Effect of Phenylalanine and Nitrogen on accumulation of Capsaicin alkaloid and some physiological parameters in two pepper *Capsicum annuum* L cultivars

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Abstract

The experiment was carried out in a greenhouse belongs to Department of Biology / faculty of Education for Girls / University of Kufa for the season 2016-2017. The study was conducted to evaluate the effect of Amino acid Phenylalanine and nitrogen on accumulation of Capsaicin and other contents in the fruits of two pepper Capsicum annuum L cultivars, chilli and sweet namely Ninar and Mystro respectively. Factorial experiment in Completely Randomized Design (C.RD) with three replicates for each parameter was used, and the means were compare using Duncan's Multiple Range Test (DMRT) at the probability level of 0.05. The experiment included 5 treatments i.e. the interaction between two pepper Mystro and Ninar cultivars sweet and chilli respectively with five spray treatments T1 control treatment(distilled water), T2, T3 Phenylalanine at concentration of 100 and 200 ppm respectively, T4, T5 Nitrogen at concentration of 200 and 300 ppm respectively . The Results from this study show that T3 treatment (200ppm Phenylalanine) affected positively on enhancing all the parameters under study, in term of Phenylalanine, Valine, Capsaicin, Dihydrocapsaicin and Vitamin C. Maximum values for these traits were found in Ninar chilli pepper 551.7 mg. g⁻¹ dry weight, 380.7 mg. g⁻¹ dry weight, 1266.1 mg. g⁻¹ dry weight, 719.1 mg. g⁻¹ dry weight, 426.5 mg. g⁻¹ fresh weight. Moreover, highest pungency level was found in Ninar cultivar (20257.6 SHU) which classified as moderately pungent based on pungency scale.

Keywords: Capsaicin, Phenylalanine, Nitrogen, Valine, pepper

1. Introduction

Pepper (*Capsicum annuum* L.) belongs to Solanaceae family which is known as night shade family. This family contains large numbers of plants such as eggplant, tomato and pepper among others.

Pepper fruits are rich source of health-related compounds such as provitamin A, C, tocopherols (vitamin E) and other antioxidant compound like Carotenoids pigments, Polyphenols as well in addition to other macro and micro nutrients (González-Zamora et al.;2013).

N fertilizer is an essential for improving yield and fruit for many economic crops such as tomato Law-Ogbomo and Egharevba (2009) and peppers Tumbare et al. (2004); Aminifard et al (2012); Marhoon and Abbas(2015). Plants absorbed and assimilate N in a form of ammonium (NH4+), nitrate (NO3–), urea in addition to the amino acid as another source of N (Liu and Lee,2012). Amino acid Phenylalanine and Valine are the precursors in path ways of many compounds including Capsaicin, flavonoids and a large numbers of phenyl propanoids compounds. Capsaicin alkaloid well famous with its activity in food pungency, and the medical applications of capsaicin make this compound very popular (Amruthraj et al, 2014).beside its physiological and pharmaceutical properties in the curing from rheumatism, analgesic, and using as antifungal, bacteria, as well as in the prevention of certain types of cancer or reducing cholesterol Capsaicin used for burning body fats (Arora et al., 2011; Gudeva and Veselinovska 2011).

Several factors affected on the accumulation of Capsaicin alkaloid in pepper fruits such as the soil climate, growing condition and fertilization also. A number of researchers studied the effect of the amino acid in the form of foliar applications on plant efficiency yield and productivity. (Mendes et al, 2016) focused on the role of mineral nutrition on the productivity and quality of pepper. Amino acids are a good source of nitrogen which is essential for improving pepper fruits and yield. Foliar application is a complementary side by side to the fertilizing done in the ground (Mendes et al, 2016). A remarkable increase in Capsaicin alkaloid was found when chilli pepper (Jalapeno) sprayed with Nitrogen (Medina-Lara et al., 2008; Johnson and Decoteau, 1996).While Henderson (2008) found a positive increase in Capsaicin in (Jalapeno) pepper cultivar when treated with nitrogen.

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The study aimed to evaluate the response of amino acids and nitrogen as foliar application on accumulation of Capsaicin, Valine and amino acids in two chilli and sweet pepper cultivars.

Materials and Methods

The experiment was carried out for the 2016-2017 season in the plastic house of the Department of Life Sciences Department at the faculty of Education for Girls/ University of Kufa. Seeds of two pepper cultivars, sweet (Mystro) and chilli (Ninar) were planted in pots (30) cm diameter filled with a mixture of (peat-moss and sand at 1:1 ratio). The chemical and physical properties of the soil experiment shown in Tabl .1. Factorial experiment carried out in Completely Randomized Design (C.R.D) with three replicates, means were compared at Duncan's multiple range tests at probability level of 0.05.

The experiment included two pepper cultivars, sweet (Mystro) and chilli (Ninar) Phenylalanine and nitrogen were used in the form of 5 treatments.

T1 control treatment = distilled water T2= 100 ppm Phenylalanine T3= 200 ppm Phenylalanine T4 = 200 ppm Nitrogen T5=300 ppm nitrogen

The first foliar application occurred 30days after the transplant, and the subsequent in breaks of 10 days after the first application. The spraying solution mixed with Tween 20 (AL-Zubaidi and Al-Hamzawi .2016). Data collected and analyzed by using the GenStat softwere program. The chemical compounds were separated by using High performed liquid chromatography (HPLC) shimadzu (LC-2010 AHT-AUTO). Capsaicin and DihydroCapsaicin, were extracted from the fruits of both pepper cultivars and estimating according to the procedure of Al Othman et al. (2011). Amino acids were estimated according to method of Jones and Gilli (1986) and Ascorbic acid was estimated according to Mitic et al. (2011). In order to classify the pepper cultivars according to their various pungency levels, the capsaicinoid contents obtained from both cultivars were converted to Scoville heat units, (SHU) based on Tilahun et al (2013).

Table 1 Chemical and physical properties of experimental
field

Physical properties g . kg ⁻¹ soil			
Soil texture			
Clay		18.8	
Green		49.2	
Sand		93.2	
Chemical properties		measruing unit	
Available Nitrogen	20.76	mg/kg of soil	
Organic Matter 4.8			

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EC	0.914	dS .m ⁻¹
PH	6.65	-
Diss	olved ions	mmol. liter ⁻¹
Ca		20.17
Mg		7.2
К		4.58
Na		7.34
Cl		10
Р		2.3

Results and discussion

The result in table (2 and 3) showed that T3 caused increase in Phenylalanine and Valine amino acid 338.0 and 265.0 mg.g⁻¹ dry weight respectively. Moreover, Ninar was superior to give 341.24, 242.67 mg.g⁻¹ dry weight respectively compare to 93.41, 106.13 mg.g⁻¹ in Mystro respectively. The interactions between the treatments and pepper cultivars had significant effect, T3 treatment increased Phenylalanine and Valine amino acid in Ninar reached up to 551.7 mg.g⁻¹ and 380.7 mg.g⁻¹ respectively.

Data in (table 4) showed the highest the highest accumulation for capsaicin (671.2 mg, g^{-1}) and the lowest (224.1 mg. g⁻¹) in T3 and T1 respectively and Ninar chilli pepper was superior as compare to the sweet pepper Mystro. Moreover, a significant interaction was found 1266.1 mg. g⁻¹ in capsicin accumulations respectively. Furthermore, data in (table 5) showed that among the five treatments, T3 showed high pungency level with 10739.2 SHU followed by 9230.4 SHU in T2 treatment and Ninar cultivar was more pungent with 13026.56 SHU, for the interactions Ninar cultivar was more pungent with 20257.6 SHU in T3 treatment while less value was found 467.2 SHU in Mystro at control treatment. On the other hand, the mean concentration of dihydrocapsaicin (table 6) was 719.1 mg. g⁻¹ for Ninar in T3 treatment and less value was found at T1 and T4 for Mystero cultivar (107.7 mg. g $^{-1}$,108.4 mg. g $^{-1}$) respectively.

Concerning to vitamin C, data in (table 7) revealed that highest response for Vitamin C was found at T3 treatment 241.2 mg. g⁻¹ fresh weight. Moreover, highest response was found in Ninar cultivar at T3 (426.5 mg. g⁻¹) while less response were found in Mystro at T1, T4 treatment (38.0 mg. g⁻¹ and 41.1 mg. g⁻¹) respectively.

Table 2 Effect of foliar spray on Phenylalanine (mg. g $^{-1}$
dry weight) in the fruits of two pepper cultivars

Treatments	Cultivars		Means
meatments	Mystro	Ninar	Iviedits
T1	66.8 j	207.3 e	137.0 e
T2	118.5 g	364.3 b	241.4 b
Т3	124.4 f	551.7 a	338.0 a
T4	76.3 i	225.2 d	150.8 d
T5	81.1 h	357.8 c	219.4 c
Means	93.41 b	341.24 a	
* Means followed by the same letter do not differ by Duncuns Multiple			

Test at a probability level (P<0.05).

Table 3 Effect of foliar spray on Valine (mg. g⁻¹ dry weight) in the fruits of two pepper cultivars

Treatments	Cultivars		Means
reatments	Mystro	Ninar	IVIEdIIS
T1	73.6 j	81.6 i	77.6 e
T2	113.2 f	262.7 b	188.0 b
Т3	149.3 e	380.7 a	265.0 a
T4	95.1 h	222.1 d	158.6 d
T5	99.4 g	242.7 с	171.0 c
Means	106.13 b	242.67 a	

* Means followed by the same letter do not differ by Duncuns Multiple Test at a probability level (P<0.05).

Table 4 Effect of foliar spray on Capsaicin accumulation (mg. g^{-1} dry weight) in the fruits of two pepper cultivars

Cultivars		Means
Mystro	Ninar	Ivieans
29.2 j	419.1 e	224.1 e
69.9 g	1084.0 b	576.9 b
76.3 f	1266.1 a	671.2 a
46.3 i	426.0 d	236.1 d
54.2 h	875.6 c	464.9 c
55.16 b	814.16 a	
	Mystro 29.2 j 69.9 g 76.3 f 46.3 i 54.2 h 55.16 b	Mystro Ninar 29.2 j 419.1 e 69.9 g 1084.0 b 76.3 f 1266.1 a 46.3 i 426.0 d 54.2 h 875.6 c

* Means followed by the same letter do not differ by Duncuns Multiple Test at a probability level (P<0.05).</p>

Table 5: Heat value of capsaicin expressed in Scoville heat units (SHU)

Treatments	Cultivars		Means
meatiments	Mystro	Ninar	Iviedits
T1	467.2	6705.6	3585.6
T2	1118.4	17344	9230.4
Т3	1220.8	20257.6	10739.2
T4	867.2	6816	3777.6
T5	882.56	14009.6	7438.4
Means	911.232	13026.56	

Table 6 Effect of foliar spray on dihydrocapsaicinaccumulation mg.g ⁻¹ dry weight in the fruits of twopepper cultivars

Treatments	Cultivars		Means
Treatments	Mystro	Ninar	Wearis
T1	107.7 h	274.7 e	191.2 e
T2	135.1 g	531.6 b	333.4 b
Т3	215.4 f	719.1 a	467.3 a
T4	108.4 h	382.2 d	245.3 d
Т5	124.8 g	420.1 d	272.4 с
Means	138.29 b	465.55 a	

* Means followed by the same letter do not differ by Duncuns Multiple Test at a probability level (P<0.05).

Table 7 Effect of foliar spray on vitamin C mg.g⁻¹. fresh weight in the fruits of two pepper cultivars

Treatments	Cultivars		Means
meatiments	Mystro	Ninar	Wealls
T1	38.0 h	183.5 e	110.8 e
T2	53.4 fg	407.1 b	230.2 b
Т3	55.9 f	426.5 a	241.2 a
T4	41.1 h	193.4 d	117.2 d
T5	51.2 g	303.7 c	177.5 c
Means	47.91 b	302.84 a	
* Means followed by the same letter do not differ by Duncuns Multiple			

Test at a probability level (P<0.05)

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