Effect of zeatin and kinetin on growth and quality of Lilium lancifolium grown in Haripur region

Raja Ahmad Ali[1], Umar Hayat[4**], Ijaz Hussain[1], Waseem Ahmed[1], Muhammad Anas Mehboob Malik[1], Malik Faizan Shaukat[1], Sughra Bibi[2], Alexander Yesaya[3], Qingtao Wang[4], Zhenyu Yao[4], Ravelomanana Julio Stanislas[5], Husnain[6]

[1.] Department of Horticulture, The University of Haripur, Pakistan.
[2.] Department of Chemistry, Hazara University Mansehra, Pakistan.
[3.] Department of Crop & Soil Sciences, Lilongwe University Of Agriculture & Natural Resource Malawi..
[4.] Zhengzhou Institute of fruit trees, Chinese Academy of Agriculture Sciences Zhengzhou 450009, China.
[5.] Anyang Institute of Cotton Research, Crop Genetics and Breeding, CAAS, China
[6.] Government post graduate college Mansehra, Pakistan

Abstract- Due of its great commercial and decorative value, lilies are in high demand as cut flowers or landscape plants all over the world. The use of biostimulants has attracted attention in the horticulture sector as a novel and promising strategy to guarantee increased and long-lasting yields as well as superior product quality. Current study investigating the "Effect of zeatin and kinetin on growth and quality of lilium lancifolium grown in Haripur region"—was carried out at Horticulture Nursary, The University of Haripur, Pakistan during 2020-2021. Following concentrations of Zeatin and Kinetin were applied as foliar spray after 12 days after sowing. Each treatment was prepared by standard procedures: To = Control, T1= 0.1% Zeatin, T2= 0.1% kinetin, T3=0.1% Zeatin and 0.1% kinetin. The data was collected after the foliar spray of zeatin and kinetin with 10 days' interval, SD1: 10 days after spray, SD2: 20 days after spray, SD3: 30 days after spray, SD4: 40 days after spray. Results reviled that application of 0.1% Zeatin and 0.1% kinetin and data observed after 40 days of spray resulted in significantly higher total number of leaves plant-1, total number of petals plant-1, plant height (cm), flower diameter, leaf area, total number of flowers plant-1. Chlorophyll content and photosynthetic rate of lily flower showed significant result for treatments applications and days after spray, whereas, their interaction remained non-significant. Protein content was affected significantly from treatments application only. From this study it is concluded that foliar application of 0.1% Zeatin and 0.1% kinetin helps in enhancing the growth and yield of lily flower and also found very effective in maintaining the quality of lily fruits under Agro-climatic conditions of Haripur region.

Keywords: Lily, Kinetin, Zeatin, biostimulants, lilium lancifolium , landscape

I. INTRODUCTION

The Liliaceae family of attractive plants includes the lily (Lilium longifolium). Horticulturists greatly value plants from the genus Lilium for its exceptional scent, variety of colors, resilience, and capacity to thrive in a variety of environmental situations[1]. Due to their enormous, fascinating, fragrant, and colourful flowers with a wide range of colours and a long shelf life, lilies are among the most significant, beautiful, and economically viable blossoming plants that can be found anywhere in the world. They are also one of the three most significant commercial bulb crops[2]. Since more than 2000 years ago, lilies have been used for a variety of occasions, such as making bouquets and decorating homes, hotels, and other high-end structures for weddings, funerals, and religious rituals[3]. It is produced commercially for cut flowers and potted plants and is mostly vegetatively propagated using bulbs. A few Easter lily varieties are offered for sale commercially[4]. In a variety of climates, lilies are widely cultivated. It is one of the top 10 cut flowers in the global floriculture market, coming in at number four[5]. About 10–12 thousand tonnes of cut flowers are produced in Pakistan each year, and floriculture is quickly becoming a successful business for the nation's small growers. However, Pakistan's participation in the global commerce in fresh cut flowers is negligible because of the low quality of its produce, which is caused by a lack of technology and formulas for nutrients and growth regulators[6]. To increase the stem length, quantity, quality, and shelf life of lily blooms, a variety of procedures are employed. Higher plants' growth, development, metabolism, and morphogenesis are all regulated by plant hormones[7].

A precise hormonal balance is needed for plants to reach their maximum growth potential, and auxins, gibberellins, abscisic acid, and cytokinins all play important roles in this process[8]. Derived from adenine, cytokinins affect a variety of plant functions, such as triggering parthenocarpy and controlling blossoming, senescence, and apical dominance[9]. Genetic engineering advances enable for precise gene editing for better fruit quality, as demonstrated by CRISPR/Cas9 technology[45]. Preharvest fruit qualities are improved by creative methods such as the synergistic application of chitosan, salicylic acid, and calcium chloride[46]. Plants such as Amaranthus hybridus can benefit from the use of nanoparticles, as demonstrated by the biogenic CuO and ZnO applications[47].

In various horticultural crops, including apple, pear, kiwi, watermelon, and others, the effects of some synthetic CKs have been investigated[10,11]. Thidiazuron and forchlorfenuron are two examples of synthetic CKs, and according to[12], they are the two most commonly utilised in viticulture. The highest leaf area ratio, absolute and relative growth rates, and net assimilation

rates were found in Basil. Due to the CK kinetin's action on these, which led to an increase in leaf area and dry matter, these underwent increased growth[13]. Additionally, cytokinins control the enzymatic activity, controlling the induction and activation of the protein synthesis required for the development of the photosynthetic system, as well as the creation and protection of cellular structures[14].

The most efficient PGRs for delaying senescence are cytokinins, which have been proven to prevent the breakdown of chlorophyll and photosynthetic proteins and to prevent the ageing of leaves and fruits[15]. One of these is zeatin, which promotes plant growth and productivity even in adverse environmental conditions. Given that this PGR cannot replace fertilizers but performs better when treated with other fertilizers, it was suggested that Zeatin exogenous therapy might be utilised in conjunction with other fertilizers. Zeatin was discovered to have a concentration that was wider than that of any other cytokinins in terms of concentration[16]. Zeatin is also a component in the delivery and distribution of carbs to the sink, where additional carbohydrates are needed to support the body's constantly expanding growth demands. This was demonstrated by[17, 18], who came to the conclusion that Zeatin riboside has a greater influence on the movement of carbs than it does on the movement of proteins. Thus keeping in mind the facts, the current study was undertaken with following objectives:

Objectives

This study intends to investigate how well Lily (Lilium longifolium) performs when cultivated in Haripur, Pakistan, which has a special climatic environment. We also want to clarify the possible advantages of two essential plant growth regulators, Zeatin and Kinetin, on the development and quality of Lily flowers. Furthermore, we try to distinguish between Zeatin and Kinetin's effects on Lily flowers, with a focus on how they affect flower growth, quality, and performance in general. These goals guide our research as we discover insightful information on lily cultivation and flower development, ultimately advancing our knowledge of how to regulate plant growth and use horticultural techniques in many environmental situations.

2. Materials and methods

The study entitled "Effect of zeatin and kinetin on growth and quality of lilium lancifolium grown in Haripur region" was carried out at Horticulture Nursery, The University of Haripur, Pakistan during 2020-2021.

2.1 Experimental Design

The study was laid in RCBD having 2 factor factorial arrangements having three replications. All agricultural operations were carried out uniformly and evenly in all treatments throughout study period. Rest of the treatments were maintained as per experimental protocol.

2.2 Preparation for Treatment

Thermo Fisher Scientific is where the zeatin and kinetin utilised in this study were purchased.

With their great purity, these plant growth regulators were ideal for experiments. To guarantee consistency and efficacy, the treatments were prepared using exacting procedures.

2.3 Treatment Application

Following concentrations of Zeatine and Kinetin were applied as foliar spray after 12 days after sowing. Each treatment was prepared by standard procedures. For the best absorption by plant tissue, zeatin and kenetin treatments were properly made. The solvent for its plant-friendly qualities was distilled water. To ensure consistency, exact cytokinin concentrations were determined using analytical balances. pH changes were made in accordance with plant conditions, and gradual addition and stirring allowed for full dissolution. Consistent and dependable experimental results were the goal of this exact process.

To = Control
T1= 0.1% Zeatin
T2= 0.1% kinetin
T3=0.1% Zeatin and 0.1% kinetin

2.4 Data Duration

The data was collected after the foliar spray of zeatin and kinetin with 10 days' interval.

1: 10 days after spray.
2: 20 days after spray.
3: 30 days after spray.
4: 40 days after spray.

2.5 Cultural Operations

2.5.1 Preparatory Cultivation

The seeds of lily plant were purchased from local market and sown in pots at research field area of University of Haripur. Pots were filled with suitable potting media.

2.5.2 Plant Protection Measures

In order to detect visual variations between the treatments and any kind of infestation by weeds, insects and diseases, the field was visited from time to time so that major pest losses could be reduced.

2.6 parameters examine

In this study, a number of parameters relating to the development, production, and quality of lily plants were systematically investigated. These parameters gave us important information about the general growth and performance of the lily plants under investigation.

2.6.1 Vegetative parameters

2.6.2 Number of Leaves:

Throughout each stage of the study, the number of leaves on each lily plant was manually counted at scheduled times. This parameter served as an indicator of the plant's foliage growth and vitality.

2.6.3 Number of Petals:

After the lily flowers had finished blooming, the number of petals on each individual flower was manually counted. This variable helped in the evaluation of floral elegance and quality.

2.6.4 Number of Roots:

At the time of harvest, the number of roots per lily plant was manually counted. This parameter revealed information on the growth of the plant's root system and potential nutrient uptake.

2.6.5 Plant Height:

Throughout every stage of the experiment, the height of each lily plant was measured using a scale. Tracking vertical growth and overall plant stature required constant observation of plant height.

2.6.6 Flower Diameter:

Using a Vernier caliper, the diameter of lily flowers was precisely measured and the findings were represented in centimeters. This parameter made it possible to measure the size and symmetry of flowers.

2.6.7 Leaf Area:

Using a measuring tape, the total leaf area of each lily plant was calculated. A significant indicator of the plant's ability to photosynthesize and general health was its leaf area.

2.6.8 Number of Flowers:

After the formation of flower buds, the number of flowers was counted. This metric gave crucial insight into the lily plant's ability for reproduction and potential yield.

2.7 Chlorophyll

The process of collecting samples for the chlorophyll content measurement method was improved by carefully weighing new leaves to ensure consistency. Leaves were homogenised at a regulated low temperature in 80% acetone (v/v) using a particular homogenizer model. Chlorophyll extraction was optimised throughout the next 24 to 48 hours of darkness incubation. Solution clarity was guaranteed by centrifugation. With a designated spectrophotometer model, precise findings were obtained from spectrophotometric measurements at 663 nm and 646 nm. In order to calculate the overall content of the chlorophyll, the quantities of chlorophyll-a and chlorophyll-b were added together using validated equations[19].

2.8 Protein Content

We have precisely optimised the approach to increase the rigour of our protein content investigations. Accuracy in sample collection was guaranteed by carefully weighing fresh leaves using Panasonic Japan analytical balance. Consistency in protein extraction was maintained by using a phosphate-buffered saline solution and a "BeadBugTM homogenizer." Using an Eppendorf 5810R centrifuge, centrifugation ensured the removal of detritus for precise protein quantification. In accordance with[20] standard protocols, the Bradford test used a spectrophotometer to measure absorbance at the right wavelength. The protein concentration determination was made more precise by adding a standard curve that was calibrated using accepted protein standards in accordance with[21] standard methodologies.

2.8.1 Photosynthetic Rate

Photosynthetic Rate were computed by[43] (2004) with some amendments Utilising infrared spectroscopy and an infrared gas analyzer, measurements of the amount of CO2 entering and leaving the system were made in order to calculate the photosynthetic rate. How much CO2 is used in photosynthesis is shown by the variation in these measurements. The observed changes in CO2 concentration throughout the designated time period were used to compute the rate of photosynthesis using established formulae or standard calibration curves.

2.9 Statistical Analysis:

Analysis of variance was performed to confirm variability of data and validity of results using computer based software Statistix 8.1. The differences amongst treatments were separated using least significance difference test (LSD) at 0.05 probability level[22].

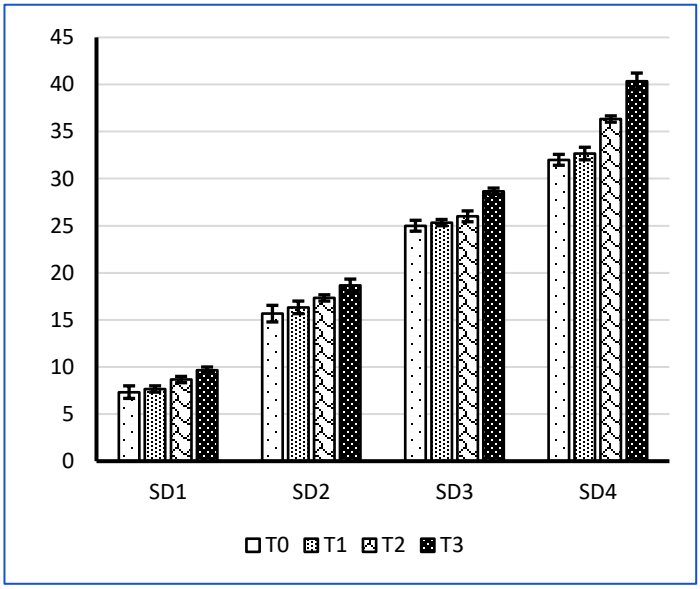
3. Results and Discussion

3.1 Total number of leaves plant-1

The total number of leaves on plant-1 was significantly affected by the foliar application of zeatin and kinetin, as well as by their interaction days following application (figure 3.1). The maximum number of leaves plant-1 (24.3) was obtained from the application of 0.1% kinetin and 0.1% zeatin, which differed significantly from other treatments ($p < 0.05$).

Zeatin and kienetin applied together had significant influence on Lilium lancifolium growth traits. Zeatin and Kinetin may work in concert to promote Lilium lancifolium's general development, as seen by the observed increase in flower number, taller plants, and larger flowers. These growth regulators probably work in concert to activate complimentary pathways or processes that improve various aspects of plant development[23]. According to earlier research, zeatin and kinetin have different functions in plant growth; zeatin stimulates cell division, while kinetin affects blooming and bud formation[42]. The improvements in grow abundance, plant height, and floral diameter that have been reported may have been caused by a balanced response resulting from the combined application. Further investigation could focus on the specific molecular and physiological mechanisms behind these synergistic effects[23].

Figure 3.1: Effect of zeatin and kinetin on number of leaves plant-1 of lilium lancifolium



LSD for treatments = 0.57 LSD for spraying days = 0.57
LSD for treatments x spraying days = 1.24

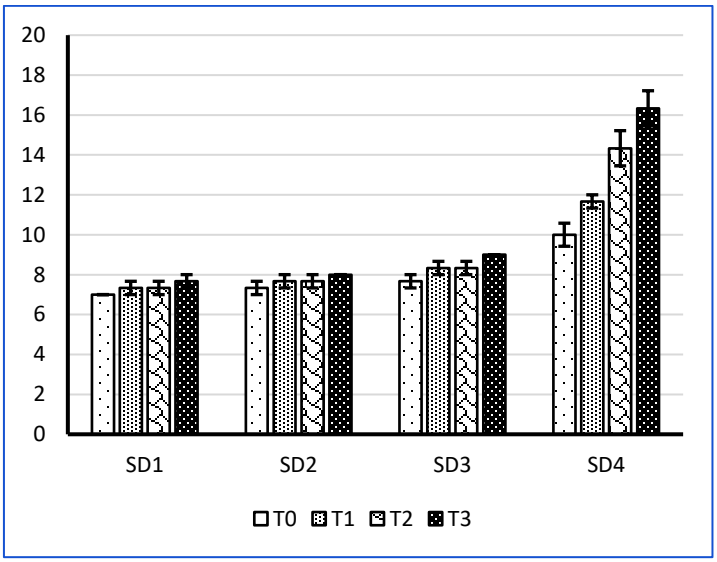
3.2 Number of petals plant-1

Figure 3.2 demonstrates the number of petals plant-1 of Lilium lancifolium enhanced by kinetin and zeatin foliar applications. The analysis of variance demonstrated that foliar application of kinetin and zeatin, days after application, and their interaction had a significant impact on the total number of petals plant-1. The highest number of petals plant-1 (10.25) was obtained with the application of 0.1% Zeatin and 0.1% kinetin, followed by (9.41) with the application of 0.1% kinetin. Regarding application days, the largest number of petals plant-1 was observed in SD4 (40 days after spray), followed by 30 days after

spray, and the lowest number of petals plant-1 was reported in plants sprayed10 days after.

In line with previous findings[24], which indicated that cytokinin administration reduced the total amount of phenolic compounds in Diospyros lotus fruits, and as reported by[25], where cytokinins were shown to be potent growth promoters, our findings suggest that Zeatin and Kinetin have a positive effect on the number of petals plant-1. The combined activity of these growth regulators, especially after 40 days of spraying, resulted in highest petals plant-1.

Figure 3.2: Effect of zeatin and kinetin on number of petals plant-1 of lilium lancifolium

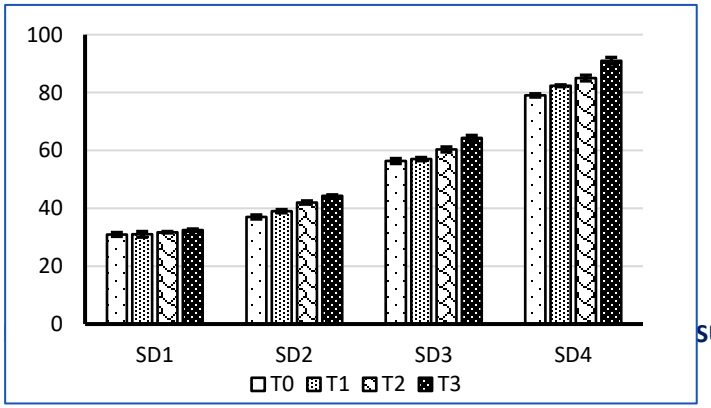


LSD for treatments = 0.62 LSD for spraying days = 0.62
LSD for treatments x spraying days = 1.24

3.3 Plant height (cm)

The effect of zeatin and kinetin on the plant height (cm) of Lilium lancifolium is shown in Figure 3.3. The analysis of variance results reveal that foliar applications of kinetin and zeatin, as well as their interaction, have significant effects on plant height. The tallest plants (58.05 cm) were observed with 0.1% Zeatin and 0.1% kinetin application, followed by (54.76 cm) with 0.1% kinetin application. In terms of application days, the tallest plants (84.33 cm) were observed in SD4 (40 days after spray), followed by (59.5 cm) 30 days after spray, and the smallest plants (10 days after spray). The interaction of zeatin and kinetin after 40 days of spraying resulted in the maximum plant height (91 cm). These findings are consistent with recent investigations on peace lily[26] and croton plants[27].

Figure 3.3: Effect of zeatin and kinetin on plant height (cm) of lilium lancifolium



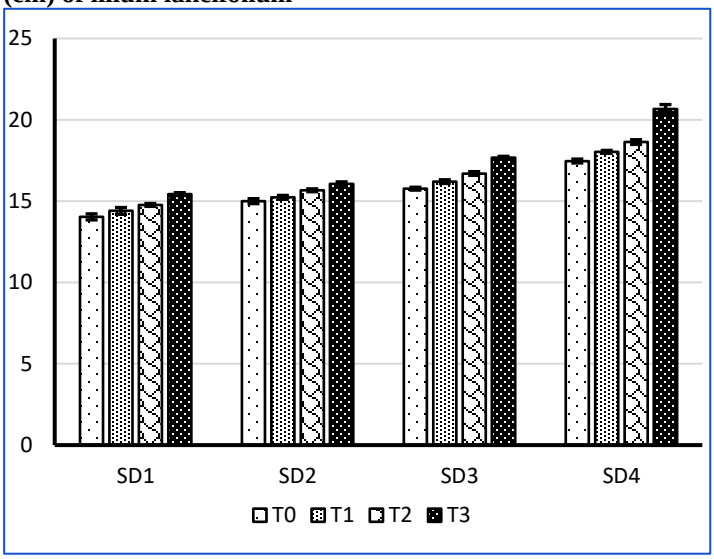
LSD for treatments = 1.07 LSD for spraying days = 1.07
LSD for treatments x spraying days = 2.15

3.4 Flower diameter (cm)

Figure 3.4 illustrates the effect of zeatin and kinetin on the flower diameter (cm) of Lilium lancifolium. The analysis of variance results show that the foliar application of kinetin and zeatin, as well as the number of days after application, had a significant influence on flower diameter. The highest flower diameter (17.45 cm) was found when 0.1% Zeatin and 0.1% kinetin were applied, followed by (16.42 cm) when 0.1% kinetin was applied.

In regard to application days, the greatest flower diameter (18.7 cm) was noted at SD4 (40 days after spray), followed by (16.58 cm) at 30 days after spray, and the smallest flower diameter (10 days after spray). In terms of interaction, 0.1% zeatin and 0.1% kinetin applied after 40 days of spraying resulted in the largest flower diameter (20.66 cm). The use of these plant growth regulators lengthened the vegetative period of the plants, resulting in the development of more photo assimilates and healthier flowers. Similar effects of cytokinins on flower growth have previously been reported[28]. Furthermore, the use of cytokinins has been linked to a decrease in the overall amount of phenolic compounds in specific fruits[24].

Figure 3.4: Effect of zeatin and kinetin on flower diameter (cm) of lilium lancifolium



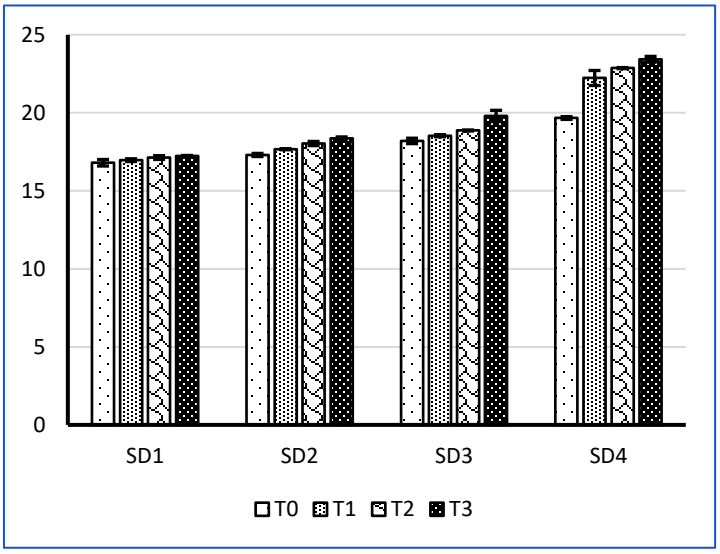
LSD for treatments = 0.20 LSD for spraying days = 0.20
LSD for treatments x spraying days = 0.40

3.5 Leaf area

The effect of zeatin and kinetin on the leaf area of Lilium lancifolium can be seen in Figure 3.5. The results of the analysis of variance show that the foliar application of kinetin and zeatin, as well as the number of days after treatment, had a significant effect on leaf area. The application of 0.1% Zeatin and 0.1% kinetin resulted in the greatest leaf area (19.7), followed by (19.22) with the application of 0.1% kinetin. In terms of application days, the maximum leaf area (22.05) was obtained at

SD4 (40 days after spray), followed by (18.85) at 30 days after spray, and the smallest leaf area (17.03) at 10 days after spray.
Regarding the effectiveness of the interaction, the maximum leaf area (23.43) was observed after 40 days of spraying with 0.1% zeatin and 0.1% kinetin, whereas the smallest leaf area was recorded without kinetin and zeatin application. In several species, stress has been found to reduce the export of cytokinins such as kinetin from the root to the shoot. However, sufficient shoot kinetin is essential for healthy growth and development[29]. As a result, exogenous cytokinin administration is expected to attenuate the consequences of stress. These findings are similar with previous studies that showed kinetin treatment increases leaf area, as shown by[30], who discovered that foliar KIN treatment increased wheat leaf yield and chlorophyll content.

Figure 3.5: Effect of zeatin and kinetin on leaf area of lilium lancifolium

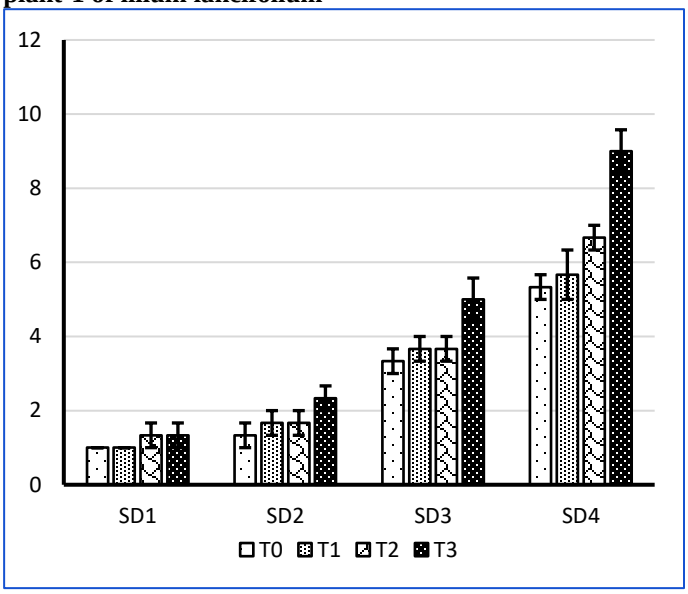


LSD for treatments = 0.27 LSD for spraying days = 0.27
LSD for treatments x spraying days = 0.54

3.6 Number of flowers plant-1

The effect of kinetin and zeatin on the number of flowers per plant in Lilium lancifolium can be observed in Figure 3.6. The total number of flowers per plant was significantly influenced by the foliar application of zeatin and kinetin as well as the number of days after treatment, according to the analysis of variance data. The greatest number of flowers per plant (4.41) were obtained from the application of 0.1% kinetin and 0.1% zeatin. This was followed by (3.3) from the application of 0.1% kinetin.
With regard to application days, the highest number of flowers per plant (6.66) was noted at SD4 (40 days after spraying), the lowest number of flowers per plant (1.1) was noted at 10 days after spraying, and the highest number of flowers per plant (3.91) at 30 days after spraying. Regarding the interaction, after 40 days of spraying, the application of 0.1% zeatin and 0.1% kinetin resulted in the greatest number of the flowers per plant (9). The application of cytokinins results in more flowers, which is consistent with earlier findings in gladiolus and tuberose[31,32]. Particularly, among the cytokinins, gladiolus has been shown to produce more florets when exposed to kinetin and zeatin[33].

Figure 3.6: Effect of zeatin and kinetin on number of flowers plant-1 of lilium lancifolium



LSD for treatments = 0.55 LSD for spraying days = 0.55
LSD for treatments x spraying days = 1.11

3.7 Chlorophyll content

Table 3.1 [25] demonstrates how the application of kinetin and zeatin affected the amount of chlorophyll in Lilium lancifolium. Chlorophyll concentration in lily flowers was found to be significantly influenced by the number of days after spraying and the foliar application of zeatin and kinetin, according to the results of the analysis of variance. Applying 0.1% kinetin and 0.1% Zeatin resulted in the highest chlorophyll content (3.91 mg g-1) and 0.1% kinetin resulted in the lowest (3.79 mg g-1). The results of this study are supported by earlier research showing that cytokinins cause lily leaves to display higher quantities of chlorophyll [34,35].

Table 3.1: Effect of zeatin and kinetin on chlorophyll content of lilium lancifolium

	T0	T1	T2	T3	Means
SD1	3.33 NS	3.4567	3.3867	3.57	**3.4358 c**
SD2	3.74	3.89	3.8033	3.9667	**3.8500 b**
SD3	3.7767	3.9467	3.86	4.1367	**3.9300 a**
SD4	3.8067	3.89	3.8867	3.97	**3.8883 ab**
Means	**3.6633 d**	**3.7958 b**	**3.7342 c**	**3.9108 a**	

LSD for treatments = 0.06 LSD for spraying days = 0.06
LSD for treatments x spraying days = Non-significant

3.8 Photosynthetic rate

Table 3.2 shows the fascinating effects of kinetin and zeatin on the photosynthetic activity of Lilium lancifolium. It is clear from a thorough variance study that the combination of kinetin and zeatin, as well as the days after spraying, compose a botanical symphony of great importance.

At 0.1% Zeatin and 0.1% Kinetin, the summit of this verdant crescendo is attained, with an astonishing 2.14 photosynthetic rate peak. 0.1% kinetin follows closely behind, producing an impressive rate of 2.03. From a temporal perspective, the peak occurs at SD4 (40 days after the spray), where the rate peaks at 2.41. On the other hand, after 10 days post-spray, the early stage experiences a low rate of 1.69, which is suggestive of the dynamic transformation in the photosynthetic symphony.

Zeatin and kinetin work in concert after 40 days of spraying, adding a resounding rhythm that drives the photosynthetic rate to its peak at 2.41—a very high example of the biochemical concerto these growth regulators have organised. According to research on biostimulants, higher photosynthetic rates are a sign of stimulated primary metabolism. These results are consistent with that conclusion. The fundamental substances increase the production of chlorophyll, which is one of the reasons for the increased levels of photosynthetic activity and chlorophyll seen in this botanical odyssey[36,37,38].

Table 3.2: Effect of zeatin and kinetin on photosynthetic rate of lilium lancifolium

	T0	T1	T2	T3	Means
SD1	**1.6367 NS**	**1.69**	**1.7067**	**1.76**	**1.6983 d**
SD2	1.8067	1.646	1.9233	1.9967	**1.8432 c**
SD3	1.93	1.9567	2.03	2.22	**2.0342 b**
SD4	2.3333	2.25	2.4733	2.6167	**2.4183 a**
Means	**1.9267 c**	**1.8857 c**	**2.0333 b**	**2.1483 a**	

LSD for treatments = 0.09 LSD for spraying days = 0.09
LSD for treatments x spraying days = Non-significant

3.9 Total proteins

We examine the effects of zeatin and kinetin on the total protein content of Lilium lancifolium in Table 3.3 [25]. The findings, which read like a biochemical sonnet, demonstrate the significant impact that these growth regulators have on the plant's protein orchestra.

0.1% Zeatin and 0.1% Kinetin compose a masterpiece at the pinnacle of this protein symphony, resulting in the greatest total protein content at 27.07. The 0.1% kinetin solo act comes in right after with an amazing 24.47. These results don't only change over time; they hold true throughout the spraying days. The composition of this botanical concerto is stable and develops without any notable interactions. Cytokinins have a balanced effect on the amount of protein in lilies, as demonstrated by earlier research[39]. With kinetin and zeatin strokes, this chapter of the biochemical tale comes to a harmonious close with an abundance of total protein, adding to the plant's grandeur[40, 41,44].

Table 3.3: Effect of zeatin and kinetin on Total proteins of lilium lancifolium

	T0	T1	T2	T3	Means
SD1	17.967NS	24.367	21.267	26.933	**22.633 NS**
SD2	18.033	24.4	21.2	26.9	**22.633**
SD3	18.1	24.433	21.533	27.1	**22.792**
SD4	18.267	24.7	21.333	27.333	**22.908**
Means	**18.092 d**	**24.475 b**	**21.333 c**	**27.067 a**	

LSD for treatments = 0.45 LSD for spraying days = Non-significant
LSD for treatments x spraying days = Non-significant

3.10 A review of prior research and new approaches is presented in "Comparative Insights and Synergistic Effects of Zeatin and Kinetin on Lilium lancifolium Growth"

In comparison to previous studies, our findings indicate that zeatin and kinetin have a significant impact on Lilium lancifolium growth traits. Notably, the combination of 0.1% zeatin and 0.1% kinetin produced excellent results, promoting higher flower number, taller plants, larger flowers, and improved leaf characteristics. These findings are similar with past study on peace lily and croton plants, demonstrating that these cytokinins have a consistent favourable effect across multiple plant species. Our findings support the idea that zeatin and kinetin interact in tandem, activating complimentary pathways that contribute to overall plant development. Notable studies include the CRISPR/Cas9 editing of lycopene epsilon-cyclase in banana fruit by[45] and the preharvest applications of chitosan, salicylic acid, and calcium chloride on date palm fruit by[46]. These investigations offer further insights into genetic engineering techniques and alternative methods to enhance plant growth. Additionally, nanoparticles—which[47] explored—offer a novel avenue for sustainable growth. The variety of studies supports the robustness of our findings and creates avenues for additional

research into the molecular and physiological mechanisms underlying the synergistic effects observed.

4. Conclusions

Our study concludes through demonstrating the substantial advantages of foliar applications of 0.1% zeatin and 0.1% kinetin on the growth and quality of Lilium lancifolium under the specific agro-climatic conditions of the Haripur region. The findings show that these biostimulants not only enhance vegetative characteristics like quantity of leaves and flowers, but also support increased plant height and flower diameter. In addition, the treatment significantly increases leaf area, chlorophyll content, photosynthetic rate, and total protein content—all vital indicators of plant health and productivity.

These results highlight the potential of Zeatin and Kinetin as useful tools for farmers and horticulturists looking to maximize the output and quality of lily flowers in similar environments. These biostimulants present a viable approach for improving lily cultivation outcomes by extending the vegetative period and reducing the effects of stress on chlorophyll and photosynthetic activity. Additional study may explore the precise mechanisms underlying these enhancements and their applications in other floral crops.

Conflict of Interest

The authors declare no conflict of interest

Author contributions

Manuscript title: **Effect of zeatin and kinetin on growth and quality of *Lilium lancifolium* grown in Haripur region**

Conceived and designed the experiments: Raja Ahmad Ali, Umar Hayat ,

Performed the experiments: ……Raja Ahmad Ali

Analyzed the data: … Umar Hayat, Ijaz Hussain, Waseem Ahmed, Alexander Yesaya, Qingtao Wang.

Contributed reagents/materials/analysis tools: ……Muhammad Anas Mehboob Malik[1], Malik Faizan Shaukat, Zhenyu Yao, Ravelomanana Julio Stanislas, Husnain.

Wrote the paper: Raja Ahmad Ali, Umar Hayat, Sughra Bibi

Name, address etc. of corresponding author: Umar Hayat, Zhengzhou Institute of fruit trees, Chinese Academy of Agriculture Sciences Zhengzhou 450009, China.

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References

1. Rafiq, S., Rather, Z.A., Bhat, R.A., Nazki, I.T., Al-Harbi, M.S., Banday, N., Farooq, I., Samra, B.N., Khan, M.H., Ahmed, A.F. and Andrabi, N., 2021. Standardization of in vitro micropropagation procedure of Oriental Lilium Hybrid Cv.'Ravenna'. *Saudi Journal of Biological Sciences*, *28*(12), pp.7581-7587.
2. Youssef, N.M., Shaaban, S.A., Ghareeb, Z.F. and Taha, L.S., 2019. In vitro bulb formation of direct and indirect regeneration of Lilium orientalis cv."Starfighter" plants. *Bulletin of the National Research Centre*, *43*(1), pp.1-9.
3. Askari, N., Visser, R.G. and De Klerk, G.J., 2018. Growth of lily bulblets in vitro, a review. *International Journal of Horticultural Science and Technology*, *5*(2), pp.133-143.
4. Alam, A., Iqbal, M. and Vats, S., 2013. Cultivation of Some overlooked Bulbous Ornamentals-A review on its commercial viability. Report and opinion, 5, pp.9-34.
5. Bhandari, N.S. and Aswath, C., 2018. Standardization of an effective protocol for in vitro culture of Lilium longiflorum Thunb. cv. Pavia. International Journal of Current Microbiology and Applied Sciences, 7(04), pp.1183-1190.
6. Sajid, G.M., Kaukab, M. and Ahmad, Z., 2009. Foliar application of plant growth regulators (PGRs) and nutrients for improvement of lily flowers. Pakistan Journal of Botany, 41(1), pp.233-237.
7. Ataklı, S.B., Şahin, S. and Alkaç, O.S., 2021. The Effect of Humic Acid Applications on the Development of Lilium Candidum Plant, Phenological and Pomological Observations in the Plant. Turkish Journal of Agriculture-Food Science and Technology, 9(sp), pp.2670-2674.
8. de Moura, F.B., Vieira, M.R.D.S., Simoes, A.D.N., Ferreira-Silva, S.L., de SOUZA, C.A., de Souza, E.S., da ROCHA, A.T., da SILVA, L.F. and Miguel, A., 2018. Physiological effect of kinetin on the photosynthetic apparatus and antioxidant enzymes activities during production of anthurium. Horticultural Plant Journal, 4(5), pp.182-192.
9. Hassan, F.E., Alyafei, M.A., Kurup, S., Jaleel, A., Al Busaidi, N. and Ahmed, Z.F., 2023. Effective Priming Techniques to Enhance Ghaf (Prosopis cineraria L. Druce) Seed Germination for Mass Planting. Horticulturae, 9(5), p.542.
10. Huitrón, M.V., Diaz, M., Diánez, F., Camacho, F. and Valverde, A., 2007. Effect of 2, 4-D and CPPU on triploid watermelon production and quality. HortScience, 42(3), pp.559-564.
11. Aremu, A.O., Fawole, O.A., Makunga, N.P., Masondo, N.A., Moyo, M., Buthelezi, N.M., Amoo, S.O., Spíchal, L. and Doležal, K., 2020. Applications of cytokinins in horticultural fruit crops: Trends and future prospects. Biomolecules, 10(9), p.1222.
12. Ahanger, M.A., Aziz, U., Alsahli, A.A., Alyemeni, M.N. and Ahmad, P., 2020. Combined kinetin and spermidine treatments ameliorate growth and photosynthetic inhibition in Vigna angularis by up-regulating antioxidant and nitrogen metabolism under cadmium stress. Biomolecules, 10(1), p.147.
13. Chen, L., Zhao, J., Song, J. and Jameson, P.E., 2020. Cytokinin dehydrogenase: a genetic target for yield improvement in wheat. Plant Biotechnology Journal, 18(3), pp.614-630.
14. Khalil, H.A., El-Ansary, D.O. and Ahmed, Z.F., 2022. Mitigation of salinity stress on pomegranate (Punica granatum L. cv. Wonderful) plant using salicylic acid foliar spray. Horticulturae, 8(5), p.375.

15. Pospíšilová, J., Synková, H. and Rulcová, J., 2000. Cytokinins and water stress. Biologia plantarum, 43(3), pp.321-328.
16. Al-Hussein, S., Shibli, R. and Karam, N.S., 2006. Regeneration in African Vilet (Saintpaulia ionantha Wendl.) Using Different Leaf Explants, Cytokinins Sources, and Lighrt Regimes. Jordan Journal of Agricultural Sciences.
17. Letham, D.S., 2019. Cytokinins as phytohormones—sites of biosynthesis, translocation, and function of translocated cytokinin. In Cytokinins (pp. 57-80). CRC press.
18. Wang, X.L., Guo, X.L., Hou, X.G., Zhao, W., Xu, G.W. and Li, Z.Q., 2014. Effects of leaf zeatin and zeatin riboside induced by different clipping heights on the regrowth capacity of ryegrass. Ecological research, 29, pp.167-180.
19. Nadeem, A., Ahmed, Z.F.R., Hussain, S.B., Omar, A.E.D.K., Amin, M., Javed, S., Ali, A., Ullah, S., Razzaq, K., Rajwana, I.A. and Nayab, S., 2022. On-tree fruit bagging and cold storage maintain the postharvest quality of mango fruit. Horticulturae, 8(9), p.814.
20. Rieder, A., Afseth, N.K., Böcker, U., Knutsen, S.H., Kirkhus, B., Mæhre, H.K., Ballance, S. and Wubshet, S.G., 2021. Improved estimation of in vitro protein digestibility of different foods using size exclusion chromatography. Food Chemistry, 358, p.129830.
21. Wider, G. and Dreier, L., 2006. Measuring protein concentrations by NMR spectroscopy. Journal of the American Chemical Society, 128(8), pp.2571-2576.
22. Gomez, K.A. and Gomez, A.A., 1984. Statistical procedures for agricultural research. John Wiley & Sons.
23. Janowska, B. and Jerzy, M., 2003. Effect of gibberellic acid on post-harvest. Journal of Fruit and Ornamental Plant Research, 100, pp.69-76.
24. Zhang, G., Sakai, H., Yoshimoto, M., Wakatsuki, H., Tokida, T., Ikawa, H., Arai, M., Nakamura, H. and Hasegawa, T., 2021. Effect of foliar spray of kinetin on the enhancement of rice yield by elevated CO2. Journal of Agronomy and Crop Science, 207(3), pp.535-543.
25. Vankova, R., 2014. Cytokinin regulation of plant growth and stress responses. Phytohormones: a window to metabolism, signaling and biotechnological applications, pp.55-79.
26. Mohammadipour, R., 2012. Effect of Fe fertilization by soil or foliar application on growth traits of Spathiphyllum (Doctoral dissertation, M. Sc. Thesis, Islamic Azad University, Rasht Branch, Rasht, Iran.(In Persian)).
27. Ibrahim, S.M., Taha, L.S. and Farahat, M.M., 2010. Vegetative growth and chemical constituents of croton plants as affected by foliar application of benzyl adenine and gibberellic acid. Journal of American Science, 6(7), pp.126-130.
28. Andrew, I.K.S., Storkey, J. and Sparkes, D.L., 2015. A review of the potential for competitive cereal cultivars as a tool in integrated weed management. Weed Research, 55(3), pp.239-248.
29. Bakhtavar, M.A., Afzal, I., Basra, S.M.A., Ahmad, A.U.H. and Noor, M.A., 2015. Physiological strategies to improve the performance of spring maize (Zea mays L.) planted under early and optimum sowing conditions. PloS one, 10(4), p.e0124441.
30. Lalarukh, I., Ashraf, M.A., Azeem, M., Hussain, M., Akbar, M., Ashraf, M.Y., Javed, M.T. and Iqbal, N., 2014. Growth stage-based response of wheat (Triticum aestivum L.) to kinetin under water-deficit environment: pigments and gas exchange attributes. Acta Agriculturae Scandinavica, Section B—Soil & Plant Science, 64(6), pp.501-510.
31. Hassanpour Asil, M., Roein, Z. and Abbasi, J., 2011. Response of tuberose (Polianthes tuberose L.) to gibberellic acid and benzyladenine. Horticulture, Environment, and Biotechnology, 52(1), pp.46-51.
32. Patil, R.H., Babu, R.L., Naveen Kumar, M., Kiran Kumar, K.M., Hegde, S.M., Ramesh, G.T. and Chidananda Sharma, S., 2015. Apigenin inhibits PMA-induced expression of pro-inflammatory cytokines and AP-1 factors in A549 cells. Molecular and Cellular Biochemistry, 403, pp.95-106.
33. Sajid, M., Anjum, M.A. and Hussain, S., 2015. Foliar application of plant growth regulators affects growth, flowering, vase life and corm production of Gladiolus grandiflorus L. under calcareous soil. Bulgarian Journal of Agricultural Science, 21(5), pp.982-989.
34. Faraji, S., Naderi, R., Ibadli, O.V., Basaki, T., Gasimov, S.N. and Hosseinova, S., 2011. Effects of post harvesting on biochemical changes in Gladiolus cut flowers cultivars [White prosperity]. Middle East Journal of Scientific Research, 9(5), pp.572-577.
35. Emami, H., Saeidnia, M., Hatamzadeh, A., Bakhshi, D. and Ghorbani, E., 2011. The effect of gibberellic acid and benzyladenine in growth and flowering of Lily (Lilium longiflorum). Advances in Environmental Biology, pp.1606-1612.
36. Bulgari, R., Franzoni, G. and Ferrante, A., 2019. Biostimulants application in horticultural crops under abiotic stress conditions. Agronomy, 9(6), p.306.
37. Yaméogo, C.W., Bengaly, M.D., Savadogo, A., Nikiema, P.A. and Traore, S.A., 2011. Determination of chemical composition and nutritional values of Moringa oleifera leaves. Pakistan Journal of Nutrition, 10(3), pp.264-268.
38. Mbandlwa, N.P., Fotouo-M, H., Maboko, M.M. and Sivakumar, D., 2020. Stomatal conductance, leaf chlorophyll content, growth, and yield of sweet pepper in response to plant growth regulators. International Journal of Vegetable Science, 26(2), pp.116-126.
39. Gulzar, S., Tahir, I., Amin, I., Farooq, S. and Sultan, S.M., 2003, August. EFFECT OF CYTOKININS ON THE SENESCENCE AND LONGEVITY OF ISOLATED FLOWERS OF DAY LILY (HEMEROCALLIS FULVA) CV ROYAL CROWN SPRAYED WITH CYCLOHEXIMIDE. In VIII International Symposium on Postharvest Physiology of Ornamental Plants 669 (pp. 395-404).
40. Ejaz, B., Sajid, Z.A. and Aftab, F., 2012. Effect of exogenous application of ascorbic acid on antioxidant enzyme activities, proline contents, and growth parameters of

Saccharum spp. hybrid cv. HSF-240 under salt stress. Turkish Journal of Biology, 36(6), pp.630-640.

41. Dawood, M.G., Abdel-Baky, Y.R., El-Awadi, M.E.S. and Bakhoum, G.S., 2019. Enhancement quality and quantity of faba bean plants grown under sandy soil conditions by nicotinamide and/or humic acid application. Bulletin of the National Research Centre, 43(1), pp.1-8.
42. Biles, S.P. and Cothren, J.T., 2001. Flowering and yield response of cotton to application of mepiquat chloride and PGR-IV. Crop Science, 41(6), pp.1834-1837.
43. Teng, S., Qian, Q., Zeng, D., Kunihiro, Y., Fujimoto, K., Huang, D. and Zhu, L., 2004. QTL analysis of leaf photosynthetic rate and related physiological traits in rice (Oryza sativa L.). Euphytica, 135(1), pp.1-7.
44. Bakry, B.A., El-Hariri, D.M., Sadak, M.S. and El-Bassiouny, H.M.S., 2012. Drought stress mitigation by foliar application of salicylic acid in two linseed varieties grown under newly reclaimed sandy soil. J Appl Sci Res, 8(7), pp.3503-3514.
45. Kaur, N., Alok, A., Kumar, P., Kaur, N., Awasthi, P., Chaturvedi, S., Pandey, P., Pandey, A., Pandey, A.K. and Tiwari, S., 2020. CRISPR/Cas9 directed editing of lycopene epsilon-cyclase modulates metabolic flux for β-carotene biosynthesis in banana fruit. Metabolic engineering, 59, pp.76-86.
46. Ahmed, Z.F., Kaur, N., Maqsood, S. and Schmeda-Hirschmann, G., 2022. Preharvest applications of chitosan, salicylic acid, and calcium chloride have a synergistic effect on quality and storability of date palm fruit (Phoenix dactylifera L.). HortScience, 57(3), pp.422-430.
47. Francis, D.V., Sood, N. and Gokhale, T., 2022. Biogenic CuO and ZnO Nanoparticles as Nanofertilizers for Sustainable Growth of Amaranthus hybridus. Plants 2022, 11, 2776.

AUTHORS

Raja Ahmad Ali[1], Umar Hayat[4*], Ijaz Hussain[1], Waseem Ahmed[1], Muhammad Anas Mehboob Malik[1], Malik Faizan Shaukat[1] , Sughra Bibi[2], Alexander Yesaya[3], Qingtao Wang[4], , Zhenyu Yao[4], Ravelomanana Julio Stanislas[5], Husnain[6]

[1]Department of Horticulture, The University of Haripur, Pakistan.
[2]Department of Chemistry, Hazara University Mansehra, Pakistan.
[3]Department of Crop & Soil Sciences, Lilongwe University Of Agriculture & Natural Resource Malawi..
[4] Zhengzhou Institute of fruit trees, Chinese Academy of Agriculture Sciences Zhengzhou 450009, China.
[5]Anyang Institute of Cotton Research, Crop Genetics and Breeding, CAAS
[6]Government post graduate college Mansehra, Pakistan

Correspondence Author –Umar Hayat, Zhengzhou Institute of fruit trees, Chinese Academy of Agriculture Sciences Zhengzhou 450009, China.