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Optimisation of the amount of nitrogen enhances quality and yield of pepper

SHUANG HAN, XIAOQIN ZHU, DONGMEI LIU, LIBO WANG, DONGLI PEI*

Henan Provincial Key Laboratory of Plant-Microbe Interactions, Shangqiu Normal University, Shangqiu, Henan, P.R. China

*Corresponding author: peidongli@126.com

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Abstract: The goals of this study were to explore the characteristics of nitrogen (N) absorption and utilisation of chilli peppers (*Capsicum annuum* L.), improve the utilisation rate of nitrogen, and provide a theoretical basis for scientific fertilisation. In this experiment, pepper cv. Huoyanjiaowang was used as the material, and potted sand cultures and field randomised block experiments were conducted to study the effects of fertilisation of different forms of nitrogen on the photosynthetic characteristics, chlorophyll, nitrate nitrogen, alkaline nitrogen, capsaicin, dihydrocapsaicin and yield. In the pot experiment, the nitrogen application rates were 0, 10, 100, 320 and 600 mg/L, a level of nitrogen of 100 mg/L significantly inhibited the growth of pepper. With the increase in the application of nitrogen, the photosynthetic capacity gradually decreased, and 10 mg/L was the optimal nitrogen level. Under 0 and 10 mg N/L nitrogen levels in the field experiment, the content of chlorophyll of this group was significantly lower than those of other treatment groups, indicating that the plot lacked nitrogen. With the increase in the level of application of nitrogen, the contents of nitrate nitrogen and alkaline hydrolysis nitrogen in the soil increased. The yield of 153.18 kg/ha and 230 kg/ha nitrogen treatments was relatively high. Therefore, among the five nitrogen treatment levels, treatment with 153.18–230 kg N/ha was the most effective at stimulating the growth and yield of pepper.

Keywords: macronutrient; nitrogen use efficiency; photosynthesis; growth and development; crop production

The area in Henan province in which peppers are planted is large, and the problem of excessive application of nitrogen (N) fertiliser in production is common, which not only wastes resources and causes environmental pollution but also reduces the quality of pepper. Therefore, it is of substantial significance to study the appropriate amount of fertiliser and improve the rate of utilisation of nitrogen fertiliser. Nitrogen is the main nutrient and component of most macromolecules and signalling compounds, such as nucleic acids, proteins and hormones (Krapp 2015). The excessive and unreasonable use of nitrogen fertiliser not only leads to the waste of nitrogen

fertiliser resources but also leads to a decrease in the rate of nitrogen utilisation and pollution (Zhang et al. 2018). An appropriate amount of nitrogen fertiliser can significantly increase nitrogen use efficiency and decrease losses owing to the leaching of nitrate (Duan et al. 2019). As the amount of fertilisation increases, the mineral nitrogen in the soil may increase (Rodríguez et al. 2020). Cui et al. (2020) showed that as the amount of nitrogen increased in different soil layers (0–20, 20–40 and 40–60 cm), that soil nitrate-nitrogen also increased.

Nitrogen is one of the most important mineral nutrients in plant growth, and it is considered one

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of the primary limiting factors for crop production (Glass 2003). Over-fertilisation over the previous few decades has caused serious environmental problems. This problem is now being addressed by reducing the applications of fertiliser to limit pollution. However, N is involved in the biosynthesis of many important compounds, and its lack could cause a serious imbalance, resulting in reduced growth and a lower yield of plants (Rubio-Wilhelmi et al. 2011). N efficiency affects many aspects of plants, including growth (Zhu et al. 2014), photosynthesis (Pérez-Jiménez et al. 2019), the maximum potential quantum efficiency of photosystem II (Cetner et al. 2017), and content of chlorophyll (Wen et al. 2019). An excessive constraint in the uptake of N limits photosynthesis alters the metabolic composition (Sperling et al. 2019) and lowers the yield of pepper fruit (de Ávila Silva et al. 2019). The application of nitrogen fertiliser affects the structure of rice starch, and excessive levels of nitrogen reduce the quality of grain (Pérez-Jiménez et al. 2019). *Capsicum chinense* Jacq. tended to decrease its rate of CO₂ assimilation, harvest index and fruit weight under high N conditions (de Ávila Silva et al. 2019). The application of moderate nitrogen levels reduces yield losses and the deterioration of grain quality (Wang et al. 2020).

Pepper (*Capsicum annuum* L.) is one of the most important vegetables throughout the world (Li 2010). Nitrogen is a key restrictive factor for continuous pepper output (Bar-Tal et al. 2001). In recent years, peppers, like other crops, have been subjected to increased applications of nitrogen as the primary agricultural measure to increase production. However, the excessive use of nitrogen fertiliser will not only delay the maturity of the pepper and reduce the spiciness but also aggravate diseases and deleteriously affect the yield and quality. Previous studies have primarily focused on the responses of switch grass biomass to the application of N nutrients. The effects of various levels of N deficiency limit the uptake of nitrogen and photosynthetic parameters and the production of chlorophyll, capsaicin and dihydrocapsaicin in peppers. In this study, the conventional cv. Huoyanjiaowang was used as the experimental material, and the relationship between the level of fertilisation of pepper and the growth and yield of the pepper was studied in two ways: pot planting and field tests to provide theoretical support for rational fertilisation and lay the foundation for the efficient use of pepper for nitrogen breeding.

MATERIAL AND METHODS

Overview of the test area. The experimental test site was located in Zhecheng county, Shangqiu city, Henan province. The experimental park was located in a temperate monsoon climate, with an average annual temperature of approximately 14.4 °C and an average annual rainfall of 700.7 mm. The precipitation from May to September in 2021 was 30% more than that in previous years. The basic physical and chemical properties of the surface soil (0–10 cm) before planting were pH 8.5, 203.14 us/cm conductivity, 19.4 mg/kg ammonium nitrogen, 14.30 mg/kg nitrate nitrogen, 0.15% total nitrogen, 163.16 mg/kg available potassium, 13.09 mg/kg available phosphorus, and 19.90 g/kg organic matter.

Plant materials and experimental design. The conventional pepper (*C. annuum* L.) cv. Huoyanjiaowang was used for the experiment. Seeds that were free of diseases and insect pests were selected, soaked in water, and placed in an artificial climate room. The average maximum/minimum temperature, photoperiod (day/night), and air humidity were 28 °C/21 °C, 14/10 h, and 70%, respectively. After approximately 30 days, the pepper seedlings were transplanted into 3 L pots that contained a 1:1 mixture of vermiculite and perlite with no added fertiliser, and they were then randomly assigned to five nitrogen levels. The N application rates were 0, 10, 100, 320 and 600 mg/L. The nitrogen source was NH₄NO₃. The nutrient solution was prepared with deionised water. The concentration of phosphorus, potassium, calcium, and magnesium in the nutrient solution was modified with Hewitt nutrient solution, which contains K₂SO₄ 2.5 mmol/L, MgSO₄ 1.5 mmol/L, and NaH₂PO₄ 1.33 mmol/L, CaCl₂ 2 mmol/L, and the pH was adjusted to 6.5. The trace elements were formulated with a modified Hoagland's nutrient solution that contained H₃BO₃ 25 µmol/L, MnCl₂ 2 µmol/L, ZnSO₄ 2 µmol/L, Na₂Mo₇O₂₄ 0.065 µmol/L, Fe²⁺-EDTA 40 µmol/L, CuSO₄ 0.5 µmol/L, NiCl 0.5 µmol/L. The nutrient solution was applied every 3 days. The culture temperature was 25–28 °C during the day, 18–22 °C at night with 14 h of light per day, and the light intensity was 200 µmol/m²/s. The relative humidity was 70–80%. The pepper seedlings of each treatment were randomly assigned to three replicates. The field experimental design included sowing for 6 weeks. The pepper seedlings were planted after they had grown 5–6 true leaves. The seedlings were planted in ridges that were 80 cm wide with

<https://doi.org/10.17221/123/2021-PSE>

2 rows per ridge, 40 cm row spacing, 25 cm plant spacing, and 96 plants per plot. Five concentrations of nitrogen application were assigned (N-0, N-I, N-II, N-III, N-IV with three replicates for each treatment).

The duration of the experiment was 2019, 2020 and 2021 for a total of 3 years. A completely random design was used in the field experiment of the study with three replicates. The applications of the N-0, N-I, N-II, N-III and N-IV treatments of nitrogen element were 0, 92, 153.18, 230 and 306.36 kg/ha, respectively. The amount of phosphate element [50% of $\text{Ca}(\text{H}_2\text{PO}_4)_2$] was 39.75 kg/ha, and it was applied as the base fertiliser two weeks after planting. Urea was applied at a ratio of 7:3 in two portions. The amount of KCl was 172.85 kg/ha, and it was applied twice in a ratio of 3:7.

Determination of the photosynthetic parameters

Pot culture experiment. After 21 days of treatment, the fourth fully expanded blade from the top was selected, and photosynthetic parameters were measured by controlling the light intensity using a LI-6400XT photosynthesis instrument (LI-COR, Lincoln, USA). Three biological replicates were established per treatment.

Field experiment. After two weeks of nitrogen treatment, the first leaf at the base of the lateral branch was selected to measure the photosynthetic parameters.

Determination of pigments. At the end of the experiment (21 days), the fourth fully expanded blade from the top was selected. Leaf disks (0.2826 cm^2 in size) were collected at noon on highly sunny days and immediately placed in 80% acetone. The solution was inverted a few times each day until the pigment had completely eluted into the solution. Chlorophyll (*Chl*) and carotenoids (*Car*) were assayed as described by Lichtenthaler (1987). There were three replicates per treatment (four disks from the same leaf per replicate).

Determination of soil nitrate-nitrogen and alkaline hydrolysis nitrogen. One week before the peppers were harvested, soil samples 5–10 cm deep were collected between two rows of peppers on the ridge to determine the soil-related parameters using a tubular soil drill. A UV spectrophotometric correction factor method was used to determine the soil and fruit nitrate nitrogen. Ten grams of fresh soil samples (accurate to 0.01 g), considered to be wet soil, was weighed into a screw-top polyethylene

bottle. A volume of 50 mL of 2 mol/L potassium chloride was added, and the bottle cap was screwed on tightly. The sample was then oscillated at room temperature for 30 min and filtered through qualitative filter paper into a 10 mL centrifuge tube. The potassium chloride extract was used as the reference solution, and its absorbance was measured using a UV spectrophotometer (UV2300II, Shanghai Tianmei Scientific Instruments, Shanghai, China).

Determination of the total nitrogen in leaves. At the end of the experiment (21 days), the fourth fully expanded blade from the top was selected. One leaf was collected from each plant for a total of 15 leaves. The leaves were dried at 80°C and ground into a powder. The Kjeldahl method was used to measure the total content of nitrogen (Bao 2000).

Contents of capsaicin and dihydrocapsaicin peppers at different developmental stages. Fifteen plants were selected in each plot. One flower bud was selected for each plant, and then samples were taken every 14 days. The developmental stages of peppers sampled at each time are shown in Figure 1. The peppers were taken from each plot four times. The peppers removed were dried at 60°C , and the seeds were removed and crushed through a 40-mesh sieve to prepare for the extraction of capsaicin and dihydrocapsaicin.

Soxhlet extraction of pepper. Three grams of paprika were added to 60 mL of absolute ethanol. The pepper extract was purified to 20 mL after siphoning for 15 times and centrifuged at 6 000 rpm for 15 min to collect the supernatant. The capsaicin and dihydrocapsaicin analyses were conducted on an Agilent Technologies 1260 Infinity II HPLC system (Santa Clara, USA). The column was a Shim-pack GIST C18 column (Shimadzu, Tokyo, Japan) ($4.6 \times 250 \text{ mm}$, $5 \mu\text{m}$ particle size). The mobile phase con-



Figure 1. Different developmental stages of pepper

sisted of methanol:water (70:30 *v/v*). The total run time was 30 min at a flow rate of 1 mL/min. The detection wavelength was set at 280 nm, and the injection volume was 10 μ L.

Test production. The points were randomly selected to test their production. In each plot, random samples of 2 m² were taken by measuring samples 1 m wide perpendicular to the ridge and then measuring 2 m long along the ridge. All the peppers were removed from each plant, weighed fresh, and repeated three times for each treatment. The number of plants was recorded at each sampling point and then brought back to the laboratory for drying at 60 °C and subsequent weighing.

RESULTS

Effects of different levels of nitrogen on the photosynthetic characteristics of pepper. On the 21st day, the net photosynthetic rate (P_n , Figure 2A) under the 600 mg/L nitrogen treatment was significantly lower than those of the 0, 10 and 100 mg/L levels of application. The 320 mg/L treatment group did not reach a level of significance. The stomatal conductance (g_s , Figure 2B) was significantly lower

than when nitrogen levels of 0 mg/L and 10 mg/L were applied. The highest g_s , intercellular CO₂ concentration (C_i , Figure 2C) and transpiration rate (E , Figure 2D) were observed in plants under the 10 mg/L treatment. As a result, the 600 mg/L and 320 mg/L treatments had a serious impact on the growth of peppers. Treatments at 10 mg/L were the most suitable for the growth of peppers. However, the 0 mg/L nitrogen deficiency treatment did not have a significant impact on the pepper plants.

After two weeks of nitrogen treatment, the photosynthetic parameters of pepper were measured, and neither P_n , g_s , C_i nor E reached significant levels. The results of the three-year study are shown in Figure 3. The photosynthetic rate decreased under the N-IV nitrogen level of application, indicating that excessive fertilisation has a negative impact on photosynthesis. This figure also shows the results for different treatments in the same year.

The effect of different levels of nitrogen on the contents of chlorophyll and carotenoid in pepper leaves. The contents of Chl *a*, Chl *b* and Car in the N-0 and N-I groups were significantly lower than those in the other nitrogen concentration treatments, and the contents of these pigments in the

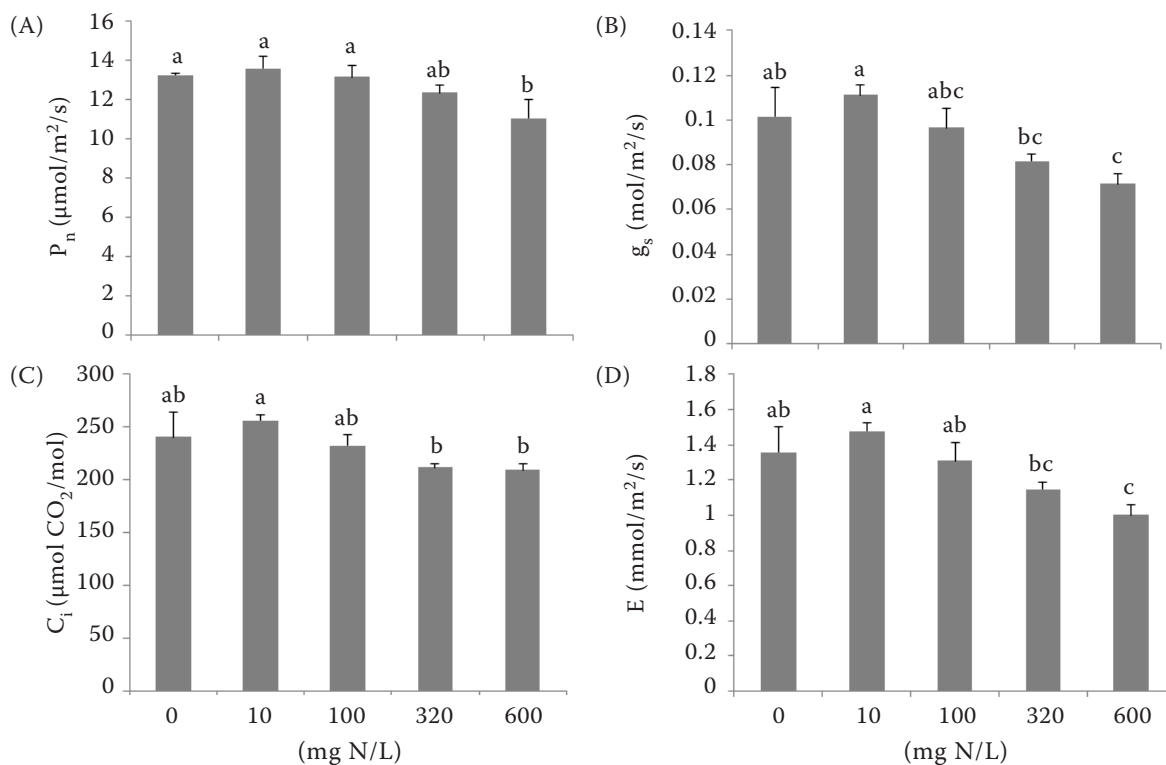


Figure 2. Effects of different levels of application of nitrogen on pepper photosynthetic parameters. (A) net photosynthetic rate (P_n); (B) stomatal conductance (g_s); (C) intercellular CO₂ concentration (C_i), and (D) transpiration rate (E)

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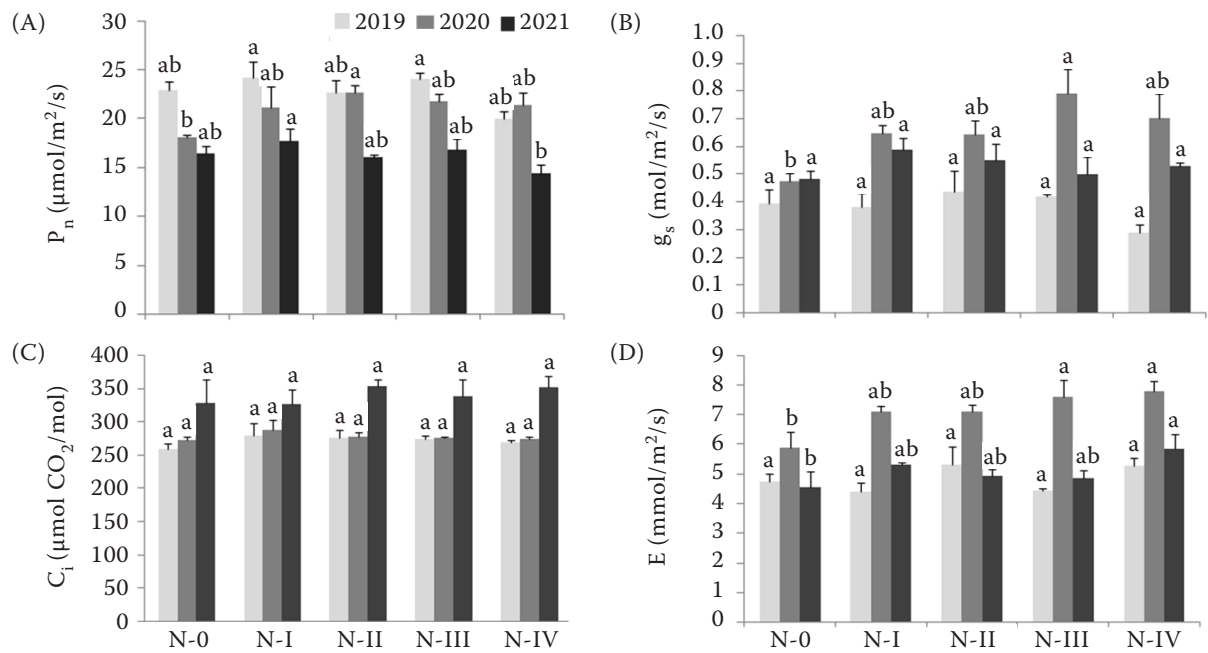


Figure 3. Effects of different levels of nitrogen application on pepper photosynthetic parameters. (A) net photosynthetic rate (P_n); (B) stomatal conductance (g_s); (C) intercellular CO_2 concentration (C_i), and (D) transpiration rate (E). N-0 – 0, N-I – 92, N-II – 153.18, N-III – 230, N-IV – 306.36 kg N/ha

N-II, N-III and N-IV groups did not reach significant levels (Figure 4).

The effect of different levels of nitrogen on the soil nitrate-nitrogen and alkaline hydrolysis

nitrogen. The nitrate-nitrogen content in the N-IV group was significantly higher than those in the N-0, N-I, N-II, N-III and N-IV treatments, which showed no significant difference in the content of soil nitrate-

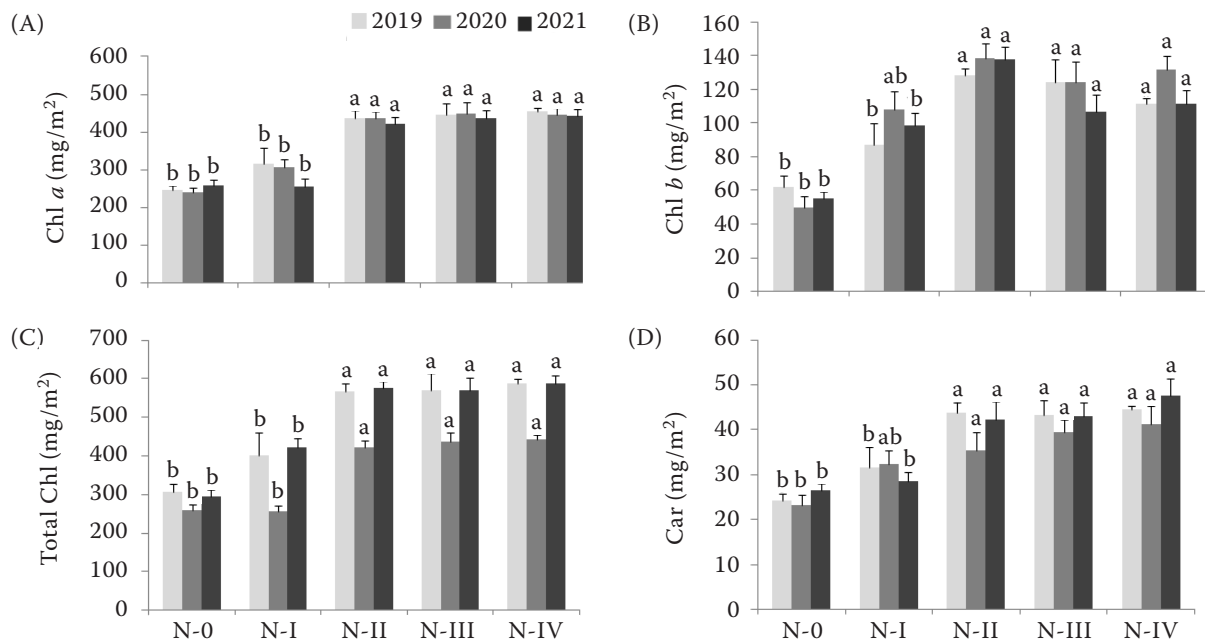


Figure 4. Effects of different levels of application of nitrogen on chlorophyll (Chl) and carotenoid (Car) concentrations in pepper leaves. (A) Chl a; (B) Chl b; (C) total Chl, and (D) Car). N-0 – 0, N-I – 92, N-II – 153.18, N-III – 230, N-IV – 306.36 kg N/ha

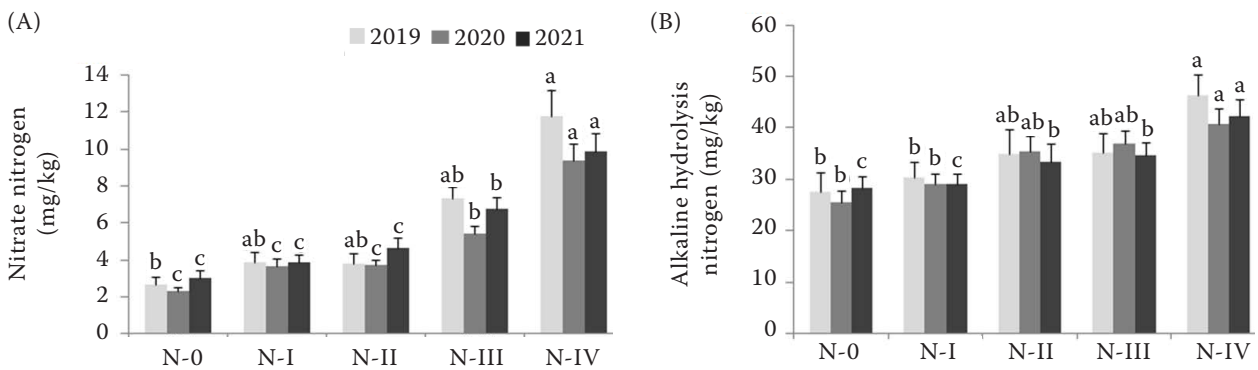


Figure 5. Effects of different levels of application of nitrogen on (A) soil nitrate nitrogen and (B) alkaline hydrolysis nitrogen content. N-0 – 0, N-I – 10, N-II – 100, N-III – 320, N-IV – 600 mg N/L

nitrogen (Figure 5A). The content of alkali hydrolysis nitrogen in soil of N-IV treatment was significantly higher than those in the N-0 and N-II groups. In addition, the difference in the content of alkaline hydrolysis nitrogen between the N-II and N-III treatments did not reach a significant level (Figure 5B).

The 56-days pepper in Figure 1 showed that as the amount of nitrogen increased, the nitrate-nitrogen content in the fruit increased significantly.

The effect of different levels of nitrogen on total nitrogen in leaves. As shown in Figure 7, as the application of nitrogen increased, the content of total nitrogen of the leaves also increased correspondingly. However, the total nitrogen content of the leaves at the IV application level of nitrogen was significantly lower than that of the III level of nitrogen application. Therefore, the application of excessive amounts of nitrogen affected its accumulation.

Effects of different levels of application of nitrogen on capsaicin and dihydrocapsaicin. The developmental stage of peppers sampled at each time

is shown in Figure 1. Capsaicin and dihydrocapsaicin were not detected until 14 and 28 days after flowering. With the increase in the application of nitrogen, the contents of capsaicin and dihydrocapsaicin increased first and then decreased. The contents of capsaicin and dihydrocapsaicin in the N-0 and N-I treatment group were significantly lower than those in the N-II and N-III treatment group at 42 days after and reached an extremely significant level by 56 days after flowering; both compounds were significantly lower in the N-IV treatment group at 56 days after flowering than those in the N-III treatment group (Figure 8). It showed that the application of insufficient or excessive nitrogen fertiliser deleteriously affected the accumulation of capsaicin and dihydrocapsaicin.

Test production. Based on the analysis of the three-year yield data, we found that the yield of pepper from the N-II group was the highest, and the yield of pepper from the N-IV high-nitrogen treatment group was significantly lower than those of the other nitrogen levels, indicating that the N-II group was the best nitrogen

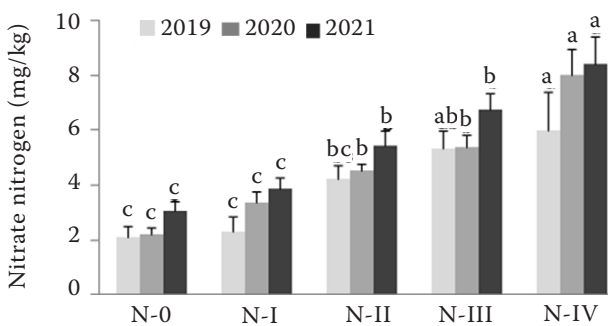


Figure 6. Effects of different levels of application of nitrogen on the levels of fruit nitrate nitrogen. N-0 – 0, N-I – 92, N-II – 153.18, N-III – 230, N-IV – 306.36 kg N/ha

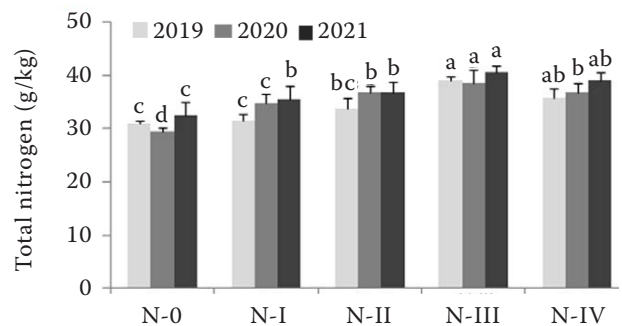


Figure 7. Effects of different levels of application of nitrogen on the total nitrogen in leaves. N-0 – 0, N-I – 92, N-II – 153.18, N-III – 230, N-IV – 306.36 kg N/ha

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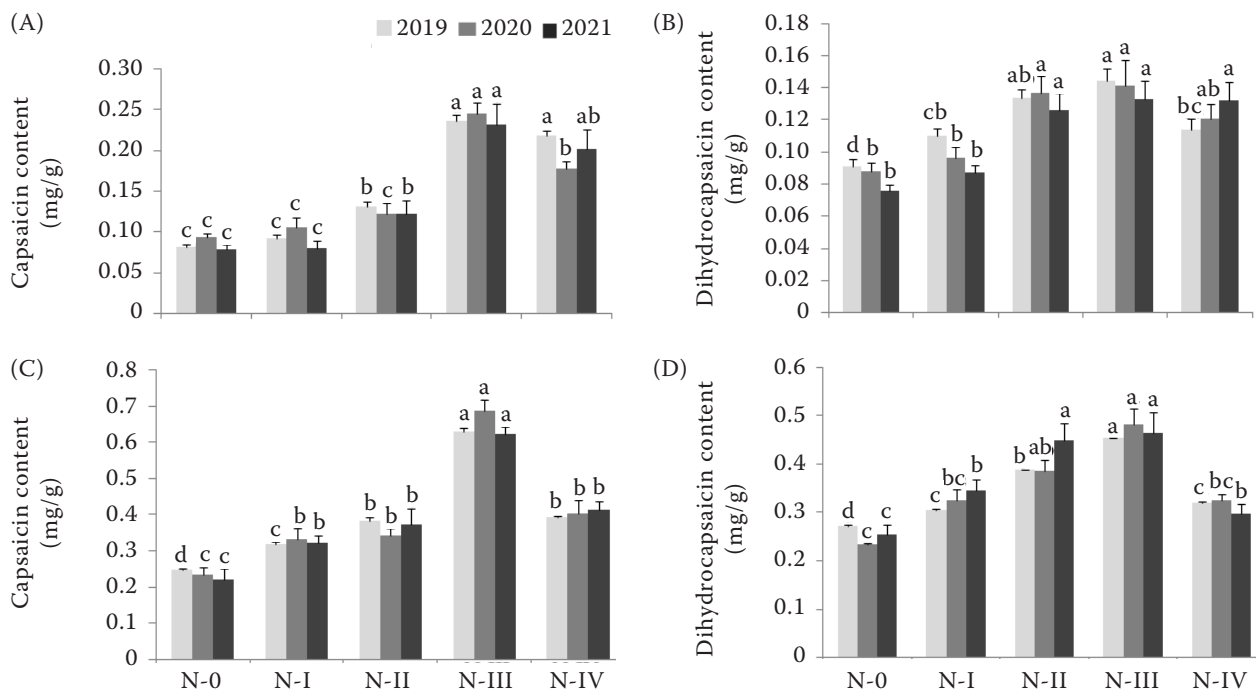


Figure 8. Effects of different nitrogen application levels on capsaicin (A, C) and dihydrocapsaicin (B, D) (A and B – 42 days after flowering; C and D – 56 days after flowering). N-0 – 0, N-I – 92, N-II – 153.18, N-III – 230, N-IV – 306.36 kg N/ha

level. The analysis of data from the third year indicated that the peppers in group N-III had the highest yield. Such a phenomenon could be related to the loss of nitrogen caused by excessive rain during the growing season.

Table 1. Effect of different levels of nitrogen on yield

Treatment	Year	Area (m ²)	Number of plants	Fresh weight		Yield (kg/ha)
				Dry weight (kg)		
N-0	2019	2	14	1.759	0.69	3 450 ± 325 ^b
	2020	2	14	1.823	0.755	3 775 ± 213 ^b
	2021	2	12	1.534	0.543	2 715 ± 124 ^c
N-I	2019	2	15	2.619	0.790	3 950 ± 335 ^a
	2020	2	14	2.702	0.806	4 030 ± 121 ^a
	2021	2	13	2.534	0.674	3 370 ± 122 ^a
N-II	2019	2	13	2.168	0.84	4 200 ± 417 ^a
	2020	2	14	2.356	0.882	4 460 ± 321 ^a
	2021	2	12	1.840	0.687	3 435 ± 213 ^a
N-III	2019	2	14	2.628	0.82	4 100 ± 326 ^a
	2020	2	13	2.702	0.733	3 665 ± 202 ^b
	2021	2	12	2.074	0.698	3 490 ± 123 ^a
N-IV	2019	2	13	2.602	0.65	3 250 ± 301 ^b
	2020	2	13	2.193	0.702	3 510 ± 234 ^b
	2021	2	13	1.875	0.643	3 215 ± 124 ^b

N-0 – 0, N-I – 92, N-II – 153.18, N-III – 230, N-IV – 306.36 kg N/ha

Thus, it was considered that the rate of application of 158.13–230 kg/ha nitrogen is the most effective treatment. The excessive application of nitrogen fertiliser not only caused environmental pollution but also reduced yield and quality.

DISCUSSION

Many studies have reported that there is a relationship between photosynthesis and the supply of nitrogen (Mu and Chen 2021). N deficiency hinders plant growth and development and reduces photosynthesis and plant productivity (Mu et al. 2016). Low N stress significantly decreases the content of chlorophyll in maize (Wu et al. 2019). When nitrogen was lacking, the nitrogen was 2.3 times that of the control treatment in rice (Zhong et al. 2019). It was found that in the pot experiment, the photosynthetic rate did not differ significantly from the control after 2 weeks of nitrogen deficiency treatment. This conclusion was inconsistent with the conclusions of other researchers, which may be owing to processing time. There was no noticeable inhibition of photosynthetic capacity in the field experiment. It could be that the nitrogen in the soil can meet the requirements of the early growth of the pepper. This cultivar is tolerant of low nitrogen.

The application amount of nitrogen fertiliser significantly or positively correlates with the accumulation of nitrate in vegetables (Oliver et al. 2017). Reducing the accumulation of NO_3^- in plants would help to increase the yield and quality of crops (Gou et al. 2020). Many researchers have reported that the accumulation of excessive nitrate can reduce agricultural productivity owing to a reduction in photosynthesis and enzyme activity (Aydinsakir et al. 2019, Matsumura et al. 2020). Excessive NO_3^- in the soil causes ion toxicity and water deficits, and thus, inhibits plant photosynthesis and growth (Ju and Gu 2014). High nitrogen fertiliser inhibits root growth (Comfort et al. 1988). Nitrogen losses from vegetables owing to NO_3^- leaching is usually considerable (Padilla et al. 2018). This study found that with the increase in the rate of nitrogen application, the nitrate-nitrogen and alkaline hydrolysis nitrogen in the soil increased; the accumulation of total nitrogen in the leaves increased significantly, and the corresponding yield decreased significantly. This finding is consistent with the results of other researchers.

Nitrogen directly affects the chlorophyll content of plants (Van Wallendael et al. 2020). This study

found that the relative chlorophyll content of the leaves showed a trend of first increasing and then decreasing with the increase in the rate of application of nitrogen. When the nitrogen element rate was 153.18 kg/ha, the chlorophyll content reached its maximal value. An excessive supply of nitrogen leads to excessive vegetative growth and relatively weakened reproductive growth, which was one of the important reasons for the decline in yield. This conclusion is consistent with the findings that the relative chlorophyll content of switchgrass leaves reached their peak value under the medium nitrogen treatment but decreased under the high nitrogen treatment (He et al. 2021).

The optimal N management of sweet pepper required recommendations of the optimal N supply, which maximised fruit production with a minimal supply of N (Rodríguez et al. 2020). The lower level of nitrogen aided in the accumulation of cinnamic acid and coumaric acid, which are the intermediate products of capsaicin synthesis (Chen et al. 2013). The results of this study found that too much or too little nitrogen will reduce the contents of capsaicin and dihydrocapsaicin. The long-term application of chemical fertilisers will accelerate the mineralisation of organic matter in the soil and decrease the total amount of organic matter (Khan et al. 2007). Zhang et al. (2012) concluded that the application of chemical fertilisers basically kept the level of soil organic matter unchanged. In this study, we found that different amounts of nitrogen applied had no significant effect on the content of organic matter in the soil.

In this study, with the increase in the rate of application of nitrogen, the nitrate-nitrogen and alkaline hydrolysis nitrogen content in the soil increased, and the total nitrogen content of the leaves increased first and then decreased. The most suitable potted nitrogen application rate was 10 mg/L NH_4NO_3 , and the field nitrogen element application rate was 153.18–230 kg/ha. An excessive or insufficient amount of nitrogen element hindered the photosynthetic capacity and growth of pepper. If the amount of nitrogen element exceeds this value, the yield and spiciness of the pepper will decrease.

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