



Potential effect of varying concentration of L-adenine on the growth promotion of Maize (*Zea mays* L.) Crop

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Abstract

Maize (*Zea mays* L.) is not only the most important cereal crop in the world in terms of production but also a significant commodity for ensuring food security. Intensive maize production is heavily reliant on chemical fertilizers which when used indiscriminately can have adverse effects on soil structure and crop quality. Such concerns have given rise to increased interest in the use of natural growth promoting agents called bio stimulants. These substances have the potential to enhance crop production by altering hormonal pathways that are responsible for nutrient uptake and root development. Cytokinins are a group of plant hormones that play key roles in various aspects of growth & development; adenine is a purine derivative and serves as a natural precursor to cytokinins such as L-adenine (L-ADE). In the study reported here the authors examined how different levels of L-ADE affected maize growth & physiology under controlled conditions. Six concentrations of L-ADE (Control, 10^{-2} M, 10^{-3} M, 10^{-4} M, 10^{-5} M, 10^{-6} M) were tested at the Soil Bacteriology Section Ayub Agricultural Research Institute Faisalabad. Maize seeds were soaked in respective L-ADE solutions prior to potting; 26 days post-planting growth parameters including root length shoot length root/shoot dry weights and chlorophyll content (a&b) were measured. Results showed that administration at low concentrations stimulated overall growth in maize seedlings particularly those at 10^{-5} M; here shoot weight increased more than two-fold compared with the control (4. 40 g vs 2. 60 g) while root weight peaked (1. 98 g vs 1. 35 g). Most notably chlorophyll content rose significantly when L-adenine levels reached 10^{-5} M; both chlorophyll a and b were higher than in untreated plants. Conversely high concentrations especially 10^{-2} M and 10^{-3} M had an inhibitory effect such as root lengths were reduced and there was only modest stimulation of shoot growth.

Keywords Adenine, Biostimulator, Cytokinin precursor, sustainable agriculture

Introduction

Maize (*Zea mays* L.) or corn, is considered one of the most important cereal food crops on a global scale today because: to its ability to grow well in most environments across all continents; its short life cycle combined with high yields; role in helping feed an ever-growing world population (presently more than seven billion) plus expected increase of same to about ten billion by 2050. Research by FAO (2022) emphasizes that while demand for food, including maize, must rise with a larger world population (expected to top 9.7 billion in 2050 according to recent estimates), this increase in production has to be realized taking into account the need for greater environmental sustainability than conventional maize farming, which relies heavily on fertilizer chemicals.

It is these latter two issues of environmental degradation plus declining soil health or things growing plants need, being harmed by conventional agricultural methods, causing low overall agricultural sustainability i.e. having sufficiently

fertile soils as well as other natural resources available to produce crops which humans regard as nutritious food that bio stimulants could play a major future role in overcoming. Traditional methods employing synthetic fertilizer chemicals carry their own negatives, though. For instance, they promote growth of plants that take heavy metals from soil, which then find their way into humans via eating crop food. Also, crops themselves under these farming systems frequently suffer deficiencies of mineral nutrients plus reduced crop grain quality overall (Abdiani et al., 2019; Maqbool et al., 2020).

Recently, there is an increased worldwide interest (e. g., Ertani et al., 2013; Nardi et al., 2016) in so-called bio stimulants, natural substances used in growing plants and crops, that can have pronounced favorable effects on plant growth. Research over this last decade specifically into various purine derivatives, e. g., adenine, shows how much promise they hold; adenine in particular serves a role in regulating plant hormone pathways. L-Adenine (adenine)

serves as the foundation from which cytokinins arise. Though L-ADE by itself doesn't set off cytokinin activities, when applied from outside can enhance the inside levels of auxins, specifically indole-3-acetic acid (IAA), which promotes maize's ability to take in nutrients & increase its resistance (Kieber et al., 2018). Because maize is both globally significant, sensitive to its environment, and a staple food, a major challenge for sustainable agriculture is finding strategies that improve productivity without adversely affecting food security.

The plant growth regulators known as cytokinins are needed to make cells divide & grow. Cytokinin formation requires the presence of L-adenine, which is present naturally in plants. Externally applying L-adenine can boost an endogenous auxin called indole-3-acetic acid (IAA) in maize; this helps the corn plant resist environmental stress better and also improve its uptake of nutrients (Kieber et al., 2018). Given maize's worldwide importance as a staple food crop, there is a need for sustainable agriculture practices that enhance productivity without hurting food security. Yet little research in Pakistan has focused on using L-adenine for enhancing maize growth & development via improved physiological responses, an area this study seeks to explore. By boosting certain enzymes involved in nitrogen metabolism (nitrate reductase & glutamine synthetase), prior work has shown that L-adenine application can also enhance the nitrogen use efficiency (NUE) of maize plants.

In addition, by changing levels of various cytokinins, L-adenine leads to several other important effects: a higher number of lateral roots that helps take in more phosphorus & micronutrients (Krouk et al., 2011), as well as greater translocation of sucrose to the maize kernels (Riefler et al., 2006). This happens because L-adenine causes cytokinin levels to rise and fall in ways that favor root development while promoting grain filling by helping move carbon around. Indeed, all these attributes may make adenine a suitable candidate as a bio-stimulant for sustainable production systems of maize, so this experiment examined how various doses of L-adenine affected maize growth.

MATERIAL AND METHODOLOGY:

A pot experiment was conducted (on 19th March 2025) at the wire house of the Soil Bacteriology Section, Ayub Agriculture Research Institute (AARI), Faisalabad.

The experiment consisted of six treatments with three replications each. The treatments included:

T1	Control
T2	L-ADE (10^{-2} M)
T3	L-ADE (10^{-2} M)
T4	L-ADE (10^{-4} M)
T5	L-ADE (10^{-5} M)

T6

L-ADE (10^{-6} M)

Figure 1. Maize seeds soaked in different concentrations of L- adenine



Before planting the seeds, we included the L-ADE concentrations. To prepare a 10^{-2} M stock solution, 0.135 g of L-ADE was dissolved in 100 mL of distilled water. A 10^{-3} M solution was obtained by taking 10 mL out of the stock and adding it to 90 mL of distilled water. Similarly, the 10^{-4} M solution was prepared by diluting 10 mL of the 10^{-3} M solution with 90 mL of distilled water. Again, this was continued by taking 10 mL from the 10^{-4} M solution and making it up to 100 mL with distilled water added at first 90 mL then another 90 mL for a 10^{-5} M solution; otherwise referred to as 10^{-6} M when distilled water was pipetted in. Five seeds per pot or fifteen seeds per treatment were used as the seed rate (5 seeds/pot or 15 seeds/treatment). The specified concentrations of these L-ADE solutions were employed in maize seed applications involving L-ADE solution. The application of these solutions followed seed placement in appropriate pots. The following data would have been collected by the end of the experiment (April 14th).

Measurements of growth parameters:

- Shoot length (cm)
- Root length (cm)
- Fresh weights of shoot and roots (g/plant)

Chemical analysis:

Leaf samples of maize were collected and kept fresh for each treatment to be used for chlorophyll determination. Estimation of Chlorophyll-a and Chlorophyll-b was performed using a UV-Visible spectrophotometer according to the method developed by Usman et al. (2013).

Figure 2. Effect of L-ADE on maize growth in a pot experiment



Chlorophyll

Determination Procedure:

Sample Preparation

Weigh 1 gram of fresh leaf plant material from each treatment.

Extraction

Using a mortar and pestle, break up the leaf tissue in 10 mL of 80% acetone until a uniform green solution is produced.

Filtration

Strain the homogenate through Whatman filter paper to get a clear extract free of solids.

Spectrophotometric Analysis

Use a spectrophotometer to measure the absorbance of the

Results and Discussion

extract at 663 nm (which is for chlorophyll a) and 645 nm (which is for chlorophyll b).

Dilution (if necessary)

If the absorbance reading is greater than 2.5, then add 10 mL of distilled water to the extract, mix the solution together, and re-measure the absorbance.

Calculation:

$\text{Chl.a (mg g}^{-1} \text{ f.wt)} = [12.7 (\text{O.D } 663) - 2.69 (\text{O.D } 645)] \times V/1000 \times W$

$\text{Chl.b (mg g}^{-1} \text{ f.wt)} = [22.9 (\text{O.D } 645) - 4.68 (\text{O.D } 663)] \times V/1000 \times W$

Where; V= Volume of sample

f wt. = Fresh weight of tissue

Table 1. Maize growth parameters under different concentrations of L-ADE

Treatment	Root Length (cm)	Shoot Length (cm)	Root Weight(g)	Shoot Weight (g)
Control	49.33	45.67	1.35	2.6
10^{-2} M	44.00	41.33	1.25	2.2
10^{-3} M	49.67	47.00	1.37	2.8
10^{-4} M	51.00	49.00	1.63	4.1
10^{-5} M	56.00	52.00	1.98	4.4
10^{-6} M	53.00	50.67	1.86	4.2

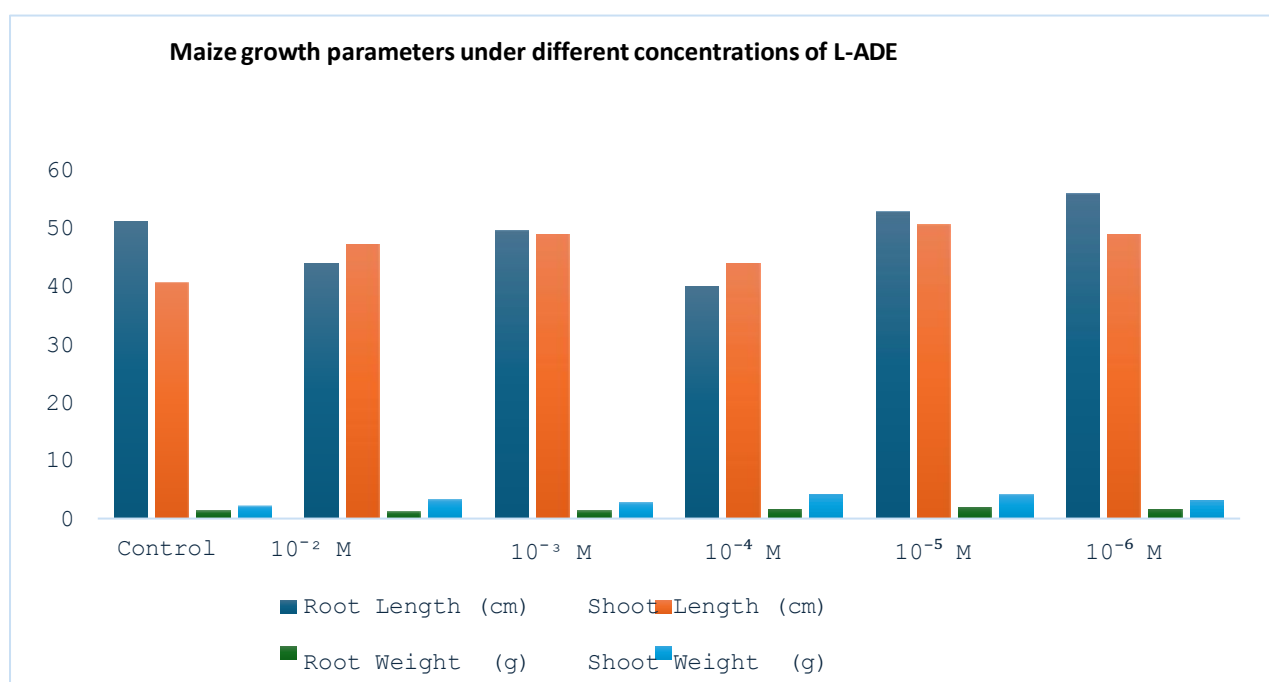
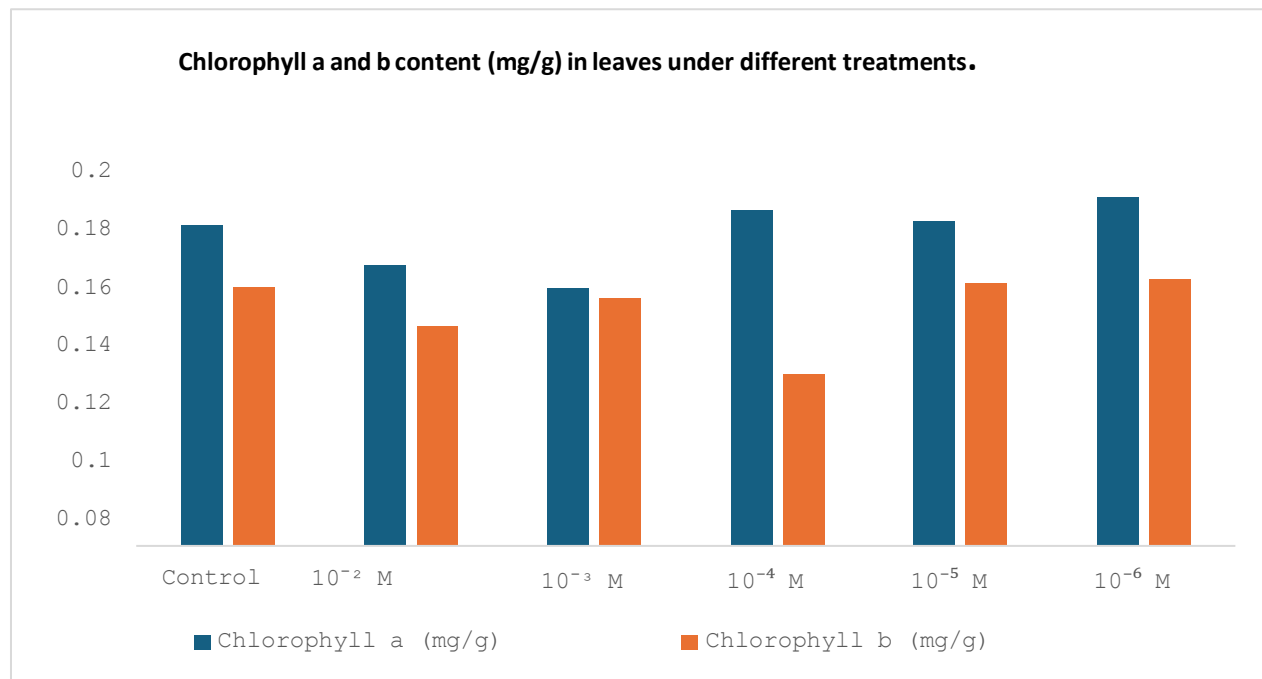
As shown in Table 1, the application of L-ADE at varying concentrations demonstrated a clear dose-dependent effect on plant growth and physiological parameters. Root length was highest at 10^{-5} M (56.00 cm), followed by 10^{-6} M (53.00 cm), both exceeding the control (49.33 cm), whereas higher concentrations (10^{-2} M and 10^{-3} M) resulted in reduced root length. Shoot length increased in all

treatments compared to the control, with the highest value recorded at 10^{-5} M (52.00 cm). Root weight peaked at 10^{-5} M (1.98 g), while shoot weight was greatest at 10^{-5} M (4.40 g), nearly twice that of the control. Overall, the most pronounced positive effects on plant growth were observed at lower L-ADE concentrations (10^{-5} M to 10^{-6} M), while higher concentrations tended to exert inhibitory effects.

Table 2. Chlorophyll a and b content (mg/g) in leaves under different treatments

Treatments	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)
Control	0.172	0.139
10 ⁻² M	0.138	0.118
10 ⁻³ M	0.152	0.133
10 ⁻⁴ M	0.174	0.137
10 ⁻⁵ M	0.187	0.143
10 ⁻⁶ M	0.181	0.141

As shown in Table 2, the chlorophyll analysis revealed that chlorophyll a content was highest at 10⁻⁵ M (0.187 mg/g), 10⁻⁶ M (0.181 mg/g) and 10⁻⁴ M (0.174mg/g), both higher than the control (0.172 mg/g). Chlorophyll b was most elevated at 10⁻⁵ M (0.143 mg/g), followed by 10⁻⁶ M (0.141 mg/g) and control (0.139 mg/g), while lower values were observed at 10⁻² M (0.118 mg/g) and 10⁻³ M (0.133 mg/g). These findings suggest that L-ADE, particularly at lower concentrations, positively influences both morphological and physiological parameters, enhancing plant growth and chlorophyll contents.



Conclusion

According to the results of the current study, the growth and physiology of maize showed a clear dependence on the concentration of applied L-adenine. The best results were observed at the two lowest concentrations of 10^{-5} and 10^{-6} mol L⁻¹, where shoot & root biomass, root elongation, and chlorophyll a & b contents were significantly improved as compared to the control & all other treatments. Such enhancements ultimately led to increased vegetative growth coupled with improved photosynthetic performance. However, applying higher doses such as 10^{-2} and 10^{-4} mol L⁻¹ resulted in inhibition of root length. This suggested that applying L-ADE at certain levels does not promote but restricts root growth; an observation consistent with any hermetic effect caused by cytokinin-like substances.

Based on these observations, it can also be concluded that L-ADE works as a natural biostimulant by acting upon auxin and cytokinin signaling pathways, which enhances nutrient uptake mechanisms, overall root system, and better source-to-sink (carbon) translocation, thereby promoting growth of maize plants. Being a natural, inexpensive compound, L-ADE may prove helpful while addressing two pertinent issues relating to crop production, which are the need of cheap inputs for resource-poor farmers (a target group especially in countries like Pakistan) plus minimizing environmental impact of farming activities due to synthetic inputs. If application protocols are developed following thorough pot experiments, it will create the possibility of improving the productivity of maize grown using conventional practices but requiring low-cost chemical inputs.

In future, investigations into different soils, agro-climatic zones, and crop response parameters, for instance performance indicators such as growth, yield, nutrient efficiency etc. along with residual profile on the soil and plant due to L-ADE, would greatly help expand information on farming practices. Potential exists to upscale beneficial effect of this chemical fertilizer both economically (input vs outputs balance) & technically viable.

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