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# EFFECT OF GROWING CONDITIONS AND BIO INOCULANTS ON ALKALINE PHOSPHATASE ACTIVITY IN RHIZOSPHERIC SOIL OF ASIATIC LILY

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**ABSTRACT**

The experiment was conducted at Agri-tourism Centre, CCS HAU, Hisar (Haryana) during the year 2020-2021 to study the effect of bio inoculants on Alkaline Phosphatase activity in rhizospheric soil of crop Asiatic lily cv. Fangio under different growing conditions. The experiment was laid out in Randomized Block Design for bud parameters with three replications. The experiments consisted of three growing conditions (polyhouse, shadenet and open conditions) and eight different treatments of bio inoculants ($T_1$: Recommended dose of fertilizers (RDF), $T_2$: RDF + Azotobacter, $T_3$: RDF + PSB, $T_4$: RDF + Mycorrhiza, $T_5$: RDF + Azotobacter + PSB, $T_6$: RDF + PSB + Mycorrhiza, $T_7$: RDF + Azotobacter + Mycorrhiza, and $T_8$: RDF + Azotobacter + PSB + Mycorrhiza). Among the different growing conditions, the maximum Alkaline Phosphatase activity was observed under shadenet condition whereas, among the various treatments, the maximum Alkaline Phosphatase activity were recorded in $T_8$ (RDF + Azotobacter + PSB + Mycorrhiza).
***Keywords***: Asiatic Lily, Bio inoculants, PSB, Mycorrhiza, Azotobacter

## Introduction

Lilies (*Lilium species*) are significant ornamental bulbous plants belonging to the family Liliaceae. The genus *Lilium* comprises nearly 100 species and more than 9,400 cultivars (Fatmi *et al*., 2018), an essential geophyte with showy flowers, appealing colour, sturdy growth, and robust flowers. Lilium, known for its impressive size, stunning beauty, and long-lasting nature, is recognized as one of the top ten most exceptional cut flowers in the world (Thakur *et al.,* 2005).

The Asiatic Lily, scientifically known as *Lilium asiaticum*, is a popular and vibrant flowering plant known for its stunning blooms and ease of cultivation. Native to various parts of Asia, this lily is widely cultivated in gardens around the world due to its diverse color range, hardiness, and relatively low maintenance requirements.

Bio inoculants, also known as bio-fertilizers, are formulations containing active or dormant strains of microorganisms which are used to enhance plant growth without causing harm to human health or the environment (Alori *et al*., 2017). Biofertilizers are crucial in antibiotic production and the breakdown of organic matter in the soil, which enhances the soil's physical properties (Vithu *et al.,* 2018). These can be used alone or combined with algae or fungi components. They work by directly or indirectly enhancing microbial activity and increasing nutrient mobilization from the soil. These customized formulations leverage the functional characteristics of microorganisms to suit various soil systems and cropping patterns, promoting agricultural sustainability. The use of bio inoculants reduces the need for chemical fertilizers, leading to lower environmental impact. This sustainable practice supports long-term soil health and reduces the risk of

chemical runoff and pollution (Adesemoye *et al.,* 2009).

Bioinoculants improve the availability and uptake of essential nutrients like nitrogen, phosphorus, and potassium. For example, mycorrhizal fungi form symbiotic relationships with plant roots, increasing the root surface area for nutrient absorption (Smith *et al*., 2008). This results in more vigorous growth and enhanced flowering. Bio inoculants have been reported to improve the quality and yield of flowers by enhancing plant growth and vigor. This can result in larger, more colorful blooms and an increased number of flowers (Singh *et al.,* 2011). Mycorrhiza also promotes the secretion of Acc-deaminase, which inhibits the synthesis of ethylene and thus prolongs the life of the plant. It also affects the increase in the activity of antioxidant enzymes and can thus prolong the flowering life of cut flowers (Bhat *et al*., 2017)

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 *et al*., 2016). Enhanced phosphatase activity in soil increases the availability of phosphorus for plant uptake, resulting in better crop growth and yield. Thus, phosphatase activity is an essential indicator when studying phosphate-solubilizing microorganisms. Additionally, alkaline phosphatases (AKP) are specific enzymes involved in the symbiotic relationship between vesicular-arbuscular mycorrhizae (VAM) and plants.

The use of bio inoculants in different growing conditions like polyhouse and shadenet cultivation systems can significantly benefit the Asiatic Lilies. These benefits include improved nutrient uptake, enhanced growth and yield, better flower quality, increased disease resistance, and improved stress tolerance. By optimizing these controlled environments with the right bio inoculants, growers can achieve superior results in the cultivation of Asiatic Lilies.

Considering the above-mentioned points, this research aims to identify the optimal growing conditions and the most effective bio inoculant treatment to enhance phosphorus availability, thereby improving the growth and yield of Asiatic Lilies. The ultimate goal is to assist farmers in Haryana in increasing their income by optimizing these factors.

## Material and Methods

The present investigation entitled ″ Effect of growing conditions and bio inoculants on Alkaline phosphatase activity in polyhouse (µg PNP/g soil/h)" was carried out at Agri-tourism Centre, CCS HAU, Hisar (Haryana) during the cropping year 2020-2021. The experiments consisted of three growing conditions (polyhouse, shadenet and open conditions) with the physicochemical properties (Ph, EC, N, P and K) and microbiological parameters of soil were evaluated in the beginning of experiment (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 | 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 three replications. The experiments consisted of three growing conditions (Polyhouse, Shadenet and Open conditions) and eight different treatments of bio inoculants ($T_1$: Recommended dose of fertilizers (RDF), $T_2$: RDF + Azotobacter, $T_3$: RDF + PSB, $T_4$: RDF + Mycorrhiza, $T_5$: RDF + Azotobacter + PSB, $T_6$: RDF + PSB + Mycorrhiza, $T_7$: RDF + Azotobacter + Mycorrhiza, and $T_8$: RDF + Azotobacter + PSB + Mycorrhiza).

| | | |
|---|---|---|
| Replications | : | 3 |
| Design and layout | : | RBD |
| Spacing | : | 30 × 30 cm |
| Plot size | : | 1.2 m × 1.2 m |

Before sowing, the planting material that is bulbs were dipped in bioinoculant solution, ensuring they are fully submerged for 30 minutes followed by drying in shade for 30 minutes This soaking process helps the beneficial microorganisms adhere to the bulb surfaces and penetrate the bulb tissues. Bioinoculants were

procured from Department of Microbiology, CCS HAU, Hisar for the inoculation of Bulbs. Planting of inoculated bulbs was done in different conditions at a spacing of 30 x 30 cm.

**Alkaline Phosphatase activity:** Two 1-gram samples of oven-dry soil were placed in 50 ml Erlenmeyer flasks. To each flask, 0.2 ml of toluene and 4 ml of MUB were added. For one set of samples, 1 ml of p-nitrophenyl phosphate solution was also added. The flasks were gently swirled to mix the contents, then stoppered and incubated at 37°C. After one hour, the stoppers were removed, and 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH were added. The flasks were swirled again, and 1 ml of p-nitrophenyl phosphate solution was added to the control set. The soil suspension was then filtered through Whatman No. 1 filter paper, and the absorbance of the resulting yellow filtrate was measured at 420 nm using 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.

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

## Results and Discussion

### Alkaline Phosphatase activity in Polyhouse Conditions

The data presented in Table 2 indicates the alkaline phosphatase activity in Polyhouse conditions. The bio inoculants significantly affected the microbial activity. The Initial alkaline phosphatase activity recorded was 166.99 µg PNP released/g soil/1h. After 60 days of planting, the maximum alkaline phosphatase activity was observed in treatment $T_8$ (208.03 µg PNP released/g soil/ h) (RDF + *Azotobacter* + PSB + Mycorrhiza), whereas minimum alkaline phosphatase activity was observed in control. The microbial activity gradually decreased with crop maturity.

**Table 2 :** Effect of growing conditions and bio inoculants on Alkaline phosphatase activity in Polyhouse conditions

| Treatment | Alkaline phosphatase activity (µg PNP/g soil/h) | | | | |
|---|---|---|---|---|---|
| | 30 days | 60 days | 90 days | 120 days | 150 days |
| **$T_1$: RDF (Control)** | 167.48 | 169.38 | 169.22 | 168.56 | 168.08 |
| **$T_2$: RDF + *Azotobacter*** | 170.01 | 201.32 | 177.94 | 177.28 | 173.29 |
| **$T_3$: RDF + PSB** | 170.52 | 202.10 | 178.22 | 178.31 | 173.44 |
| **$T_4$: RDF + Mycorrhiza** | 173.36 | 205.45 | 181.18 | 180.14 | 175.35 |
| **$T_5$: RDF + *Azotobacter* + PSB** | 171.15 | 204.14 | 180.16 | 179.17 | 174.12 |
| **$T_6$: RDF + PSB + Mycorrhiza** | 175.01 | 207.23 | 183.27 | 182.37 | 177.63 |
| **$T_7$: RDF + Azo + Myco** | 174.38 | 206.17 | 182.02 | 181.45 | 176.34 |
| **$T_8$: RDF + Azo + PSB + Myco** | 176.37 | 208.03 | 185.40 | 183.38 | 178.07 |
| C.D. | 0.24 | 0.13 | 0.26 | 0.41 | 0.23 |

### Alkaline phosphatase activity in shadenet conditions

The data presented in Table 3 indicates the alkaline phosphatase activity in shadenet conditions. The bio inoculants significantly affected the microbial activity. The Initial alkaline phosphatase recorded was 175.77 µg PNP released/g soil/ h. After 60 days of planting, the maximum alkaline phosphatase activity was observed in treatment $T_8$ (216.18 µg PNP released/g soil/ h) (RDF + *Azotobacter* + PSB + Mycorrhiza) whereas minimum alkaline phosphatase activity was observed in control.

### Alkaline phosphatase activity in open field conditions

The data presented in Table 4 indicates the alkaline phosphatase activity in open field conditions. The bio inoculants significantly affected the microbial activity. The Initial alkaline phosphatase activity recorded was 159.43 µg PNP released/g soil/ h. After

60 days ofplanting, the maximum alkaline phosphatase activity was observed in treatment $T_8$ (200.47 µg PNP released/g soil/ h) (RDF + *Azotobacter* + PSB + Mycorrhiza) while minimum alkaline phosphatase activity was observed in control.

**Table 3 :** Effect of growing conditions and bio inoculants on Alkaline phosphatase activity in shadenet conditions

| Treatment | Alkaline phosphatase activity (µg PNP/g soil/h) | | | | |
|---|---|---|---|---|---|
| | 30 days | 60 days | 90 days | 120 days | 150 days |
| **$T_1$: RDF (Control)** | 176.26 | 178.16 | 178.00 | 177.34 | 176.86 |
| **$T_2$: RDF + *Azotobacter*** | 178.79 | 210.10 | 186.72 | 186.06 | 182.07 |
| **$T_3$: RDF + PSB** | 179.30 | 210.88 | 187.00 | 187.09 | 182.22 |
| **$T_4$: RDF + Mycorrhiza** | 182.14 | 214.23 | 189.96 | 188.92 | 184.13 |
| **$T_5$: RDF + *Azotobacter* + PSB** | 179.93 | 212.92 | 188.94 | 187.95 | 182.90 |
| **$T_6$: RDF + PSB + Mycorrhiza** | 183.79 | 216.01 | 192.05 | 191.15 | 186.41 |
| **$T_7$: RDF + Azo + Myco** | 183.16 | 214.95 | 190.80 | 190.23 | 185.12 |
| **$T_8$: RDF + Azo + PSB + Myco** | 185.15 | 216.81 | 194.18 | 192.16 | 186.85 |
| C.D. | 0.16 | 0.21 | 0.23 | 0.29 | 0.22 |

**Table 4 :** Effect of growing conditions and bio inoculants on Alkaline phosphatase activity in open field conditions

| Treatment | Alkaline phosphatase activity (µg PNP /g soil/h) | | | | |
|---|---|---|---|---|---|
| | 30 days | 60 days | 90 days | 120 days | 150 days |
| **$T_1$: RDF (Control)** | 159.92 | 161.82 | 161.66 | 161.00 | 160.52 |
| **$T_2$: RDF + *Azotobacter*** | 162.45 | 193.76 | 170.38 | 169.72 | 165.73 |
| **$T_3$: RDF + PSB** | 162.96 | 194.54 | 170.66 | 170.75 | 165.88 |
| **$T_4$: RDF + Mycorrhiza** | 165.80 | 197.89 | 173.62 | 172.58 | 167.79 |
| **$T_5$: RDF + *Azotobacter* + PSB** | 163.59 | 196.58 | 172.60 | 171.61 | 166.56 |
| **$T_6$: RDF + PSB + Mycorrhiza** | 167.45 | 199.67 | 175.71 | 174.81 | 170.07 |
| **$T_7$: RDF + Azo + Myco** | 166.82 | 198.61 | 174.46 | 173.89 | 168.78 |
| **$T_8$: RDF + Azo + PSB + Myco** | 168.81 | 200.47 | 177.84 | 175.82 | 170.51 |
| C.D. | 0.26 | 0.23 | 0.24 | 0.33 | 0.18 |

Among the growing conditions, shadenet reported the maximum (216.81 µg PNP/g soil/ h) alkaline phosphatase activity after 60 days of planting followed by polyhouse (208.03 µg PNP/g soil/ h). In contrast, minimum alkaline phosphatase activity was observed in open conditions (200.47 µg PNP/g soil/h). Alkaline phosphatase activity in rhizospheric soil ranged from (185.15-216.81 µg PNP/g soil/h), (176.37 – 208.03 µg PNP/g soil/h) and (168.81- 200.47 µg PNP/g soil/h) in treatment $T_8$ in shadenet, polyhouse and open conditions, respectively.

Phosphatases play a pivotal role in plant cell metabolism by regulating inorganic phosphate levels as the enzyme causes hydrolysis of p-nitrophenol phosphate into p-nitrophenol and inorganic phosphate (iP). This iP indirectly control the carbohydrate metabolism of plants. Phosphatases can be studied in crude cellular extracts or pure form. In the absence of the enzyme, the reaction would proceed very, very slowly. The increase in rhizosphere microbial population helps the plants to increase various enzymatic activities, including dehydrogenases, alkaline phosphatase, nitrogenase and hydrolysis activities. Dehydrogenase is involved in the oxidative cleavage of pyruvate, and it is decarboxylated to release ammonia. Phosphatase in soil plays an essential role in the mineralization process of organic phosphorus by catalyzing catalyzing hydrolytic cleavage from inorganic phosphorus compounds (Dick, 1980).

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