RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
57 (3) 1318-1325, 2025
http://doi.org/10.54910/sabrao2025.57.3.43
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

# EFFECT OF *AZOTOBACTER* AND NITROGEN FERTILIZER ON THE GROWTH, YIELD, AND ACCUMULATION OF CAPSAICIN CONTENT IN CHILI PEPPERS

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## SUMMARY

The aim of this study sought to determine the effect of *Azotobacter* and nitrogen fertilizer on the growth, yield, and accumulation of capsaicin content in fruits of two chili pepper (*Capsicum annuum* L.) hybrids. Carried out in the crop season of 2022–2023 at the University of Kerbala, District Husseiniya, Iraq, the experiment was in a randomized complete block design (RCBD) with a split-plot arrangement and three replicates. The study included two factors: the first factor was two chili pepper hybrids, i.e., Barbarian and Hyffa, and the second factor was eight fertilizer combinations (control, *Azotobacter*, urea [150 mg $L^{-1}$], urea [300 mg $L^{-1}$], urea [450 mg $L^{-1}$], *Azotobacter* + urea [150 mg $L^{-1}$], *Azotobacter* + urea [300 mg $L^{-1}$], and *Azotobacter* + urea [450 mg $L^{-1}$]). Results showed the Barbarian hybrid gave the highest total yield (18.12 t $ha^{-1}$) and capsaicin content in fruits (742.41 mg $kg^{-1}$) compared with the Hyffa hybrid. The fertilizer combination *Azotobacter* + urea (450 mg $L^{-1}$) also excelled other treatments in plant height (71.47 cm), the number of branches (9.50 branches $plant^{-1}$), stem diameter (2.37 cm), total yield (29.06 t $ha^{-1}$), and capsaicin content in chili fruits (820.20 mg $kg^{-1}$).

**Keywords:** Chili pepper (*C. annuum* L.), cultivars, *Azotobacter*, nitrogen fertilizer, growth and yield traits, capsaicin content

**Key findings:** In chili peppers (*C. annuum* L.), the hybrid Barbarian gave the maximum values in total yield and capsaicin content in fruits compared with the hybrid Hyffa. The fertilizer combination *Azotobacter* + urea (450 mg $L^{-1}$) showed the highest values for plant height, the number of main branches, stem diameter, total yield, and capsaicin content in fruits.

Communicating Editor: Dr. A.N. Farhood

Manuscript received: April 21, 2024; Accepted: October 08, 2024.


**Citation:** Farhan SS, Mutar KA (2025). Effect of *azotobacter* and nitrogen fertilizer on the growth, yield, and accumulation of capsaicin content in chili peppers. *SABRAO J. Breed. Genet.* 57(3): 1318-1325. http://doi.org/10.54910/sabrao2025.57.3.43.

## INTRODUCTION

Chili pepper (*Capsicum annuum* L.) is one of the valuable summer vegetable crops, belonging to the family Solanaceae, and a native to Central and South America. Chili has great economic, food, and health significance, playing an important role in the economy of many countries (Arora *et al.,* 2011). Its nutritional significance comes from the use of its fruits as fresh, dried, and in powder form for cooking, pickling, and the manufacture of spices. Its fresh consumption provides humans with carbohydrates and proteins, as well as various minerals (Park and Kaura, 2012).

Health care comes from vitamin-rich fruits, including vitamins C and A, as well as, from phenolic and carotene compounds acting as antioxidants. Chili also contains an effective alkaloid group called capsaicinoids, which develop the spicy taste. One of its most popular compounds is capsaicin, beneficial in treating and improving heart function, stimulating the stomach, treating rheumatism, pain reliever, and as an antibacterial and antifungal (Cortez-Baheza *et al.,* 2008).

The cultivated area of peppers was around 2,078,042 hectares, with a total production of 39.4 million tons worldwide (FAO, 2024). As for Iraq, chili growing prevails in most Iraqi governorates, and the total chili crop production reached 43,904 tons, with an area estimated at 5.086 hectares (CSO, 2024).

Nitrogen-fixing bacteria are among the most essential types of bacteria used in biofertilization. *Azotobacter* is one of the most efficient free-living nitrogen-fixing organisms. These bacteria occur in soil, water, and on the surfaces of plant roots. However, if found on plant roots' surface, it becomes more active and benefits root secretions as a source of carbon (Hindersah *et al.,* 2020).

Numerous studies have indicated that *Azotobacter* can produce various growth regulators, with the most crucial are IAA and cytokinins and gibberellins. These bacteria could greatly improve the growth and productivity of crops, enhancing a large percentage of plant length and the amount of nitrogen absorbed when fertilized (Wani *et al.,* 2013).

Nitrogen fertilization is highly significant because of its positive role in the construction of amino acids, proteins, and nucleic acids and its involvement with magnesium to form chlorophyll molecules (Kazem and Mutar, 2023). Various studies enunciated around 70% of the leaf nitrogen enters to create photosynthetic pigments, enzymes and amino acids, and IAA (Leghari *et al.,* 2016).

Foliar fertilization has an influential role in improving plant growth by ensuring the absorption of important macro- and micronutrients by the leaves (Mutar *et al.,* 2022). Urea is one of the most suitable types of nitrogen fertilizers, which can also apply for foliar addition due to its rapid absorption, transportation, non-polarity, low toxicity, high solubility, and higher nitrogen content (Bondada *et al.,* 2001). The presented study sought to investigate the effect of adding *Azotobacter* and nitrogen fertilizer on the growth and productivity of two hybrids of chili pepper and their role in the accumulation of the compound capsaicin.

## MATERIALS AND METHODS

The promising study aimed to determine the effect of *Azotobacter* and nitrogen fertilizer on the growth, yield, and accumulation of capsaicin content in fruits of two chili pepper (*Capsicum annuum* L.) hybrids. Happening in the crop season of 2022–2023 at the University of Kerbala, District Husseiniya, Iraq, the experiment layout was in a randomized complete block design (RCBD) with a split-plot arrangement and three replicates. The study included two factors: the first factor was two chili pepper hybrids, i.e., Barbarian and Hyffa, and the second factor included eight fertilizer combinations (control, *Azotobacter*, urea [150 mg $L^{-1}$], urea [300 mg $L^{-1}$], urea [450 mg $L^{-1}$], *Azotobacter* + urea [150 mg $L^{-1}$], *Azotobacter* + urea [300 mg $L^{-1}$], and *Azotobacter* + urea [450 mg $L^{-1}$]).

The experiment included 48 experimental subunits, and the area of each experimental subunit was 3 $m^2$. Eight chili plants comprised each experimental subunit, and the distance

between the plants was 40 cm. The planting of seedlings for both chili hybrids was on March 25, 2022, on raised beds, subsequently using a drip irrigation system. The first hybrid was the Barbarian, belonging to the Indian company United Genetics, while the second hybrid was Hyffa, of Dutch origin, produced by the West Frisian Seeds Company. Nitrogen fertilizer (urea) spraying continued in three batches, on April 30 and May 14 and 29, 2022, respectively. The addition of the compound chemical fertilizer (PK = 144 $K_2O$ + 160 $P_2O_5$ kg $ha^{-1}$) (Al-Dahami, 2013) and organic fertilizer (sheep manure) to the soil was according to the fertilizer recommendation at a 5% soil volume (Al-Lamy, 2015). Field service operations, including irrigation, hoeing, weed removal, and pest control, took place, as needed. *Azotobacter* mixing ensued at concentrations of 1 × 18 CFU/ml at 10 ml/hill to the treatments that require the addition in the field with the chili planted seeds. The physicochemical properties of the experimental soil before planting are presented in Table 1.

**Data recorded**

Recording the data of five plants randomly selected in each treatment focused on growth and productivity related parameters. The data recorded comprised the traits, viz., plant height (cm), the number of main branches per plant, main stem diameter (cm), early yield (t $ha^{-1}$), total yield (t $ha^{-1}$), and the determination of capsaicin content in chili fruits (mg $kg^{-1}$).

**Statistical analysis**

After collecting and tabulating the data related to the study, the data underwent the analysis of variance (ANOVA) based on RCBD with a factorial arrangement, using the GenStat15 program. The least significant difference ($LSD_{0.05}$) test used helped compare and separate the means (Steel and Torrie, 1980).

## RESULTS AND DISCUSSION

**Plant height**

Based on the results, the chili hybrids revealed no significant differences for plant height (Table 2). On fertilization treatments, these gave significant variations in plant height, and the tallest plant height appeared with the treatment combination of *Azotobacter* + urea (450 mg $L^{-1}$), at an average of 71.47 cm. Meanwhile, the shortest plant height was visible in the control treatment (57.96 cm). This could be due to the *Azotobacter* as it

**Table 1.** Physical and chemical properties of the experimental soil.

| Property | pH | EC (dS $m^{-1}$) | Total N (mg $kg^{-1}$) | $CaCo_3$ (mg $kg^{-1}$) | Organic matter (%) | Available P (mg $kg^{-1}$) | Clay-silt-sand (%) |
|---|---|---|---|---|---|---|---|
| Value | 7.42 | 3.52 | 22 | 21.43 | 1.06 | 4.1 | 43.6-20.42-36 |

**Table 2.** Effect of Azotobacter and urea and their interaction on the plant height in peppers.

| Fertilization treatments | Hybrids | | Means (cm) |
|---|---|---|---|
| | Barbarian | Hyffa | |
| Control | 57.1 | 58.8 | 57.96 |
| Azotobacter treatment | 58.91 | 59.13 | 59.02 |
| Urea (150 mg $L^{-1}$) | 64.13 | 61.72 | 62.92 |
| Urea (300 mg $L^{-1}$) | 64.35 | 62.57 | 63.46 |
| Urea (450 mg $L^{-1}$) | 66.81 | 64.22 | 65.51 |
| Azotobacter + Urea (150 mg $L^{-1}$) | 68.84 | 69.18 | 69.01 |
| Azotobacter + Urea (300 mg $L^{-1}$) | 69.24 | 69.76 | 68.5 |
| Azotobacter + Urea (450 mg $L^{-1}$) | 70.44 | 72.51 | 71.47 |
| Means (cm) | 64.98 | 64.73 | |
| $LSD_{0.05}$ Hybrids: N.S., Fertilization treatments: 9.200, Interaction: 12.23 | | | |

secretes biochemical compounds in the root growth area (rhizosphere), which are substances similar to auxins, gibberellins, cytokinins, and vitamins. These, in turn, increase root hairs and their growth, and eventually boosting the surface area of the roots and absorption, contributing an enhanced vegetative growth in chili genotypes (Singh and Sharm, 2019; Dhopavakar *et al.,* 2021). This is also because of the positive role of nitrogen in the construction of growth hormones, especially auxins and cytokinins, which stimulate cell division and elongation and increase the vegetative growth, especially plant height and the number of branches (Glawischnig *et al.,* 2000).

Outcomes also showed a significant interaction between pepper hybrids and fertilization treatments (Table 2). The taller chili plants were prevalent in the treatment combination of *Azotobacter* + urea (450 mg $L^{-1}$) and the hybrid Hyffa, with an average of 72.51 cm, while the smallest plant height resulted in the control treatment with the Barbarian hybrid (57.10 cm).

**The number of main branches**

Results showed nonsignificant differences between pepper hybrids for the number of main branches (Table 3). However, fertilization treatments showed notable changes for the main branches' number in chili. The most number of main branches emerged in the treatment *Azotobacter* + urea (450 mg $L^{-1}$), with an average of 9.50 branches $plant^{-1}$, while the least number of main branches came from the control treatment in chili (3.60 branches $plant^{-1}$). The reason may be due to the positive role of *Azotobacter* in secreting substances similar to auxins, gibberellins, cytokinins, and vitamins. Additionally, the key role of nitrogen contributes in building growth hormones to stimulate cell division and elongation, encouraging vegetative growth and raising the number of branches (Glawischnig *et al.,* 2000).

The study findings also revealed noteworthy interaction effects between pepper hybrids and fertilization treatments (Table 3). The topmost number of branches occurred with the interaction of *Azotobacter* + urea (450 mg $L^{-1}$) and the chili Hyffa hybrid (9.60 branches $plant^{-1}$). However, the lowest number of branches was apparent in the control treatment with the Hyffa hybrid (3.07 branches $plant^{-1}$).

**Stem diameter**

Results of remarkable differences appeared between pepper hybrids for the stem diameter (Table 4). The increased main stem diameter surfaced in the Barbarian hybrid (2.28 cm), while the lowest in the Hyffa hybrid (2.02 cm). This may be due to the difference in the genetic makeup of genotypes and their potential. The genotype genes, found responsible for the growth and development of the supportive and vascular tissues in the plant body, affected the stem diameter in peppers (Tripodi *et al.,* 2018).

**Table 3.** Effect of Azotobacter and urea and their interaction on branches per plant in peppers.

| Fertilization treatments | Hybrids | | Means (branches $plant^{-1}$) |
|---|---|---|---|
| | Barbarian | Hyffa | |
| Control | 4.13 | 3.07 | 3.60 |
| Azotobacter treatment | 6.27 | 5.86 | 6.06 |
| Urea (150 mg $L^{-1}$) | 6.60 | 6.13 | 6.36 |
| Urea (300 mg $L^{-1}$) | 7.53 | 7.02 | 7.27 |
| Urea (450 mg $L^{-1}$) | 7.93 | 8.07 | 8.00 |
| Azotobacter + Urea (150 mg $L^{-1}$) | 8.01 | 8.20 | 8.10 |
| Azotobacter + Urea (300 mg $L^{-1}$) | 8.60 | 8.93 | 8.76 |
| Azotobacter + Urea (450 mg $L^{-1}$) | 9.41 | 9.60 | 9.50 |
| Means (branches $plant^{-1}$) | 7.31 | 7.11 | |
| $LSD_{0.05}$ Hybrids: N.S., Fertilization treatments: 0.840, Interaction: 0.940 | | | |

**Table 4.** Effect of Azotobacter and urea and their interaction on the stem diameter in peppers.

| Fertilization treatments | Hybrids | | Means (cm) |
|---|---|---|---|
| | Barbarian | Hyffa | |
| Control | 1.81 | 1.73 | 1.77 |
| Azotobacter treatment | 1.88 | 1.79 | 1.83 |
| Urea (150 mg $L^{-1}$) | 2.01 | 1.80 | 2.91 |
| Urea (300 mg $L^{-1}$) | 2.17 | 1.84 | 2.05 |
| Urea (450 mg $L^{-1}$) | 2.20 | 2.13 | 2.16 |
| Azotobacter + Urea (150 mg $L^{-1}$) | 2.26 | 2.27 | 2.26 |
| Azotobacter + Urea (300 mg $L^{-1}$) | 2.31 | 2.28 | 2.29 |
| Azotobacter + Urea (450 mg $L^{-1}$) | 2.38 | 2.35 | 2.37 |
| Means (cm) | 2.28 | 2.02 | |
| $LSD_{0.05}$ Hybrids: 0.146, Fertilization treatments: 0.149, Interaction: 0.15 | | | |

Regarding fertilization treatments, it was noticeable that considerable variations arose among them for the main stem diameter in peppers (Table 4). The broadest main stem diameter resulted in the treatment combination of *Azotobacter* + urea (450 mg $L^{-1}$), with an average of 2.37 cm, while the narrowest main stem diameter was evident in the control treatment of the chili (1.77 cm). This could refer to the positive role of *Azotobacter* and nitrogen in secreting and building growth hormones to stimulate cell division and elongation and thus widening the stem diameter (Glawischnig *et al.,* 2000). Results further showed a significant interaction between the pepper hybrids and fertilization treatments (Table 4). The enhanced stem diameter manifested in the interaction of treatment combination *Azotobacter* + urea (450 mg $L^{-1}$) with the chili Barbarian hybrid (2.38 cm), with the lowest value recorded in the control treatment with the Hyffa hybrid (1.73 cm).

**Early yield**

Study outcomes showed substantial differences between the pepper hybrids in early yield (Table 5). The highest early yield materialized in the Barbarian hybrid (3.67 t $ha^{-1}$), while the lowest was in the Hyffa hybrid (3.04 t $ha^{-1}$). It could be because of the hybrids' ability to adapt to environmental conditions and thus providing better performance in utilizing available resources, which positively reflects on growth characteristics and yield in peppers (Tripodi *et al.,* 2018).

On fertilization treatments, significant differences occurred among them for the early yield (Table 5). The premier chili early yield was evident in the treatment *Azotobacter* + urea (450 mg $L^{-1}$), at an average of 5.89 t $ha^{-1}$, while the lowest resulted in the control treatment at an average of 1.51 t $ha^{-1}$. The combined role of bacteria and nitrogen fertilizer can play an important role in increasing crop yields by improving soil properties and providing the nutrients necessary for plant growth. Soil bacteria help decompose organic material and release nutrients into the soil in a way that enables the plant to absorb them. It also helps improve soil structure, increasing its water-holding capacity and improving aeration. As for the nitrogen, it helps improve the growth of vegetative plants, causing the improvement of the size and quality of the pepper crop (Yildirim *et al.,* 2012).

Results also showed a noteworthy interaction effect between pepper hybrids and fertilization treatments (Table 5). The ultimate early yield appeared with the interaction of the treatment combination of *Azotobacter* + urea (450 mg $L^{-1}$) and the chili Barbarian hybrid (6.25 t $ha^{-1}$), while the lowest interaction observed in the control treatment with the Hyffa hybrid (1.44 t $ha^{-1}$).

**Total yield**

Study findings revealed significant differences between pepper hybrids in the total yield (Table 6). The maximum total yield resulted in the Barbarian hybrid (18.12 t $ha^{-1}$), while the

**Table 5.** Effect of Azotobacter and urea and their interaction on the early yield in peppers.

| Fertilization treatments | Hybrids | | Means (t ha$^{-1}$) |
|---|---|---|---|
| | Barbarian | Hyffa | |
| Control | 1.57 | 1.44 | 1.51 |
| Azotobacter treatment | 2.33 | 1.93 | 2.13 |
| Urea (150 mg L$^{-1}$) | 2.50 | 2.01 | 2.25 |
| Urea (300 mg L$^{-1}$) | 2.87 | 2.42 | 2.64 |
| Urea (450 mg L$^{-1}$) | 3.33 | 2.74 | 3.03 |
| Azotobacter + Urea (150 mg L$^{-1}$) | 4.85 | 3.80 | 4.32 |
| Azotobacter + Urea (300 mg L$^{-1}$) | 5.71 | 4.50 | 5.10 |
| Azotobacter + Urea (450 mg L$^{-1}$) | 6.25 | 5.53 | 5.89 |
| Means (t ha$^{-1}$) | 3.67 | 3.04 | |
| $LSD_{0.05}$ Hybrids: 0.391, Fertilization treatments: 0.397, Interaction: 0.40 | | | |

**Table 6.** Effect of Azotobacter and urea and their interaction on the total yield in peppers.

| Fertilization treatments | Hybrids | | Means (t ha$^{-1}$) |
|---|---|---|---|
| | Barbarian | Hyffa | |
| Control | 8.39 | 7.14 | 7.76 |
| Azotobacter treatment | 11.15 | 9.33 | 10.24 |
| Urea (150 mg L$^{-1}$) | 11.12 | 9.38 | 10.25 |
| Urea (300 mg L$^{-1}$) | 13.36 | 12.59 | 12.97 |
| Urea (450 mg L$^{-1}$) | 16.67 | 14.72 | 15.69 |
| Azotobacter + Urea (150 mg L$^{-1}$) | 24.59 | 18.00 | 21.29 |
| Azotobacter + Urea (300 mg L$^{-1}$) | 29.04 | 22.29 | 25.66 |
| Azotobacter + Urea (450 mg L$^{-1}$) | 30.64 | 27.39 | 29.01 |
| Means (t ha$^{-1}$) | 18.12 | 15.10 | |
| $LSD_{0.05}$ Hybrids: 2.602, Fertilization treatments: 2.667, Interaction: 2.786 | | | |

minimum total yield emerged in the Hyffa hybrid (15.10 t ha$^{-1}$). This may refer to the genetic differences between hybrids and the potential of each hybrid to absorb nutrients and convert them into dry matter in the pepper crop (Jan *et al.,* 2020).

On fertilization treatments, remarkable variations ensued between them for the total yield (Table 6). The highest total yield in chili was prominent in the treatment *Azotobacter* + urea (450 mg L$^{-1}$), with an average of 29.01 t ha$^{-1}$, and the lowest total yield manifested in the control treatment (7.76 t ha$^{-1}$). The superiority of the treatment combination of *Azotobacter* + urea (450 mg L$^{-1}$) in total yield may be due to its positive role in enhancing vegetative growth indicators, such as the number of branches. In turn, it increased the number of fruits and the early and total yields. Similarly, the role of *Azotobacter* and spraying urea contributes to boosting nitrogen in the leaves, with a key role in producing growth hormones. Added to that, hormone-like substances (auxins and cytokinins) secreted by *Azotobacter* act as chemical vectors in the transfer of nutrients from the leaves to the fruits, enhancing fruit growth and weight (Al-Khafaji, 2014).

Moreover, results provided significant interaction between pepper hybrids and fertilization treatments (Table 6). In chili, the greatest total yield was evident in the interaction of the treatment combination of *Azotobacter* + urea (450 mg L$^{-1}$) and the Barbarian hybrid (30.64 t ha$^{-1}$), while the least was in the control treatment with the Hyffa hybrid (7.14 t ha$^{-1}$).

**Capsaicin content**

The study obtained sizable variations between pepper hybrids in capsaicin content of fruits (Table 7). The highest capsaicin content in chili fruits resulted in the Barbarian hybrid (742.41

**Table 7.** Effect of Azotobacter and urea and their interaction on the capsaicin content in pepper fruits.

| Fertilization treatments | Hybrids | | Means (mg $kg^{-1}$) |
|---|---|---|---|
| | Barbarian | Hyffa | |
| Control | 604.20 | 423.80 | 514.00 |
| Azotobacter treatment | 631.30 | 452.60 | 541.00 |
| Urea (150 mg $L^{-1}$) | 642.10 | 444.20 | 543.15 |
| Urea (300 mg $L^{-1}$) | 682.20 | 499.60 | 590.90 |
| Urea (450 mg $L^{-1}$) | 706.60 | 576.10 | 641.35 |
| Azotobacter + Urea (150 mg $L^{-1}$) | 871.60 | 611.70 | 741.75 |
| Azotobacter + Urea (300 mg $L^{-1}$) | 892.70 | 657.30 | 775.00 |
| Azotobacter + Urea (450 mg $L^{-1}$) | 908.60 | 731.80 | 820.20 |
| Means (mg $kg^{-1}$) | 742.41 | 549.63 | |
| $LSD_{0.05}$ Hybrids: 24.66, Fertilization treatments: 25.16, Interaction: 27.20 | | | |

mg $kg^{-1}$), while the lowest capsaicin content was in fruits of the Hyffa hybrid (549.63 mg $kg^{-1}$). This could be attributable to the genetic potential and differences between the pepper hybrids (Liu *et al.,* 2020).

Concerning fertilization treatments, observations revealed significant differences between them for capsaicin content in chili fruits (Table 7). The maximum capsaicin content in fruits appeared in the treatment combination of *Azotobacter* + urea (450 mg $L^{-1}$), with an average of 820.20 mg $kg^{-1}$, while the minimum value emerging in the control treatment (514.00 mg $kg^{-1}$). The reason for this is the positive role of Azotobacter and urea fertilizer foliar application in the nitrogen supply to chili plants, which raised the nitrogen content in leaves. This also reflected in an enhanced vegetative growth, causing an increase in the interception of the leaves and sunlight absorption and its positive impact on activating the process of photosynthesis. As a result, it improved the production and accumulation of manufactured materials, as reflected in the increased capsaicin content in fruits. The capsaicin building pathway also depends on the supply of nitrogen and its beneficial role in the formation of initiating amino acids (Sung *et al.,* 2005), which, in turn, activates the bio-pathway of capsaicin compound, hence, boosting the content in chili fruits (Fernandez *et al.,* 2021).

The results further showed a relevant interaction between pepper hybrids and fertilization treatments (Table 7). The highest capsaicin compound in chili fruits was visible in the interaction of the treatment combination of *Azotobacter* + urea (450 mg $L^{-1}$) and the Barbarian hybrid (908.60 mg $kg^{-1}$), while the lowest interaction effect appearing in the control treatment with the Hyffa hybrid (423.80 mg $kg^{-1}$).

## CONCLUSIONS

Study results revealed the positive role of combining biofertilizers with nitrogen fertilizers in increasing vegetative growth indicators and quantitative and qualitative yield characteristics of pepper plants. Therefore, these reduced the excessive use of chemical fertilizers, which are also harmful to the environment. Furthermore, the study recommends that the chili hybrid Barbarian should be the cultivated cultivar in the holy governorate of Kerbala, Iraq, as it suits the climatic conditions.

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