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Foliar application of asparagine and casein on biochemical and morphological attributes of garden cress (*Lepidium sativum* L.) under greenhouse conditions

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Abstract: In this study, the effect of foliar application of Asparagine (ASN) and Casein (CSN) during vegetative stage at four rates (0, 50, 100 and 150 mg l^{-1}) was investigated on garden cress (*Lepidium sativum* L.). The results showed that asparagine application, especially at a high level, could significantly increase the morpho-physiological traits such as plant height, leaf and stem fresh weights and leaf and stem dry weights, leaf pigments (chlorophyll a and chlorophyll b) and leaf nutrients content (Nitrogen and Phosphorus). Also, the results indicated that casein application at 50 mg l^{-1} rate had the best performance through in stem and root fresh weights, stem dry weight and diameter of main stem traits. Casein application at rate 100 mg l^{-1} had the highest leaf nitrogen and phosphorus content. Generally, our findings suggest that the use of asparagine and casein can be considered as an appropriate growth regulator in garden cress cultivation.

1. Introduction

Garden cress (*Lepidium sativum* L.) is an edible herb and a member of the Cruciferae (Brassicaceae) family. It is commonly cultivated throughout the temperate regions of India and Pakistan (Nadkarni, 1954). The plant is cultivated as culinary vegetable all over Asia (Nadkarni, 1976). Garden cress is an annual standing plant, growing up to 30 cm. It is a well known cookable herb and the leaves are widely used as a garnish in salads. In addition to its leaves that have medicinal properties, the seeds are aperients, diuretic, tonic, demulcent, aphrodisiac, carminative (Chopra *et al.*, 1986). Moreover, the seeds, which are used in folk therapies, have many activities like thermogenic, depurative, rubefacient, tonic, aphrodisiac, abortive, ophthalmic, diuretic (Gokavi *et al.*, 2004; Dugasani *et al.*, 2009).

Intensive farming practices, which produce high yields and quality, require the extensive use of chemical fertilizers that are both costly and create environmental problems. Therefore, there has been a recent resurgence of interest in environmentally friendly, sustainable and organic agricultural practice (Orhan *et al.*, 2006). Thus, it is necessary to supply the plant requirement to nutrient through proper procedure. There are different ways to supply plant nutrient's requirement such as soil feeding and foliar application. Of these, foliar feeding is an effective method for improving soil deficiencies and overcoming the soils inability to transfer nutrients to the plant. It has reported the foliar feeding can be 8 to 10 times more effective than soil feeding and up to 90% of a foliar fed nutrient solution can be found in the smallest root of a plant within 60 minutes of application (Garcia and Hanway, 1976).

Amino acids are the major building element for proteins (Andrews, 1986). Amino acids is a well-known biostimulant which has positive influence on plant growth, yield and significantly decrease the damages caused by abiotic stresses (Kowalczyk and Zielony, 2008). ASN is widely used as a source of organic nitrogen in the media upon which certain bacteria are grown (Long and Seibert, 1926). ASN is thought to play a clearly important role in the transportation and storage of nitrogen (Lehmann and Ratajczak, 2008), because of their relatively stable nature and high N/C ratio (Ireland and Lea, 1999; Masclaux-Daubresse *et al.*, 2006). In plants, ASN, one of the most prevalent amides, has been reported to be the primal source of Nitrogen for protein synthesis, particularly in actively growing tissues (Brouquisse *et al.*, 1992). ASN aggregation in plants in response to environmental stress could be an ammonium detoxification mechanism and a means to stock up Nitrogen when protein synthesis is impaired in plants due to stressful environments (Herrera-Rodríguez *et al.*, 2007).

CSN is a very rich source of essential amino acids (Sarode *et al.*, 2016). There are four different CSN proteins (α_{s1}, α_{s2}, β and Κ), which are different in their amino acid composition (Dalgleish, 1989). Among proteins, CSN has been vastly used in artificial rearing diets because it contains all the essential amino acids, is soluble in water, and does not coagulate or precipitate after heating (Parra, 1979). CSN also contains important substances such as fatty acids, cholesterol, sugars, vitamins, and minerals (Vanderzant, 1974). CSN contains 0.7-0.9% phosphorus. So, CSN is known as a phosphoprotein (Sarode *et al.*, 2016). Several pre-harvest factors like climatic conditions and available nutrients can influence on yield and quality of vegetables (Lee and Kader, 2000).

In spite of wide rang of properties, there are a few study about the effect of amino acids on medicinal plants. So this study was conducted to evaluate the effects of ASN and CSN foliar application on vegetative and reproductive growth, physiological and biochemical traits of garden cress.

2. Materials and Methods

In order to investigate the response of *Lepidium sativum* to foliar application of asparagine (ASN) and casein (CSN) two separate pot experiments were conducted at research greenhouse of University of Guilan, Iran. The experiments were carried out based on two randomized complete design with three replications. The treatments in this study were different levels of ASN and CSN (0, 50, 100 and 150 mg l^{-1}). Foliar application of experimental solutions was started from the four-leaf stage and done once every two weeks in the morning, up to the early May. In each spraying date, 20-25 ml of corresponding solution was applied per pot. Garden cress seeds which were prepared from Isfahan-PakanBazr Company were planted in 6th January. The sowing was done in pots with the 33 cm diameter and 22 cm height. The seed bed was a mixture of soil, cow manure and sand with the ratio of 2:1:1. The main properties of soil used in the pots are shown in Table 1. Except for cow manure, no fertilizer was used during the plants growth cycle. Plants were irrigated every 10 days. Plant thinning was performed 15 days after emergence, so that, 6 plants were kept in each pot. During plant growth the greenhouse temperature was 25˚C on the day and 15°C during the nights. In addition, CO_2 concentration was 350 ppm, relative humidity was 40% and photoperiod adjusted as 16 hours' light and 8 hours of darkness.

Table 1 - Some chemical indices of soil used in pots for garden cress cultivation

Organic carbon (%)	Nitrogen (%)	Calcium (%)	Phosphorous (%)	Potassium (%)	pH	Texture
1.2	1	0.48	0.19	0.55	7.1	Loam sandy

In 4th May, four plants were lifted form the soil in each pot for determination of some above- and below-ground vegetative indices. The measured indices were plant height, leaf weight, root weight, stem weight and main stem diameter (by a ruler). The remained two plants were used to measure some reproductive traits including number of florets. The pigments content (carotenoid, chlorophyll a and chlorophylls b) was measured by the method has been explained by Minguez-Mosquera and Prez-Galvez (1998). To determine the leaf nitrogen, phosphorous and calcium percentages, the method of Jones (2001) was used.

The method of Bakhshi and Arakava (2006) also was used for the extraction of leaf extract to determine phenolic compounds and antioxidant capacity. Then, phenols were analyzed by using the method of Folin-Ciocalteau reported by Tavarini *et al*. (2008). For this purpose, the absorbance at 760 nm was measured using a spectrophotometer (T80+PG Instrument UV/Vis Spectrometer) and the values were expressed as mg gallic acid/100 g fresh weight. Antioxidant capacity was determined using DPPH free radical scavenging method which has been described by Sanchez-Moreno *et al*. (1999). Finally, data analysis was done using SAS 9.2 software and means were separated via Tukey's (honest significant difference) test at the 5% level of probability.

3. Results and Discussion

Vegetative and reproductive growth

The analysis of variance of the investigated morphological traits was presented in Table 2. The results revealed that both ASN and CSN significantly affected the plant length, leaf and stem fresh weight, leaf and stem dry weight. So that, the greatest plant height was observed at a rate of 150 mg l^{-1} of ASN. Moreover, applying ASN at rate of 100 mg l^{-1} had the highest leaf and stem fresh weights and leaf and stem dry weights.

Casein application significantly affected the stem and root fresh weights, stem dry weight and diameter of main stem (Table 2). So that, the plants grown under CSN spray at rate of 50 mg l^{-1} had the best performance due to stem dry and fresh weight, root fresh weight and main stem's diameter.

Asparagine and CSN foliar spraying could improve the morphological characteristics in garden cress. These results are in agreement with the previously studies by Kaya *et al.* (2013) on maize, Rasmia *et al.* (2014) on palm, Saeed *et al.* (2005) on soybean, Akladious and Abbas (2013) and El-Desouky *et al*. (2011) on tomato, Shafeek and Helmy (2012) on onion. Also, El-Zohiri and Asfour (2009) on potato found that spraying of amino acids at 0.25 ml/L significantly increased vegetative growth expressed as plant height and dry weight of plant. Their finding indicated that amino acids could improve the vegetative and reproductive traits in plants. These ability my be due to their important role in plant metabolism and protein assimilation which is necessary for cell formation and consequently increase the fresh and dry matter (Fawzy *et al*., 2012).

Amino acids contribute to the synthesis of growth hormones; therefore, it can be concluded that an increase in cell division and cell enlargement is the reason behind enhanced growth parameters (Shafeek and Helmy, 2012). The positive effect of amino acids on growth was stated by Goss (1973) who indicated that amino acids can serve as a source

Table 2 - Mean squares for the effect of different levels of Aspargine (ASN) and Casein (CSN) on vegetative and reproductive indices of garden cress

Traits	Source of Variation					
	Treatment		Error		CV	
	ASN	CSN	ASN	CSN	ASN	CSN
Plant height	24.22 *	5.86 NS	3.75	10.41	5.75	10.38
Leaf fresh weight	0.66 *	1.008 NS	0.09	0.25	5.09	7.97
Leaf dry weight	0.27 **	0.04 NS	0.03	0.02	11.69	12.28
Root fresh weight	0.17 NS	0.2 **	0.06	2	8.52	5.22
Root dry weight	0.002 NS	0.004 NS	0.01	0.005	8.54	5.94
Stem fresh weight	2.65 **	2.001 **	0.04	0.16	3.62	6.83
Stem dry weight	0.5 **	0.22 *	0.02	0.04	9.66	14.13
Diameter of main stem	0.04 NS	0.11 *	0.05	0.01	10.98	5.87
Number of florets	0.22 NS	0.75 NS	0.66	0.5	11.66	10.47

NS, *, ** Non-significant, significant at 5%, and 1% probability level, respectively.

of carbon and energy when carbohydrates become deficient in the plant's releasing the ammonia and organic acid form which the amino acid was originally formed (Table 3). The organic acids then enter Kerb's cycle, to be broken down to release energy through respiration (Goss, 1973). Serna *et al*. (2012) found that the spray of pepper plants with a mixture of amino acids led to raise the efficiency of photosynthesis, and thus, give the best vegetative growth.

Biochemical traits and nutrients content

The mean squares of physiological and mineral content are presented in Table 4. Total phenolic compounds and antioxidants activity were not affected by foliar spraying of ASN and CSN (Table 5). Chlorophyll a and b content significantly increased in response to foliar ASN treatments (Table 4). The highest contents of chlorophyll a and b observed in plants grown under spraying of ASN at rate of 100

Table 3 - Means comparison for the effect of different levels of Asparagine and Casein on some vegetative and reproductive growth parameters of garden cress

Treatments (mg l^{-1})	Plant height (cm)	Leaf fresh weight (g $plant^{-1}$)	Leaf dry weight (g $plant^{-1}$)	Root fresh weight (g $plant^{-1}$)	Root dry weight (g $plant^{-1}$)	Stem fresh weight (g $plant^{-1}$)	Stem dry weight (g $plant^{-1}$)	Diameter of main stem (mm)	Number of florets
Aspargine									
0	29.66 b	5.47 b	1.18 b	2.87 a	1.20 a	4.77 c	1.15 c	1.99 a	7.00 a
50	34.66 ab	6.05 ab	1.81 a	2.97 a	1.25 a	5.73 b	1.44 bc	2.08 a	6.66 a
100	34.00 ab	6.57 a	1.83 a	3.42 a	1.26 a	6.96 a	2.06 a	2.25 a	7.00 a
150	36.33 a	6.31 a	1.62 ab	3.12 a	1.26 a	6.38 ab	1.86 ab	2.02 a	7.33 a
Casein									
0	29.66 a	5.47 a	1.18 a	2.87 b	1.2 a	4.77 b	1.15 b	1.99 b	7.00 a
50	31.00 a	6.75 a	1.45 a	3.39 a	1.22 a	6.64 a	1.80 a	2.37 a	6.33 a
100	30.66 a	6.23 a	1.38 a	2.82 b	1.15 a	6.22 ab	1.58 ab	2.01 b	6.33 a
150	33.00 a	6.65 a	1.43 a	3.14 ab	1.25 a	6.16 ab	1.55 ab	2.3 ab	7.33 a

Means with the same letter(s) within a column are not significantly different (P≤0.05) based on Tukey's test.

Table 4 - Mean squares for the effect of different levels of Aspargine (ASN) and Casein (CSN) on leaf nutrient and pigments content and some biochemical parameters in garden cress

Traits	Source of variation					
	Treatment		Error		CV	
	ASN	CSN	ASN	CSN	ASN	CSN
Chlorophyll a	0.13 **	31.66 NS	0.006	0.008	4.9	6.75
Chlorophyll b	0.05 **	0.01 *	0.001	0.002	5.83	7.89
Carotenoid Content	0.0004 NS	0.003 NS	0.001	0.002	8.32	12.96
Leaf Nitrogen Content	0.09 **	0.18 **	0.006	0.01	4.79	6.72
Leaf Phosphorous Content	0.01 *	0.03 **	0.003	0.002	8.86	6.66
Leaf Calcium Content	0.007 NS	0.0007 NS	0.007	0.003	9.45	6.95
Total Antioxidants	21.41 NS	6.97 NS	12.75	6.16	6.64	4.7
Total Phenol	31.66 NS	6.97 NS	7.33	17.33	4.99	7.81

NS, *, ** Non-significant, significant at 5%, and 1% probability level, respectively.

Table 5 - Means comparison for the effect of different levels of Asparagine and Casein on biochemical parameters of garden cress

Treatments (mg l^{-1})	Chlorophyll a	Chlorophyll b	Carotenoid content	Leaf nitrogen content	Leaf phosphorous content	Leaf calcium content	Total antioxidants	Total phenol
Aspargine								
0	1.38 b	0.56 c	0.39 a	1.45 b	0.57 b	0.89 a	51.6 a	50.3 a
50	1.53 b	0.73 b	0.4 a	1.6 ab	0.66 ab	0.83 a	53 a	52.6 a
100	1.87 a	0.89 a	0.37 a	1.81 a	0.71 ab	0.95 a	57.6 a	57.3 a
150	1.64 ab	0.71 b	0.35 a	1.81 a	0.73 a	0.87 a	52.6 a	56.3 a
Casein								
0	1.36 a	0.56 b	0.39 a	1.45 b	0.57 c	0.89 a	51.66 a	50.3 a
50	1.41 a	0.73 a	0.37 a	1.62 ab	0.60 bc	0.86 a	52 a	54.3 a
100	1.45 a	0.70 a	0.44 a	2.01 a	0.78 a	0.82 a	55 a	54.6 a
150	1.38	0.65 ab	0.43 a	1.88 a	0.74 ab	0.87 a	52.3 a	53.6 a

Means with the same letter(s) within a column are not significantly different (P≤0.05) based on Tukey's test.

mg l^{-1} (Table 5). Generally, application of ASN had a significant effect on N and P% content, However, there was no significant difference in leafs calcium content (Table 4). Meanwhile, the highest amount of N% was belonging to application of ASN at rates of 100 and 150 mg l^{-1}. Moreover, the highest leaf content of P% was observed at rates of 150 mg l^{-1} ASN (Table 5).

Casein application could influence significantly on chlorophyll b content and leaf's Nitrogen and phosphorous content (Table 4). Lowest chlorophyll b content were observed in plants that treated with distilled water, while the highest chlorophyll b content were in plants that treated with 50 and 100 mg l^{-1} of CSN (Table 5). The plant treated with 100 and 150 mg l^{-1} CSN had the highest amount of nitrogen, and the highest Phosphorous content were in plants treated with 100 mg l^{-1} of CSN (Table 5). These results are in Conformity with previous study in maize (Cazetta *et al*., 1999; Kaya *et al*., 2013), palm (Rasmia *et al*., 2014), almond (Youssefi *et al*., 2000), Datura (Hussein *et al*., 1992) and strawberry (Abo Sedera *et al*., 2010).

Youssefi *et al*. (2000) reported that leaf-nitrogen concentrations were related positively to concentrations of applied amino acids (especially asparagine and glutamine). On the other hand, increasing the nitrogen supply led to increase in the activity of certain enzymes, starch and the levels of nitrogen compounds (total nitrogen, soluble protein and free amino acids) and decreased the levels of carbon metabolites (sucrose and reducing sugars) in the tested plant (Cazetta *et al*., 1999). Exogenous application of ASN was improved the phosphorus content of maize (Kaya *et al*., 2013).

This increase in chlorophyll contents might be due to the availability of higher levels of amino acids in treated plants, because amino acids help to increase the chlorophyll content and this may lead to the increase in different growth criteria (Awad *et al*., 2007).

4. Conclusions

The major problem with the use of conventional chemical fertilizers is the way in which ions enter the plant cell. The ions dissipate calcium from the cell wall and damage it, while amino acid chelates enter the space in the cell with the least resistance and without damaging the cell membrane. At the last stage, the binding of the chelate and desired atom is broken down into the plant cell, and the nutritional needs of the cells are provided with the lowest losses. Results of the current study revealed that foliar application of ASN and CSN could increase the growth and yield of garden cress. It could be recommended that spraying garden cress plants by ASN (100 and 150 mg l^{-1}) increased the vegetative growth traits (plant height, leaf fresh weight, number of florets), nutrients uptake (leaf nitrogen and phosphorus content) and pigments concentration (chlorophyll a and chlorophyll a) and also application of CSN at 50 mg l^{-1} level could improved some morphological and biochemical traits of garden cress such as: root fresh weight, stem fresh weight, Chlorophyll b, Leaf nitrogen and phosphorus content in comparison control treatment. Therefore, it can be concluded that application of ASN and CSN is a good strategy in garden cress cultivation, but their effectiveness must be also evaluated under environmental stresses, in the future studies. Also spraying different nutrient solution in combination with each other may show interaction and contrary behavior in plants in comparison applying them solely, so further research with higher concentrations and also combined spraying of these amino acids, applying on different vegetables in greenhouse and farm conditions is suggested.

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