



Physiological Effect of Adding Concentrations of Humic Acid on Growth and Protein Percentage of *Zea mays* L. Plant Genotypes

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ABSTRACT

Background: The global trend at present is to utilise organic fertilisers of various types and sources to minimise the adverse effects of chemical fertilisers as much as possible. Humic acid is an organic fertilizer widely used due to its many benefits in improving the properties of soil and its effect on plant growth and production. The global production of hybrid varieties has been at a steady pace for years, driven by their productivity and the number of genetic combinations introduced, which increases the capacity of their genetic base and thus helps them adapt to environmental conditions or growth factors.

Methods: The experiment conducted in Tikrit University, Salah Al-Din, Iraq, to investigate the effect of varying concentrations of organic fertilizer (0, 1, 2 and 3 ml L⁻¹ humic acid) on the three *Zea mays* plant genotypes (Toro, Vito and Jooly) growth characteristics and protein percentage, in the autumn season of 2025 using an RCBD design repeated three times.

Result: Humic acid with 3 ml L⁻¹ superior in plant height, leaves number plant⁻¹, leaf area, dry weight plant⁻¹, leaf chlorophyll content (SPAD) and protein percentage, by percentage increase of 3.96%, 7.89%, 21.43%, 12.25%, respectively and leaf chlorophyll content reached 42.10 SPAD and protein percentage 8.6%. Jooly genotype was superior in the same traits by percentage increase of 15.84%, 2.72%, 8.35% and 9.78%, respectively and leaf Chlorophyll content 46.56 SPAD and protein percentage 8.70%. The interaction between 3 ml L⁻¹ humic acid concentrations and Jooly genotype superior in the same traits by percentage increase of 29.73%, 10%, 30.15% and 3.29%, respectively and leaf chlorophyll content of 48.25 SPAD and protein percentage 8.90 %. It can be concluded from this experiment that adding of humic acid helps in enhancing growth and protein percentage of new *Zea mays* L. plant genotypes.

Key words: Genotypes, Organic fertilizer, Physiological traits, *Zea mays*.

INTRODUCTION

A *Zea mays* L. plant from the Poaceae family and is an important economic cereal plant (Raheem *et al.*, 2023). In terms of area and production, it ranks third after wheat (Al-Asady, 2015 and Idan and Hindi, 2023). It is a staple food in most parts of the world (Priyadarshini *et al.*, 2025), containing approximately 9% protein, a high percentage of carbohydrates (up to 70%) and about 4% oil (License, 2008; Amanah and Hadi, 2022). In addition to containing vitamins B1, B2 and E (Al-Badry, 2019). Corn varieties are used as a fodder crop in poultry and livestock feed, in addition to the use of its leaves in the paper industry (Al-Kaisy and Al-Heety, 2017 and Saja *et al.*, 2024) and it was called the king of crops for its use in food industries, its use as animal feed and in the manufacture of dyes, in addition to its use as cars fuel (Mohmmad and Akhtar, 2001).

The study and research of varieties suitable for the local growing conditions in Iraq are required, as well as searching for safe technology, including safe and more effective alternative fertilizers that increase production, such as the trend towards manufactured organic fertilizers. The aforementioned reasons necessitate the search for modern methods and techniques that increase plant productivity and reduce the negative impact of chemical fertilizers therefore, on human health. (Shehata *et al.*, 2011).

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There is a global trend at the moment to use organic fertilizers of various types and sources to reduce as much as possible the negative effects of chemical fertilizers. Humic acid (HA) is considered among the organic fertilizers widely used due to its many benefits in improving soil properties and its effect on plant growth and production (Nassour and Hediwa, 2016 and AL-Azee *et al.*, 2023), including the use of humic acid increases the availability of nutrients to plants and has a high chelating capacity for nutrients which improves soil properties and increases

water and nutrient absorption, in addition to its role similar to the growth regulator gibberellin (Sabet, 2023 and Taha *et al.*, 2025). Several studies have demonstrated the importance of humic acid fertilization for increasing the plant growth and productivity of various plants, especially under environmental stress conditions, as it enhances the availability, absorption and utilization of nutrients by crops (Al-Aswad, 2021 and Raheem, 2024), as well as its role in increasing the root system and increasing the plant's protein content (Muscota *et al.*, 2007).

Global production of the hybrid variety has been at a steady pace for years and for several locations, depending on the productivity rate of the genetic combination and the number of genetic combinations entering (Ali *et al.*, 2025), which increases the capacity of its genetic base and thus helps to adapt to environmental conditions or growth factors. Studies have indicated that most growth characteristics of most genetic combinations may differ according to the genetic makeup of varieties (Raheem, 2024 and Raheem and Ali, 2025). Therefore, this study was conducted to test different concentrations of liquid humic acid organic fertilizer to determine the concentration that yields the highest values for the corn studied traits, aiming to reduce the use of chemical fertilizers. It also aimed to test different imported genotypes of corn to identify the most suitable genotype under the local environmental conditions of the study area.

MATERIALS AND METHODS

The factorial experiment with two factors was conducted at Tikrit University, Salah Al-Din, Iraq, located 34°62'N, 43°65'E and 137 m.s.l., using an RCBD design repeated three times by using 3 plastic pots per experimental unit, which were filled with clay loam soil, during the autumn season of 2025. The first factor was different concentrations of humic acid (1, 2 and 3 ml L⁻¹) added to the soil in addition to the control (without addition). The second factor included three genotypes of *zea mays* (Jooly, Vito and Toro). The seeds were sown on 20/7/2025 and fertilized with superphosphate and urea according to the recommended quantity (Guidance bulletin No. 18, 2006). Humic acid treatments were sprayed on the soil and plants twice, the first time one month after planting and the second time one month after the first application. The pots were placed in an open, sunny location.

The following characteristics were studied at the flowering stage: Plant height (cm): measured from the soil surface to the plant top. Leaf number plant⁻¹: The leaves for each plant were counted for the three repeaters and the average was taken. Leaf area (cm): measured by using the equation of McKee (1964): Leaf area (cm² plant⁻¹) = leaf length below ear × maximum leaf width × 0.75. Shoot dry weight (gm): It was weighed after the plant's stems and leaves dried by oven at 70°C for 72 h. Tacked by weighing the stem and leaves of the plant in each experimental unit using a digital sensitive scale and then the average was taken. Grain protein percentage (%): The nitrogen percentage

was estimated by using the Micro Kjeldahl apparatus and then the percentage of protein (%) was estimated as follows: percentage of protein (%) = Nitrogen percentage × 6.25 (Hart and Fisher, 1971). The data collected were analyzed by the ANOVA analysis technique for a factorial experiment in a randomized complete block design (RCBD) with three replications per treatment. Duncan's multiple range test at 0.05 a probability level was used to compare the treatment means.

RESULTS AND DISCUSSION

Plant height

Table 1 results show that using different humic acid concentrations showed a significant effect on plant height. Adding the concentration of 3 ml L⁻¹ HA resulted in the highest plant height (2018 cm), by an increase of 3.96% compared with the control treatment (210 cm). Furthermore, the genotypes differed significantly in plant height; the highest plant height was observed for the Jooly genotype (228 cm), with an increase of 15.84% compared with the Toro genotype, which registered the lowest height (197 cm). The interaction between the humic acid treatments and genotypes showed a significant effect on the plant height, as the interaction between the HA treatment at 3 ml L⁻¹ concentration and the Jooly genotype had the highest plant height (240 cm), with an increase of 29.73%, compared to the interaction of no humic acid treatment with the Toro genotype that registered the lowest plant height (185 cm). These results were due to the humic acid role, which works to increase cell division and root branching, thereby increasing their capacity to absorb nutrients from the soil. HA also plays a crucial role in physiological processes by stimulating enzyme activity and its role in division and elongation of the cell, leading to increased plant height (Chen *et al.*, 2022 and AL-Azee *et al.*, 2023). This activity stimulates photosynthesis by increasing the activity of the Rubisco enzyme, which is responsible for CO₂ fixation, thus increasing growth.

Plant leaves number

The results in Table 2 show that adding different concentrations of HA showed an insignificant effect on the leaves plant⁻¹. Jooly genotype also showed an improvement in the number of leaves plant⁻¹ (13.1 leaves plant⁻¹), which increased by 2.72% compared with Toro genotype, which registered the lowest leaves plant⁻¹ (12.2 leaves plant⁻¹).

Table 1: Humic acid, genotypes and their interactions effect on plant height (cm).

Genotypes	Humic acid (ml L ⁻¹)				Mean
	0	1	2	3	
Toro	185k	205h	210g	190j	197c
Veto	215f	220e	200i	225d	215b
Jooly	230c	210g	235b	240a	228a
Mean	210b	212b	215ab	218a	

The interaction between adding different concentrations of HA and the genotypes showed a significant impact on the number of leaves plant⁻¹, as the interaction between 3 ml L⁻¹ of HA and the Jooly genotype showed highest number of leaves plant⁻¹ (13.2 leaves plant⁻¹), which did not differed significantly with the interaction of Jooly genotype and all HA concentrations, with 10% increasing percentage compared to the interaction of treatment without adding the humic acid and adding 1 ml L⁻¹ of HA with the Toro genotype which registered the lowest number of leaves plant⁻¹ (12.0 leaves plant⁻¹). Humic acid contributes to ion chelating, which enhances the availability of nutrients for efficient plant uptake. Furthermore, it promotes the presence of beneficial microorganisms in the soil, resulting in increased nutrient availability and supporting growth and production (Channab *et al.*, 2023). It also contains large amounts of nutrients, plant hormones and amino acids, which contribute to increasing the soil fertility and promoting plant growth (Alfarisy *et al.*, 2021), thereby reflecting on number of leaves plant⁻¹ increasing.

Plant leaf area (cm²)

Table 3 shows that adding different concentrations of the HA showed a significant impact on the plant leaf area. The highest concentration of 3 ml L⁻¹ HA resulted in the highest leaf area plant⁻¹ (736.9 cm² plant⁻¹), with an increase percentage reached of 21.43%, opposite the control treatment, which recorded the lowest leaf area plant⁻¹ (680.1 cm² plant⁻¹). Furthermore, the genotypes significantly differed in their effect on plant leaf area. The Jooly genotype was superior (774.6 cm² plant⁻¹), with an increase of 8.35%, opposite the Toro genotype, which was the lowest value (637.8 cm² plant⁻¹). From the same table, it is notes that the interaction between the addition of different concentrations from humic acid and the genotype show an effect on the plant leaf area, as the 3 ml L⁻¹ HA with the Jooly genotype had the high plant leaf area (794.1 cm² plant⁻¹) with an increase percentage reached of 30.15%, opposite the interaction of treatment without adding the humic and the Toro genotype, which recorded the lowest value of leaf area plant⁻¹ (610.1 cm² plant⁻¹). Humic acid provides easily absorbed macro and micronutrients, promoting vegetative growth and increasing photosynthesis, thereby contributing to an expansion of leaf area and increased leaf productivity (Sindhu *et al.*, 2022). Humic acids also contribute to increased root growth and vegetative growth and humic acids increase leaf area (Nardi *et al.*, 2002). The differences between genotypes are due to internal genetic factors for each genotype.

Plant dry weight (gm)

From Table 4, observe a significant effect of HA on the plant dry weight of the vegetative parts. The treatment with 3 ml L⁻¹ HA resulted in the highest plant dry weight (422.5 g) by 15.25% increasing, opposite the treatment without HA, which gave the lowest plant dry weight (409 gm). Also, differences among genotypes show a significant impact; the Jooly genotype had a higher increase percentage

19.78% (460.2 gm) than the Toro genotype (399.3 gm). From the same table notes that there is a significant influence of interaction between the addition of different concentrations of HA and the *Zea mays* genotypes in the plant dry weight, as for the interaction between the 3 ml L⁻¹ concentration of HA and the Jooly genotype was showed greater increase by 3.29% (456.2 g), opposite the treatment of not adding the humic acid and the Toro genotype (380.9 gm), which recorded the lowest plant dry weight.

Humic acid contributes to rising the ease of use of soil nutrients to the plant and improving cell division (Nardi *et al.*, 2002), it plays a vital role in improve plant ability to absorb nutrients and this contributes to stimulating the activity of hormones, such as auxin, which directly affects germination of seed and vegetative growth and this is reflected in the plant's dry weight. (Chen *et al.*, 2022 and Wijesinghe *et al.*, 2023) and differences among genotypes are due to internal genetic factors.

Leaves' total chlorophyll content (SPAD)

Fig 1 show that HA had a significant positive impact on the plant leaf total chlorophyll content. Using 3 ml L⁻¹ of humic acid gave the highest leaves chlorophyll content of 46.08 SPAD, compared to the non-humic acid addition treatment, which gave the lowest leaf chlorophyll content of 42.10 SPAD. Furthermore, different genotypes of the *Zea mays* plant showed a significant difference in the total chlorophyll

Table 2: Humic acid, genotypes and their interactions effect on plant leaves number.

Genotypes	Humic acid (ml L ⁻¹)				Mean
	0	1	2	3	
Toro	12.0c	12.0c	12.1bc	12.6b	12.2b
Veto	12.6b	12.7b	12.7b	12.8ab	12.7ab
Jooly	12.9ab	13.1a	13.1a	13.2a	13.1a
Mean	12.5a	12.6a	12.6a	12.5a	

Table 3: Humic acid, genotypes and their interactions effect on plant leaf area (cm² plant⁻¹).

Genotypes	Humic acid (ml L ⁻¹)				Mean
	0	1	2	3	
Toro	610.1k	624.0g	650.5i	666.7h	637.8c
Veto	670.1g	687.2f	710.0e	750.0d	704.3b
Jooly	760.1c	760.8c	784.6b	794.1a	774.6a
Mean	680.1d	690.7c	715.0b	736.9a	

Table 4: Humic acid, genotypes and their interactions effect on plant shoots dry weight (gm).

Genotypes	Humic acid (ml L ⁻¹)				Mean
	0	1	2	3	
Toro	380.9h	395.2g	426.1d	395.0g	399.3c
Veto	400.1f	436.0c	400.0f	416.2d	413.1b
Jooly	446.1b	410.1de	435.4c	456.2a	460.2a
Mean	409.0d	413.8c	420.5d	422.5a	

content of the leaf. The Jooly genotype recorded the highest chlorophyll content of the leaf, 46.56 SPAD. However, the lowest the leaf chlorophyll content was registered for the Toro genotype, 43.38 SPAD. The differences among genotypes are due to the differences in their internal genetic factors. The interaction between different concentrations of HA and genotypes showed a significant impact on leaf chlorophyll content. The interaction of 3 ml L⁻¹ HA with the Jooly genotype resulted in the highest total chlorophyll content, 48.25 SPAD, compared to the interaction of no humic acid treatment with the Toro genotype, which recorded the lowest total chlorophyll content, 42.1 SPAD. The increase in total chlorophyll content is due to the role of HA in promoting growth and increasing the plant's physiological efficiency, which is reflected in growth, increased leaf area and chlorophyll development (Al-

Khafaji, 2015). In addition, the levels of plant hormones such as cytokinins and auxins may increase. This led to improved physiological processes and increased chlorophyll accumulation (Alzamel *et al.*, 2022). The differences among genotypes are due to different genetic makeup among them.

Grain protein percentage (%)

Fig 2 show that treatment with humic acid showed a significant impact on grain protein percentage. The concentration of 3 ml L⁻¹ from HA showed the highest percentage, reaching 8.67%, compared to the non-addition humic acid 8.60%. The same figure also shows that the genotypes varied significantly, with the Jooly genotype recording the highest protein percentage, 8.70%, compared to the Toro genotype, which recorded 8.53%. The interaction between different concentrations of humic acid and genotypes shows a

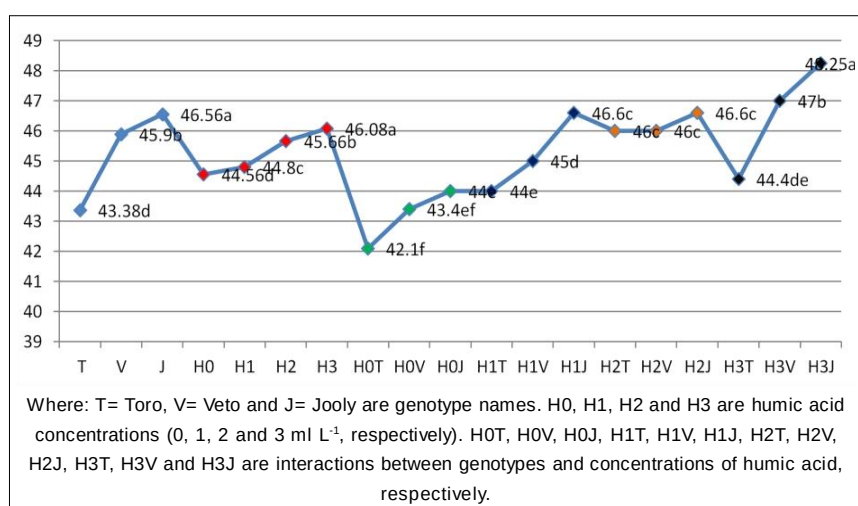


Fig 1: Humic acid, genotypes and their interactions effect on leaf chlorophyll content (SPAD Reading).

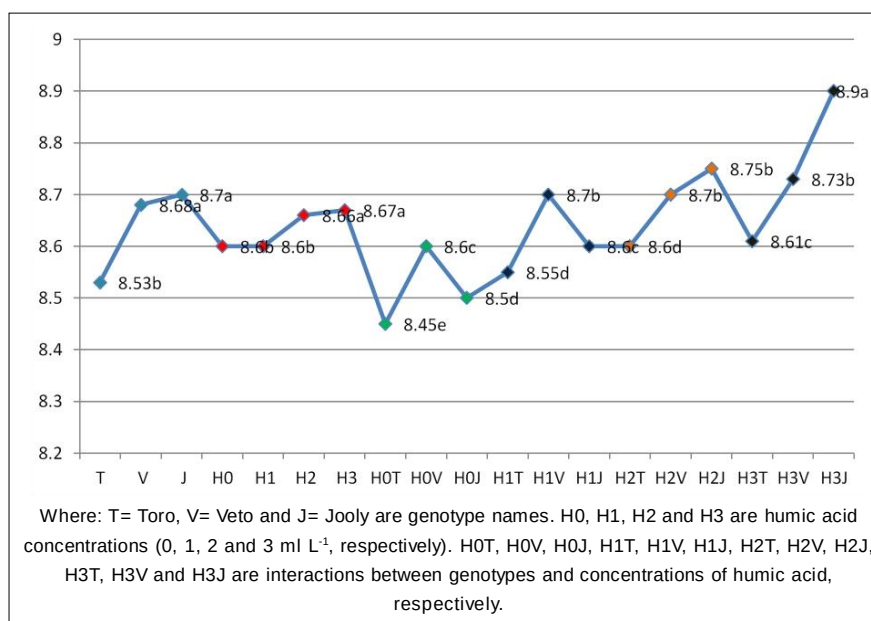


Fig 2: Humic acid, genotypes and their interactions effect on grain protein (%).

change in the protein content in the grain. The interaction between a 3 ml L⁻¹ concentration of humic acid and the Jooly genotype resulted in the highest protein content, reaching 8.90%, opposite the interaction of check treatment with the Toro genotype, which gave the lowest protein percentage, reaching 8.45%. Protein percentage increasing in *zea mays* grains is due to the effect of HA, which increases the fertility of the soil and improves the availability of nutrients. Humic acid also enhances the activity of microorganisms in the soil, which increases the absorption of nitrogen, an essential element in protein synthesis, as it is involved in the amino acids, which are the building blocks of protein (AL Azzi and AL-Obaidy, 2019). Organic acids also increase the yield and its quality (Al-juboury, 2023).

CONCLUSION

The study results showed that treatment with organic fertilizer humic acid, especially high concentrations, had a significant positive impact on growth traits and protein content in *Zea mays* grains and the different genotypes of *Zea mays* plants showed variation in growth characteristics and in grain protein content, with the Jooly genotype outperforming the other genotypes in this study.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

Experiment on plants, not animals.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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