenase enzymes in the soil essentially reflect the population of beneficial microbes, as these enzymes are produced by these microorganisms. By facilitating the breakdown of organic materials into simpler inorganic forms, dehydrogenase activity directly impacts the availability of essential elements such as carbon and nitrogen, which are crucial for plant uptake and microbial use. Higher dehydrogenase activity is often associated with an abundance of beneficial microbes, leading to enhanced nutrient availability, improved soil health, and ultimately, increased crop productivity (Kaur and Kaur, 2021), (Kumar *et al*., 2013).

In the context of gladiolus cultivation, the planting time plays a significant role in optimizing plant growth and flower yield. The planting time affects various environmental factors, including soil temperature and light duration, both of which influence plant physiological processes such as photosynthesis and nutrient uptake. Proper time ensures that plants are exposed to optimal conditions for growth, which enhances their ability to fix carbon and improve root development. Approximately 11% of the net carbon fixed during photosynthesis is released into the rhizosphere as root exudates, which can stimulate microbial activity and support beneficial soil processes (Yaygin *et al*., 2022). The interaction between planting time and soil enzyme activity is particularly relevant for gladiolus. Optimal planting conditions not only support healthy plant growth but also influence the activity of soil enzymes, including dehydrogenases. By improving the timing of planting, it is possible to enhance soil enzyme functions and, consequently, nutrient availability, which contributes to better plant health and increased flower yield.

So, this research was conducted to investigate the impact of different planting times on dehydrogenase enzyme activity and their subsequent effects on the growth and yield of gladiolus. Additionally, the study will evaluate how these factors interact with bioinoculant treatments to enhance phosphorus availability and overall crop performance under the specific conditions of Haryana. The goal is to provide practical recommendations for optimizing planting schedules and bioinoculant applications, thereby helping farmers improve crop yields and economic returns.

Material and Methods

The current study, titled "Impact of Staggered Planting and Bioinoculant Application on Dehydrogenase Enzyme Activity in the Rhizospheric Soil of Gladiolus cv White Prosperity," was carried out at the Agri Tourism Centre, CCS Haryana Agricultural University, Hisar during the 2020-21 period. The investigation was performed under open field conditions. The soil type was sandy loam with following physicochemical properties (pH, electrical conductivity, nitrogen, phosphorus, and potassium) and microbiological parameters of the soil were as follows (see Table 1).

Table 1 : Physico chemical and biological properties of soil

Soil properties	Value	Microbiological parameters	Value
Soil texture	Sandy loam	Dehydrogenase (µg TPF/g soil/24 h)	73.81
Ph	8.10		
EC dSm^{-1}	0.68		
Available N (kg ha^{-1})	162.00		
Available P (kg ha^{-1})	25.00		
Available K (kg ha^{-1})	321.00		

The experiment was designed using a randomized block layout, incorporating 32 treatment combinations with 8 types of bioinoculants and 4 planting times, each replicated three times. The treatments included: T1: Recommended Dose of Fertilizers (RDF), T2: RDF + Azotobacter, T3: RDF + Phosphate

Solubilizing Bacteria (PSB), T4: RDF + Mycorrhiza, T5: RDF + Azotobacter + PSB, T6: RDF + PSB + Mycorrhiza, T7: RDF + Azotobacter + Mycorrhiza, and T8: RDF + Azotobacter + PSB + Mycorrhiza. The planting times were P1: 1st fortnight of October, P2: 2nd fortnight of October, P3: 1st fortnight of November, and P4: 2nd fortnight of November.

Prior to sowing, the corms were first peeled and then treated using the corm dip method with bioinoculants such as VAM, Azotobacter, and PSB for 30 minutes, followed by a 30-minute shade drying period (Chaudhari *et al.,* 2014). The bioinoculants were obtained from the Department of Microbiology at CCS HAU, Hisar, for the corm inoculation. The planting of the treated corms occurred on four distinct dates, with a spacing of 30 x 30 cm between them.

Soil Sampling

Soil samples are collected from the field using a clean soil auger or corer to avoid contamination. The samples are usually taken from the top 0-20 cm of the soil, which is the most biologically active layer. For each treatment, samples from five plants are taken, and the dehydrogenase enzyme activity is measured to assess the biological activity and health of the soil.

Dehydrogenase activity

Soil dehydrogenase activity was determined by using the method described by Casida *et al*. (1964).

Reagents

- Triphenyl Formazone (TPF) Standard Solution
- Methanol AR Grade
- Triphenyl Tetrazolium Chloride (TTC)

Procedure

Five grams of soil were placed in test tubes with a 25 ml capacity. Each tube received 1 ml of a 3% TTC solution and 2.5 ml of distilled water. The contents were thoroughly mixed using a glass rod. After mixing, the tubes were sealed with stoppers and incubated at 37°C for 24 hours. Once the incubation was complete, the stoppers were removed, and 10 ml of methanol was added to each tube. The mixture was shaken for one minute, and then the suspension was filtered through Whatman No.1 filter paper into a 500 ml volumetric flask. The process was repeated by adding another 10 ml of methanol to the tubes and extracting until the methanol extract no longer showed any reddish color. All extracts were combined, and the final volume of the filtrate was adjusted to 50 ml with methanol. The absorbance of the colored solution was then measured at 485 nm using a spectrophotometer, with methanol serving as the blank. The quantity of TPF produced was determined using a standard curve, and the dehydrogenase activity was expressed as µg TPF per gram of soil per 24 hours.

Calculations

µg TPF/g soil/24 h = O.D. (optical density) x 1250/weight of soil sample (g)

Statistical Analysis

The data collected on growth, flowering, yield, and corm characteristics throughout the study were analyzed using OPSTAT software with analysis of variance (ANOVA). The effects of treatments were assessed using the F test. The critical difference (CD) was calculated at a 5% significance level to determine the significance of differences between treatment means.

Results and Discussion

Dehydrogenase activity for 1st time of planting

The data presented in Table 2 revealed the activity of the dehydrogenase enzyme during the growth period of gladiolus. The table showed that the activity of the dehydrogenase enzyme differed significantly with the number of days. The maximum dehydrogenase enzyme activity (86.63 µg TPF/g soil/24 h) was observed in T_8 treatment (RDF + *Azotobacter* + PSB + Mycorrhiza) after 60 days of planting. In contrast, the minimum enzyme activity (73.81) was observed at 0 days of planting.

Table 2: Effect of 1st time of planting and bio inoculants on dehydrogenase enzyme activity in soil of gladiolus cv. White prosperity

Treatments	Dehydrogenase activity (µg TPF/g soil/ 24h)				
	30 days	60 days	90 days	120 days	150 days
T_1: Recommended dose of fertilizers (RDF)	74.83	81.14	77.33	75.79	76.79
T_2: RDF + *Azotobacter*	75.51	81.96	78.15	76.61	77.61
T_3: RDF + PSB	76.47	82.92	79.11	77.57	78.57
T_4: RDF + Mycorrhiza	78.18	84.45	80.64	79.10	80.10
T_5: RDF + *Azotobacter* + PSB	77.63	84.07	80.26	78.72	79.41
T_6: RDF + PSB + Mycorrhiza	79.98	86.15	82.98	80.82	81.52
T_7: RDF + *Azotobacter* + Mycorrhiza	78.78	85.27	82.09	79.94	80.63
T_8: RDF + *Azotobacter* + PSB + Mycorrhiza	80.30	86.63	83.46	81.30	82.00
C.D.	**0.77**	**0.87**	**1.14**	**0.88**	**0.92**

Fig. 1 : T_8 treatment samples for measuring dehydrogenase enzyme activity

Dehydrogenase activity for 2nd time of planting

The data presented in Table 3 revealed the activity of the dehydrogenase enzyme during the growth period of gladiolus. The maximum dehydrogenase enzyme activity (86.36 µg TPF/g soil/24 h) was observed in T_8 treatment (RDF + *Azotobacter* + PSB + Mycorrhiza) after 60 days of planting. In contrast, the minimum enzyme activity (72.28) was observed at 0 days of planting.

Dehydrogenase activity for 3rd time of planting

The data presented in Table 4 revealed the activity of the dehydrogenase enzyme during the growth period of gladiolus. The maximum dehydrogenase enzyme activity (84.53 µg TPF/g soil/24 h) was observed in T_8 treatment (RDF + *Azotobacter* + PSB + Mycorrhiza) after 60 days of planting. Whereas, the minimum enzyme activity (70.61) was observed at 0 days of planting.

Table 3: Effect of 2nd time of planting and bio inoculants on dehydrogenase enzyme activity in soil of gladiolus cv. White prosperity

Treatments	Dehydrogenase activity (µg TPF/g soil/ 24h)				
	30 days	60 days	90 days	120 days	150 days
T_1: Recommended dose of fertilizers (RDF)	73.58	80.98	76.51	74.79	75.22
T_2: RDF + *Azotobacter*	74.26	81.80	77.33	75.27	76.04
T_3: RDF + Phosphate solubilizing bacteria	75.22	82.76	78.29	76.23	77.00
T_4: RDF + Mycorrhiza	76.93	84.29	79.82	77.76	78.20
T_5: RDF + *Azotobacter* + PSB	76.38	83.64	79.44	77.39	78.05
T_6: RDF + PSB + Mycorrhiza	78.47	85.88	82.16	79.49	79.95
T_7: RDF + *Azotobacter* + Mycorrhiza	77.27	85.00	81.28	78.61	79.07
T_8: RDF + *Azotobacter* + PSB + Mycorrhiza	78.79	86.36	82.64	79.97	80.43
C.D.	**0.75**	**0.86**	**0.93**	**0.87**	**1.02**

Table 4: Effect of 3rd time of planting and bio inoculants on dehydrogenase enzyme activity in soil of gladiolus cv. White prosperity

Treatments	Dehydrogenase activity (µg TPF/g soil/ 24h)				
	30 days	60 days	90 days	120 days	150 days
T_1: Recommended dose of fertilizers (RDF)	71.61	79.33	74.98	73.02	73.30
T_2: RDF + *Azotobacter*	72.39	79.77	75.10	73.50	74.12
T_3: RDF + Phosphate solubilizing bacteria	73.35	80.93	76.43	74.46	75.08
T_4: RDF + Mycorrhiza	75.06	82.59	77.85	75.33	76.18
T_5: RDF + *Azotobacter* + PSB	74.68	81.88	77.63	75.25	75.58
T_6: RDF + PSB + Mycorrhiza	76.76	84.05	80.50	77.69	77.16
T_7: RDF + *Azotobacter* + Mycorrhiza	75.07	83.17	79.09	77.50	75.83
T_8: RDF + *Azotobacter* + PSB + Mycorrhiza	76.92	84.53	80.62	78.67	78.18
C.D.	**0.66**	**0.85**	**0.73**	**0.48**	**0.54**

Dehydrogenase activity for 4th time of planting

The data presented in Table 5 revealed the activity of the dehydrogenase enzyme during the growth period of gladiolus. The maximum dehydrogenase enzyme activity (83.67 µg TPF/g soil/24 h) was observed in T_8 treatment (RDF + *Azotobacter* + PSB + Mycorrhiza) after 60 days of planting. Whereas the minimum enzyme activity (68.81) was observed at 0 days of planting.

Table 5: Effect of 4th time of planting and bio inoculants on dehydrogenase enzyme activity in soil of gladiolus cv. White prosperity

Treatments	Dehydrogenase activity (µg TPF/g soil/ 24h)				
	30 days	60 days	90 days	120 days	150 days
T_1: Recommended dose of fertilizers (RDF)	70.44	78.10	72.89	71.59	71.49
T_2: RDF + *Azotobacter*	71.22	78.54	73.41	71.85	72.31
T_3: RDF + Phosphate solubilizing bacteria	72.18	79.70	74.77	72.81	73.27
T_4: RDF + Mycorrhiza	73.74	81.36	76.76	73.61	74.37
T_5: RDF + *Azotobacter* + PSB	73.51	80.65	76.57	73.56	73.77
T_6: RDF + PSB + Mycorrhiza	75.49	82.79	79.41	76.04	75.35
T_7: RDF + *Azotobacter* + Mycorrhiza	73.80	82.20	77.61	75.85	74.02
T_8: RDF + *Azotobacter* + PSB + Mycorrhiza	76.39	83.67	79.66	77.02	76.37
C.D.	**0.41**	**0.68**	**0.10**	**0.50**	**0.55**

Discussion

The data recorded revealed that the dehydrogenase activity is significantly affected by the different time of plantings. Among the different time of plantings, 1st fortnight of October recorded with maximum activity of dehydrogenase enzyme (86.63 µg TPF/g soil/ 24h). This might be due to the fact that the performance of the dehydrogenase enzyme (microbial origin) is influenced by the changing climatic conditions (Wolińska & Stępniewska, 2012). Basically, the optimum time of planting enhances soil conditions such as temperature and moisture, which are conducive to increased microbial activity. This leads to higher levels of dehydrogenase enzymes, crucial for nutrient cycling and organic matter decomposition. Additionally, the synchronization of root exudation and microbial activity during optimum planting time promotes the availability of essential nutrients like nitrogen and phosphorus.

Dehydrogenase enzyme activity is significantly affected by different bio inoculants treatment. The maximum activity of the dehydrogenase enzyme (86.63 µg TPF/g soil/ 24h) was recorded in the T8 treatment. This might be due to the addition of biofertilizers to the soil that results in an increase in soil microbiological activity (Latkovic *et al.*, 2020). Bioinoculants such as plant growth-promoting rhizobacteria (PGPR), including species like Azotobacter, Phosphorus Solubilizing Bacteria (PSB) and Vesicular Arbuscular Mycorhiza (VAM) increase microbial activity by improving nutrient availability, particularly nitrogen and phosphorus. These beneficial microorganisms stimulate the microbial communities that produce dehydrogenase enzymes, leading to higher enzyme activity in the rhizosphere.

Conclusion

The study revealed that the activity of the dehydrogenase enzyme in the rhizospheric soil is significantly influenced by both the time of planting and the application of bioinoculants. The 1st fortnight of October emerged as the most favorable planting time, recording the highest dehydrogenase enzyme activity (86.63 µg TPF/g soil/24h). This may be attributed to the synchronization of climatic conditions, such as temperature and moisture, with enhanced microbial activity during this period. Additionally, the application of bioinoculants, particularly PGPR, Azotobacter, Phosphorus Solubilizing Bacteria (PSB), and Vesicular Arbuscular Mycorrhiza (VAM), significantly boosted microbial activity in the soil. This, in turn, resulted in an increase in dehydrogenase enzyme activity, essential for nutrient cycling and organic matter decomposition.

References

Bittencourt, P. P., Alves, A. F., Ferreira, M. B., da Silva Irineu, L. E. S., Pinto, V. B., & Olivares, F. L. (2023). Mechanisms and applications of bacterial inoculants in plant drought stress tolerance. *Microorganisms*, ***11***(2), 502.

Cárceles Rodríguez, B., Durán-Zuazo, V. H., Soriano Rodríguez, M., García-Tejero, I. F., Gálvez Ruiz, B., & Cuadros Tavira, S. (2022). Conservation agriculture as a sustainable system for soil health: A review. *Soil Systems*, ***6***(4), 87.

Casida Jr, L. E., Klein, D. A., & Santoro, T. (1964). Soil dehydrogenase activity. *Soil science*, ***98***(6), 371-376.

Chaudhari, S. R., Patil, A. B., & Patel, N. K. (2014). Response of bio-inoculants, FYM and inorganic fertilizers on corms and cormels production in gladiolus cv. American beauty. *BIOINFOLET-A Quarterly Journal of Life Sciences*, ***11***(2a), 308-310.

Dhakal, K., Khanal, D., Ayer, D. K., Khanal, A. P., Pandey, L., Pant, S. S., ... & Ashmit, K. C. (2017). Effect of nitrogen and phosphorous on growth, development and vase life of gladiolus. *RRJoAST*, ***6***, 1-7.

Effect of biostimulants and biofertilizers on growth, flowering and quality of gladiolus (*Gladiolus grandiflorus* L.) Cv. American beauty under greenhouse conditions PB Pansuriya, DK Varu and RR Viradia

Elmer, W. H., & Kamo, K. K. (2018). Diseases of gladiolus. *Handbook of Florists Crops Diseases. Handbook of Plant Disease Management. Springer, Cham. https://doi. org/10.1007/978-3-319-39670-5-47*, 1289-1311.

Kamath, A., Shukla, A., Saiyed, T., Bhatt, S., Rathod, H., Makwana, V., ... & Patel, D. (2023). Bioinoculants: the agrarian avengers. *Symbiosis*, ***91***(1), 151-166.

Kaur, J., & Kaur, G. (2021). Dehydrogenase activity as a biological indicator of soil health. *Chem. Sci. Rev. Lett*, ***10***(39), 326-329.

Krasilnikov, P., Taboada, M. A., & Amanullah. (2022). Fertilizer use, soil health and agricultural sustainability. *Agriculture*, ***12***(4), 462.

Kumar, S., Chaudhuri, S., & Maiti, S. K. (2013). Soil dehydrogenase enzyme activity in natural and mine soil-a review. *Middle East J Sci Res*, ***13***(7), 898-906.

Latkovic, D., Maksimovic, J., Dinic, Z., Pivic, R., Stanojkovic, A. and Stanojkovic-Sebic, A. (2020). Case Study upon Foliar Application of Biofertilizers Affecting Microbial Biomass and Enzyme Activity in Soil and Yield Related Properties of Maize and Wheat Grains. *Biology*, **9**(12), 452.

Nagrale, D. T., Chaurasia, A., Kumar, S., Gawande, S. P., Hiremani, N. S., Shankar, R., ... & Prasad, Y. G. (2023). PGPR: the treasure of multifarious beneficial microorganisms for nutrient mobilization, pest biocontrol and plant growth promotion in field crops. *World Journal of Microbiology and Biotechnology*, ***39***(4), 100.

Negi, R., Sharma, B., Jan, T., Kaur, T., Chowdhury, S., Kapoor, M., ... & Yadav, A. N. (2024). Microbial Consortia: Promising Tool as Plant Bioinoculants for Agricultural Sustainability. *Current Microbiology*, ***81***(8), 222.

Sultana, M. N., Mehraj, H., Mahasen, M., Naznin, A., & Jamal Uddin, A. F. M. (2013). Regulation of growth and flowering of gladiolus with different gibberelic acid concentrations for summer cultivation. *International Journal Sustainable Agricultural Technology*, ***9***(1), 122-125.

Wolińska, A., & Stępniewska, Z. (2012). Dehydrogenase activity in the soil environment. *Dehydrogenases*, ***10***, 183-210.

Yayğin, T., Erden, K., & Pakyürek, A. Y. (2022). Effects Of Different Planting Times and Corm Sizes on Plant Growth, Development, and Flower Production of Gladiolus (*Gladiolus grandiflorus*) under Sanliurfa Conditions. *MAS Journal of Applied Sciences*, ***7***(1), 23-31.