Effect of staggered planting and application of bioinoculants on the phosphatase enzyme activityin the rhizospheric soil of Gladiolus cv. White prosperity

Abstract

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. The experiment was laid out in randomized block design with three replications. Among the different bio inoculants treatment, T_8 treatment (RDF + Azotobacter + PSB + Mycorrhiza) proved best with respect to maximum phosphatase enzyme activity when compared to other treatments and among different time of plantings, 1st fortnight of October was the best planting time for gladiolus in terms of maximum phosphatase enzyme activity.

Keywords: Gladiolus, Bio inoculants, PSB, Mycorrhiza, Azotobacter

Introduction:

Gladiolus, a globally commercially important cut flower, is native to the subtropical climate of South Africa. It is a perennial bulbous plant and is also known as the "queen of bulbous plants". Gladiolus, also known as the "sword lily", takes its name from its sword-shaped leaves. The inflorescence of gladiolus is a spike. The gladiolus spike is usually erect and unbranched. Its diverse spike forms and colors, makes it popular for flower arrangements. Gladiolus (*GladiolusgrandiflorusL.*) being a preferred choice in the cut-flower industry due to its elegant appearance, vibrant spikes, and extended vase life.Nitrogen and Phosphorous are two very essential major nutrient elements that influence growth and development of gladiolus to the great extent (Dhakal*etal*., 2017). However, the continuous and indiscriminate use of chemical fertilizers as a source of Nitrogen and Phosphorus for gladiolus cultivation has led to nutrient imbalances and negatively impacting soil structure and health(An *etal*., 2022 and Dhakal*etal*., 2017).

To address these concerns and achieve high-quality gladiolus spikes, corms, and cormels, there is a growing emphasis on integrated nutrient management. This involves combining bioinoculants and judicious use of chemical fertilizers to enhance soil biological and physico-chemical properties while improving nutrient uptake (Zaidi *etal*., 2016).Bioinoculants are beneficial microorganisms that are used in agriculture to enhance plant growth and productivity, improve soil health, and reduce the use of chemical fertilizers and pesticides. They include bacteria, fungi and other microorganisms that interact with plants in various ways to promote growth, nutrient uptake, and stress tolerance.Bioinoculants, particularly plant growth-promoting rhizobacteria

Comment [IM1]: On italic.

Comment [IM2]: Do not use as keywords those that are already present in the title.

Comment [IM3]: On italic.

Comment [IM4]: Check spacing.

Comment [IM5]: Check spacing.

(PGPR) like Azotobacter,PSB and fungi like VAMplays a crucial role in sustainable organic agriculture by contributing to the decomposition of organic matter, nutrient cycling, and supporting plant growth and health (Qasim *etal*., 2014). The bioinoculants application serves as a source of microbial population in the rhizosphere, which enhances the enzymatic activity in the soil. Bioinoculants are important for soil because they can improve the availability and cycling of essential nutrients such as nitrogen, phosphorus etc. They can also help in maintaining soil physico-chemical and biological properties, microbial diversity, and organic matter content. Moreover, they can protect plants from various biotic and abiotic stresses, such as drought, salinity, pests, and diseases. Interaction of AMF and PSB bioinoculants can enhance the ability of plants to obtain Phosphorus (Zaidi *etal*., 2016)By using bioinoculants, farmers can achieve sustainable crop production with lower environmental impacts and economic costs.

Comment [IM6]: On italic.

Comment [IM7]: Indicate the meaning of the acronym.

Comment [IM8]: Indicate the meaning of the acronym.

Comment [IM9]: Indicate the meaning of the acronym.

Comment [IM10]: Check score.

Phosphatase enzymes play a crucial role in the availability of phosphorus (P) for plant growth. This enzyme catalyze the hydrolysis of organic P to inorganic P, which can be taken up by plants or microorganisms (Zaidi *etal*., 2016). Increased phosphatase activity in the soil can enhance the availability of P for plant uptake, leading to improved crop growth and yield. So, Phosphatase activity is a crucial parameter for studying phosphate solubilizing microorganisms. Also the alkaline phosphatases (AKP) is a specific enzyme in the VAM and plant symbiosis system.

The time of planting plays a vital role in obtaining better growth and yield of gladiolus. The time of planting is indirectly related to the temperature and day length and both of these are influencing the photosynthesis of plants. Around 11% of the net carbon fixed during photosynthesis becomes available to microbes in the rhizosphere as root exudates. Proper planting time provides optimum growth conditions for plants for better growth by improving processes like photosynthesis and ultimately enhancing the activity of soil microbial enzymes.

Keeping in view the above-mentioned points this research is conducted to find out the best time of planting and best bioinoculant treatment so that we can increase phosphorus availability influencing the growth and yield of gladiolus and hence can help the farmer to increase their income under Haryana conditions.

Material and methods:

The present experimententitled ‘effect of staggered planting and application of bioinoculants on phosphatase enzyme activity in rhizospheric soil of gladiolus cv white prosperity’ was conducted at Agri Tourism Centre, CCS Haryana Agricultural University, Hisar during the year 2020-21. The experiment was conducted in open field conditions and the physicochemical properties (Ph, EC, N, P and K) and microbiological parameters of soil were evaluated in the beginning of experiment (Table 1).

Comment [IM11]: It's redundant.

Comment [IM12]: Correct for pH.

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	Alkaline Phosphatase µg PNP/g soil/ h	169.20
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 laid out in randomized block design with a total of 32 treatments combination with 8 Bio inoculants treatmentand 4 different time of planting taken in 3 replicates: 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, T_8: RDF + Azotobacter + PSB + Mycorrhiza, P_1:1st fortnight of October, P_2: 2nd fortnight of October, P_3: 1st fortnight of November and P_4: 2nd fortnight of November.

Before sowing, the planting material that is corms were first dehusked and then by using the corm dip method treated with bio inoculants like VAM, Azotobacter and PSB for 30 minutes followed by drying in shade for 30 minutes. Bioinoculants were procured from Department of Microbiology, CCS HAU, Hisar for the inoculation of corms. Planting of inoculated corms was done on four different dates at a spacing of 30 x 30 cm.

Alkaline Phosphatase activity: Two sets of one gram soil (oven-dry equivalent) were placed in 50 ml Erlenmeyer flasks. In all the flasks, 0.2 ml of toluene, 4 ml of MUB were added. 1 ml of p-nitrophenyl phosphate solution was added to only one set of samples. Flasks were swirled for a few seconds to mix the contents.Flasks were stoppered and placed in an incubator at 37°C. After one h, stoppers were removed, and 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH were added. Flasks were swirled for a few seconds again. One ml of p-nitrophenyl phosphate solution was added to the remaining set (control) of samples, and soil suspension was filtered through a Whatman No.1 filter paper. The absorbance of the yellow coloured filtrate was measured at 420 nm in a UV-VIS spectrophotometer. The p-nitrophenol content of filtrate was calculated with reference to the standard curve, and phosphatase activity was expressed as µg PNP released/g soil/h. (Tabatabai and Bremner (1969)

Calculations

µg PNP/g soil/h = Amount of p-nitro phenol (µg)/time of incubation (h) x dry weight of soil (g)

Statistical analysis:the investigation was subjected to statistical analysis by using randomized block design for analysis of variance (ANOVA) by using the OPSTAT. The significance of treatment effects was judged by using the F test. The critical difference (CD) was worked out at a

Comment [IM13]: Correct for pH.

Comment [IM14]: On italic.

Comment [IM15]: On italic.

Comment [IM16]: Review.

Comment [IM17]: On italic.

Comment [IM18]: On italic.

Comment [IM19]: On italic.

Comment [IM20]: What is the microbial titer of bioinoculants?

5% level of significance to judge the significance of the difference between the two treatment means.

Results and Discussion

Alkaline Phosphatase activity for 1st time of planting

The data presented in Table 1 revealed the activity of the alkaline phosphatase enzyme during the growth period of gladiolus. The table showed that the activity of the phosphatase enzyme differed significantly with the number of days. The maximum phosphatase enzyme activity (201.51 µg PNP/g soil/ h) was observed in T_8 treatment (RDF + Azotobacter + PSB + Mycorrhiza) after 60 days of planting. Whereas the minimum enzyme activity (169.20) was observed at 0 days of planting.

Alkaline phosphatase activity for 2nd time of planting : The data presented in Table 3 revealed the activity of the alkaline phosphatase enzyme during the growth period of gladiolus. The table showed that the activity of the phosphatase enzyme differed significantly with the number of days. The maximum phosphatase enzyme activity (198.97 µg PNP/g soil/ h) was observed in T_8 treatment (RDF + Azotobacter + PSB + Mycorrhiza) after 60 days of planting. Whereas the minimum enzyme activity (167.28) was observed at 0 days of planting.

Alkaline phosphatase activity for 3rd time of planting: The data presented in Table 4 revealed the activity of the alkaline phosphatase enzyme during the growth period of gladiolus. The table showed that the activity of the phosphatase enzyme differed significantly with the number of days. The maximum phosphatase enzyme activity (196.20 µg PNP/g soil/h) was observed in T_8 (RDF + Azotobacter + PSB + Mycorrhiza) treatment after 60 days of planting. Whereas the minimum enzyme activity (165.25) was observed at 0 days of planting.

Table:2Alkaline Phosphatase activity for 1st time of planting

Treatments	**Phosphatase activity (µg PNP/g soil/ h)**				Mean
	30 days	**60 days**	**90 days**	**120 days**	
T_1: Recommended dose of fertilizers (RDF)	171.24	193.11	184.76	180.90	174.67
T_2: RDF + Azotobacter	172.11	193.98	185.63	181.78	175.54
T_3: RDF + Phosphate solubilizing bacteria	173.31	195.18	186.83	182.98	176.74
T_4: RDF + Mycorrhiza	176.24	198.11	189.76	185.91	179.67
T_5: RDF + Azotobacter + PSB	175.14	197.01	188.66	184.81	178.57
T_6: RDF + PSB + Mycorrhiza	179.17	201.04	192.69	188.84	182.60
T_7: RDF + Azotobacter + Mycorrhiza	177.25	199.12	190.77	186.91	180.68
T_8: RDF + Azotobacter + PSB +	179.65	201.51	193.16	189.31	183.08

Comment [IM21]: On italic.

Comment [IM22]: On italic.

Comment [IM23]: On italic.

Comment [IM24]: On italic.

Comment [IM25]: On italic.

Comment [IM26]: On italic.

Comment [IM27]: On italic.

Mycorrhiza					
C.D	**0.15**	**0.22**	**0.23**	**0.34**	**0.22**

Table:3Alkaline Phosphatase activity for 2nd time of planting

Treatments	**Phosphatase activity (µg PNP/g soil/ h)**				**Mean**
	30 days	**60 days**	**90 days**	**120 days**	
T_1: Recommended dose of fertilizers (RDF)	169.42	190.57	182.87	178.68	172.71
T_2: RDF + *Azotobacter*	170.29	191.44	183.74	179.56	173.58
T_3: RDF + Phosphate solubilizing bacteria	171.49	192.64	184.94	180.76	174.78
T_4: RDF + Mycorrhiza	174.42	195.57	187.87	183.69	177.71
T_5: RDF + *Azotobacter* + PSB	173.32	194.47	186.77	182.59	176.61
T_6: RDF + PSB + Mycorrhiza	177.35	198.50	190.80	186.62	180.64
T_7: RDF + *Azotobacter* + Mycorrhiza	175.43	196.58	188.88	184.69	178.72
T_8: RDF + *Azotobacter* + PSB + Mycorrhiza	177.83	198.97	191.27	187.09	181.11
C.D.	**0.21**	**0.18**	**0.13**	**0.26**	**0.23**

Alkaline phosphatase activity for 4th time of planting: The data presented in Table 5 revealed the activity of the alkaline phosphatase enzyme during the growth period of gladiolus. The table showed that the activity of the phosphatase enzyme differed significantly with the number of days. The maximum phosphatase enzyme activity (194.49 µg PNP/g soil/ h) was observed in T_8 (RDF + Azotobacter + PSB + Mycorrhiza) treatment after 60 days of planting. Whereas the minimum enzyme activity (162.23) was observed at 0 days of planting.

Comment [IM28]: On italic.

Table:4Alkaline Phosphatase activity for 3rd time of planting

Treatments	**Phosphatase activity (µg PNP/g soil/ h)**				**Mean**
	30 days	**60 days**	**90 days**	**120 days**	
T_1: Recommended dose of fertilizers (RDF)	167.70	187.79	180.09	176.67	170.38
T_2: RDF + *Azotobacter*	168.58	188.66	180.97	177.54	171.25
T_3: RDF + Phosphate solubilizing bacteria	169.78	189.86	182.17	178.74	172.45
T_4: RDF + Mycorrhiza	172.71	192.79	185.10	181.67	175.38
T_5: RDF + *Azotobacter* + PSB	171.61	191.69	184.00	180.57	174.28

T_6: RDF + PSB + Mycorrhiza	175.64	195.72	188.03	184.60	178.31
T_7: RDF + *Azotobacter* + Mycorrhiza	173.71	193.80	186.10	182.68	176.39
T_8: RDF + *Azotobacter* + PSB + Mycorrhiza	176.11	196.20	188.50	185.08	178.79
C.D.	**0.24**	**0.27**	**0.11**	**0.29**	**0.21**

Table: **5Alkaline Phosphatase activity for 4th time of planting**

Treatments	**Phosphatase activity (µg PNP/g soil/ h)**				**Mean**
	30 days	**60 days**	**90 days**	**120 days**	
T_1: Recommended dose of fertilizers (RDF)	166.05	186.08	178.23	174.20	167.64
T_2: RDF + *Azotobacter*	166.93	186.96	179.11	175.08	168.51
T_3: RDF + Phosphate solubilizing bacteria	168.13	188.16	180.31	176.28	169.71
T_4: RDF + Mycorrhiza	171.06	191.09	183.24	179.21	172.64
T_5: RDF + *Azotobacter* + PSB	169.96	189.99	182.14	178.11	171.54
T_6: RDF + PSB + Mycorrhiza	173.99	194.02	186.17	182.14	175.57
T_7: RDF + *Azotobacter* + Mycorrhiza	172.06	192.09	184.24	180.21	173.65
T_8: RDF + *Azotobacter* + PSB + Mycorrhiza	174.46	194.49	186.64	182.61	176.04
C.D.	**0.16**	**0.20**	**0.33**	**0.17**	**0.26**

Conclusion:

The data revealed that the phosphatase enzyme activity varied significantly with the time of planting. Among the different times of planting, 1st fortnight of October proved best with maximum phosphatase enzyme activity (201.51 µg PNP/g soil/ h) 60 days after sowing of the crop. This might be due to the optimum climatic conditions that promote plant growth and simultaneously plant provide food for microbes. The lower phosphatase activity in later plantings might be due to the fact that the performance of PSB is hampered by environmental variables like temperature, moisture etc. Sharma *etal*., (2013).

Similarly the data recorded revealed that the phosphatase enzyme activity varied considerably with the different bio inoculants treatment. Among different bio inoculants treatment, T_8 proved best with maximum phosphatase enzyme activity (201.51 µg PNP/g soil/ h). This might be due to the presence of phosphatase enzymes in PSB and VAM.

References:

1. An, X., Liu, J., Liu, X., Ma, C., & Zhang, Q. (2022). Optimizing phosphorus application rate and the mixed inoculation of arbuscular mycorrhizal fungi and phosphate-solubilizing bacteria can improve the phosphatase activity and organic acid content in alfalfa soil. *Sustainability*, *14*(18), 11342.
2. 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.
3. Eivazi, F., &Tabatabai, M. A. (1977). Phosphatases in soils. *Soil biology and biochemistry*, *9*(3), 167-172.
4. Qasim, M., Younis, A., Zahir, Z. A., Riaz, A., Raza, H., & Tariq, U. (2014). Microbial inoculation increases the nutrient uptake efficiency for quality production of Gladiolus grandiflorus. *Pakistan Journal of Agricultural Sciences*, *51*(4).
5. Zaidi, A., Khan, M. S., Ahmad, E., Saif, S., Rizvi, A., & Shahid, M. (2016). Growth stimulation and management of diseases of ornamental plants using phosphate solubilizing microorganisms: current perspective. *Acta Physiologiae Plantarum*, *38*, 1-21.
6. Sharma, P., Singh, G., & Singh, R. P. (2013). Conservation tillage and optimal water supply enhance microbial enzyme (glucosidase, urease and phosphatase) activities in fields under wheat cultivation during various nitrogen management practices. *Archives of Agronomy and Soil Science*, *59*(7), 911-928.