Effect of nitroxin biofertilizer on morphological and physiological traits of *Amaranthus retroflexus*

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Abstract

Greenhouse experiments in a completely randomized design were carried out to investigate the effects of nitroxin fertilizer (0, 1 and 2 li/ha) on morphological and physiological traits of *Amaranthus retroflexus* in 2013. The traits in the study included plant height, fresh and dry weight of shoots, roots, stem and leaf, Chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, anthocyanin, and flavonoids. Findings showed that nitroxin biofertilizer had linear and nonlinear regression effect on the fresh and dry weights of shoot. Increase in nitroxin also increased fresh and dry weights of leaf, stem, chlorophylls a, b, total Carotenoids and anthocyanin as well as carotenoids content of the plants linearly. The results showed that the correlation between the traits there is a significant relationship. Also fresh weights shoot with each of traits dry weights shoot, fresh and dry weights of leaf, fresh and dry weights of stem and dry weights of shoot with each of traits fresh and dry weights of leaf, fresh and dry weights of stem and fresh weights of leaf with each of traits fresh and dry weights of stem, dry weights of leaf and dry weights of stem with each of traits dry weights of leaf and stem there is a significant relationship. Chlorophyll a with chlorophyll b, total, carotenoids and flavonoids and chlorophyll b whit chlorophyll total, carotenoids and flavonoids and chlorophyll total whit carotenoids and flavonoids and carotenoids with flavonoids there is a significant relationship. The results of step-wise regression analysis showed that dry weight of stem, had positive and fresh weight of shoots reduced effect on dry weight of shoots.

Keywords: Amaranthus; stepwise regression; nitroxin; correlation

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Introduction

Amaranthus belonging to the family Amaranthaceace, is a C4 plant which is highly adaptable to various climatic conditions. This plant is resistant to drought, poor nutrition conditions, and wide temperature variability. With its anatomical features and C4 metabolism, the plant can efficiently use CO2 under a wide range of both temperature and moisture stresses, and is able to grow in wide geographic regions and under diverse environmental conditions.

Amaranthus yields are extremely variable depending on cultivar, the growing season, and particularly with regard to available soil moisture. The grain yield is usually over 5 t/ha. In irrigated cultivar trials of the Montana State University Southern Agricultural Research Center at Huntley, yield was about 112 kg /ha. Yield between 450 to 700 kg /ha under dry land 1-2 t/ha in irrigated conditions are economically cost effective.

Amaranthus grains contain 77% unsaturated fatty acids most of which are linoleic

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Table 1
Soil analysis

Soil Texture	Sand %	Silt %	Clay %	EC	PH	Organic Matter %	Organic Carbon %	Total Calcareous %
Loam-clay	23	43.5	33.5	198.5	7.68	0.9	0.54	46.5

fatty acids. Compared to other cereals, amaranth seeds contain more unsaturated fatty acids. These seeds are rich with vitamin E as well as protein, carbohydrates, fats, calcium, iron and sodium. Presently, the plant is cultivated in 2000 to 3000 ha in the Great Plains area, particularly Nebraska and other areas in the United States (Stallknecht et al., 1993).

Different organisms in soil structure can provide essential food for plants. The greater the number and diversity of bacteria in soil, the higher plant growth (Mader et al., 2002). The use of fertilizers, including chemical fertilizers and manures, to enhance soil fertility and crop productivity has often negatively affected the complex system of the biogeochemical cycles (Perrott et al. 1992; Steinshamn et al., 2004). Chemical fertilizers pose danger for human health, soil, water, and environment. Moreover, supplying and manufacturing these fertilizers are expensive. Therefore, these fertilizers should not be applied so broadly (Chandrasekar et al., 2005). Use of biofertilizers is the alternate way to meet the nutrient requirements of agricultural plants and crops. Most plants cannot absorb nitrogen (N_2). On the other hand, some bacteria can join the plant roots and provide the plant with the necessary nitrogen absorbed from the air. This is what happens when nitroxin biofertilizers are applied; the biological nitrogen-fixation bacteria fixate the nitrogen present in the air, and at the same time feed the plants with nitrogen (Koocheki et al., 2008).

Table 2
Analytical response of application of nitroxin values in the variables under study

Traits	Linear Regression	Regression Grade 2	C.V. (%)
Shoot dry weight	0.18**	0.05**	9.56
Shoot fresh weight	0.58**	0.11**	16.5
Leaf fresh weight	0.17**	0.01ns	24.88
Stem fresh weight	0.39**	0.04 ns	23.77
Leaf dry weight	0.18**	0.02 ns	13.86
Stem dry weight	0.28*	0.009 ns	19.03
Plant height	1.98 ns	10.9 ns	25.11
Chlorophyll a	0.23*	0.023 ns	17.07
Chlorophyll b	0.15*	0.012 ns	16.96
Total Chlorophyll	0.43*	0.03 ns	18.17
Carotenoids	0.11*	0.007 ns	15.42
Anthocyanins	0.38*	0.01 ns	5.74
Flavonoids	0.003 ns	0.0008 ns	13.23

**, *, and ns indicate significance at 1% and 5%, and no significant difference, respectively.

Nitroxin biological fertilizer and chemical fertilizers (N, P) were reported to increase the active ingredient artemisinin in Artemisia annua L (Bijeh keshavarzi et al., 2011). In another study, application of nitroxin biological fertilizer had significant effects on most of the studied traits - including plant height, bush dry weigh, amount of head seeds, the number of bush heads and seed yield- except the number of branches (Moghimi et al., 2011). It was also reported that nitroxin has a positive effect on the quality of forage sorghum (Saeed nejad et al., 2011). Finally, the bio-fertilizer Super Nitro Plus was shown to have a significant effect on height and diameter as well as fresh and dry weight of Hyssopus officinali (Koocheki et al., 2008). The objective of this study was to determine the response of growth, yield, and some morphological and physiological traits of four species of Amaranthus to the application of nitroxin biofertilizer.

Materials and methods

The experiment was carried out as a factorial completely randomized design with 3 replications in Damavand region east of Tehran, Iran (35°43N, 52°34'E) in 2012. Seeds of *Amaranthus retroflexus* (collection of Zarand Kerman) were planted in one-litre plastic pots with the density of 10 seedlings per pot. Soil test results are shown in Table 1. Treatments consisted of nitroxin at 3 levels, namely, 0 lit/ha (control), 1 lit/ha, and 2 lit/ha.

The traits in the study included plant height, fresh and dry weight of shoots and roots, Chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, anthocyanin, and flavonoids. Lichtenthaler and Wellburn (1987) method was used for measuring Chlorophyll a, chlorophyll b, chlorophyll total, and carotenoids.

The method described by Wagner (1979) was used for measuring leaf anthocyanin. In order to measure flavonoids, the method explained by Krizek et al. (1998) was used. All data were analyzed by Kolmogorov-Smirnov method which is based on the largest vertical difference between the hypothesized and empirical distribution (Conver, 1999); the SPSS software version 14 was used for statistical calculations.

In addition to the impact of nitroxin biofertilizer on plant traits, the relationship between these traits and their effects on each

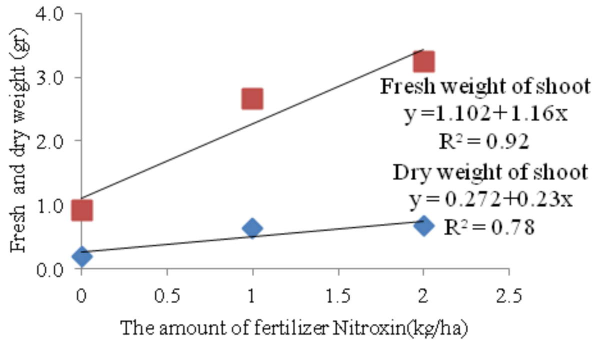

Fig. I. Effect of nitroxin biofertilizer on fresh and dry weight of shoots of *Amaranthus. retroflexus*

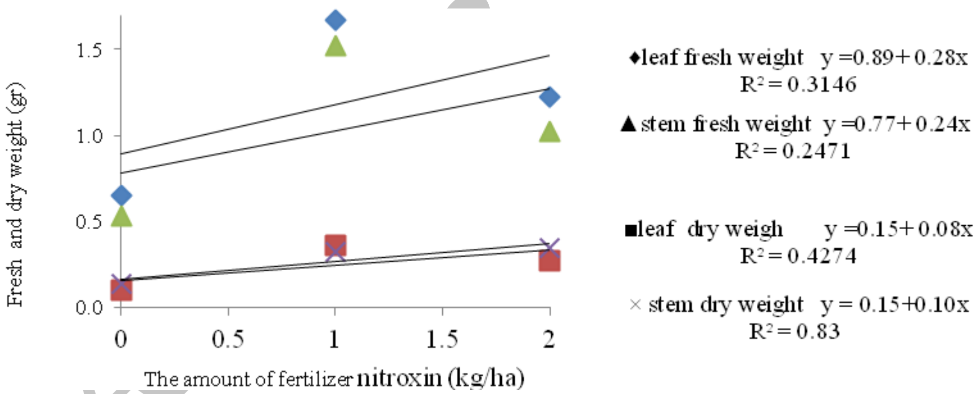

Fig. II. Effect of nitroxin biofertilizer on fresh and dry weight of leaf and stem of *Amaranthus. retroflexus*

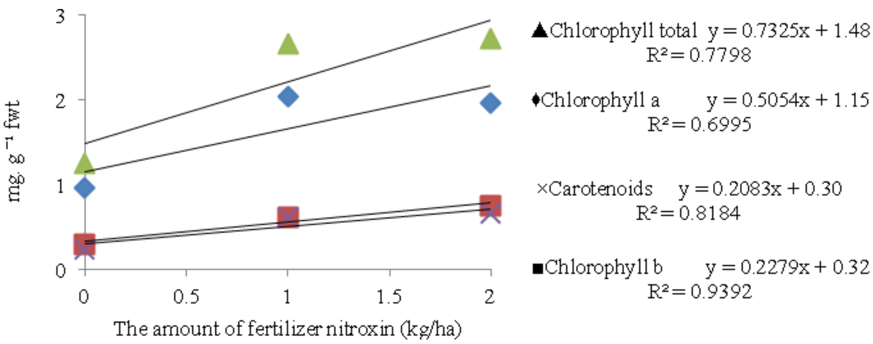

Fig. III. Effect of nitroxin biofertilizer on Chlorophyll a, b, total, and Carotenoids of Amaranthus. *Retroflexus* other were examined in the study. Relationship with and impact on other traits were also investigated. Correlation analysis and regression analysis were utilized in the study. Correlation analysis shows the possible relationships between the characteristics under study. Regression analysis on the other hand, determines the relative effect of the remaining characteristics in the model on shoot dry weight.

Results

Findings showed that nitroxin biofertilizer had linear and nonlinear regression effect on the fresh and dry weights of shoot. Increase in nitroxin also increased fresh and dry weights of leaf, stem, chlorophylls a, b, total Carotenoids, and anthocyanin content of the plants linearly (Table 2). Maximum response to biofertilizer was recorded under 2 kg/ha nitroxin treatment (Figs. I, II, III, and IV).

The findings showed significant positive correlations fresh weights shoot with each of traits dry weights shoot, fresh and dry weights of leaf, fresh and dry weights of stem. Correlations between dry weights of shoot with each of traits fresh and dry weights of leaf, fresh and dry weights of stem were significant. Leaf fresh weight also significantly correlated with stem fresh weight, leaf dry weight and stem dry weight. Stem fresh weight and leaf and stem dry weights correlated with each other. On the other hand, the chlorophyll a, b, and total carotenoids significantly correlated. Finally, flavonoids and other plant pigments showed significant negative correlation (Table 3).

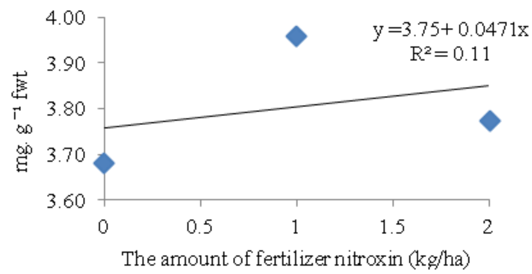

Fig. IV. Effect of nitroxin biofertilizer on Anthocyanins of *Amaranthus. retroflexus*

Step-wise regression helps determine the important characteristics and traits eliminating unimportant ones (Agrama, 1996). The results of step-wise regression analysis are presented in Table 4. Based on these results, there was a significant regression relationship between remaining traits of model and biomass (P≤0.01).

Table 3
Correlation analysis on morphological and physiological traits of *Amaranthus. retroflexus*

	Shoot dry weigh	Shoot fresh weight	Leaf fresh weight	Stem fresh weight	Leaf dry weight	Stem dry weight	Plant height	chlorophyll a	chlorophyll b	chlorophyll total	Carotenoids	Anthocyanins	Flavonoids
Shoot dry weigh	1	0.84**	0.76*	0.80**	0.81**	0.87**	0.49ns	0.55 ns	0.56 ns	0.55 ns	0.56 ns	0.61 ns	-0.40 ns
Shoot fresh weight		1	0.85**	0.98**	0.94**	0.98**	0.60 ns	0.58 ns	0.59 ns	0.57 ns	0.58 ns	0.42 ns	-0.39 ns
leaf fresh weight			1	0.85**	0.96**	0.89**	0.50 ns	0.58 ns	0.57 ns	0.58 ns	0.57 ns	0.58 ns	-0.45 ns
stem fresh weight				1	0.92**	0.97**	0.56 ns	0.59 ns	0.58 ns	0.59 ns	0.58 ns	0.32 ns	-0.39 ns
leaf dry weight					1	0.94**	0.56 ns	0.62 ns	0.63 ns	0.62 ns	0.61 ns	0.53 ns	-0.43 ns
stem dry weight						1	0.49 ns	0.63 ns	0.64 ns	0.62 ns	0.63 ns	0.50 ns	-0.46 ns
plant height							1	0.42 ns	0.43v	0.42 ns	0.40 ns	0.11 ns	-0.27 ns
chlorophyll a								1	0.99**	0.98**	0.99**	0.23 ns	-0.88**
chlorophyll b									1	0.98**	0.99**	0.24 ns	-0.87**
chlorophyll total										1	0.98**	0.24 ns	-0.89**
carotenoids											1	0.26 ns	-0.90**
Anthocyanins												1	-0.25
Flavonoids													1

**, *, and ns indicate significance at 1% and 5%, and no significant difference, respectively.

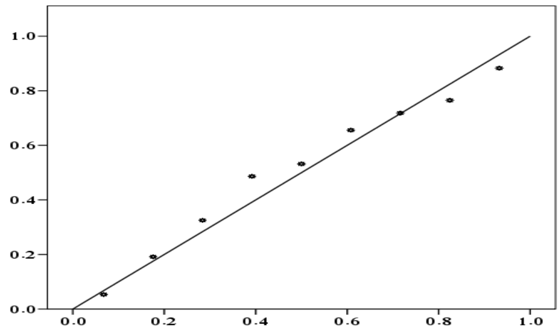

Fig. V. The data fit the regression line (on the morphological and physiological traits of *Amaranthus. Retroflexus)*

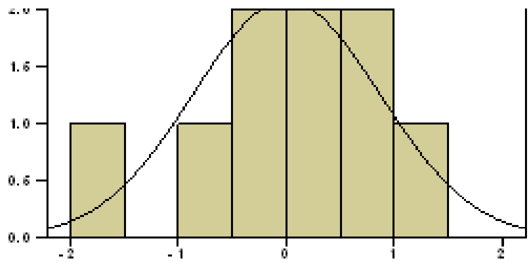

Fig.VI. Comparing the histogram data of the normal curve (on the morphological and physiological traits of *Amaranthus. Retroflexus)*

The results of step-wise regression analysis showed that dry weight of stem had positive and fresh weight of shoots negative effect on dry weight of shoots. Comparison of the data histogram (Fig. V) shows that the obtained data had suitable correspondence with normal curve (the distribution is in the range of ±2). On the other hand, Fig. (VI) shows that fitting the data to the regression line was acceptable and therefore regression model is correct (Rezaei and Soltani, 2003).

Discussion

Morphological response of the plant and plant pigments had a linear relationship with nitroxin. This suggests that application of nitroxin was beneficial for the traits under study. Verlinden et al. (2010) found that application of biofertilizers increased vegetative organs of the grassland plants. In another study Gulser et al. (2010) found that biofertilizers increased fresh weight of leaves and stems in peppers. The study by Ansari and Rosta (2008) also showed that the

Table 4
Results of step-wise regression analysis on morphological and physiological traits of *Amaranthus. Retroflexus*

S.O.V.	df	MS	R^2
Regression	3	3.06**	
Error	32	0.047	84.7%
Total	35		

**, *, and ns indicate significant at P≤0.01, P≤0.05, and not significant, respectively.

application of nitroxin biofertilizer in zea maize (C4 plant) increased plant height as well as shoot weight. Yet another research on the effect of nitroxin on maize cultivars KSC704, N.S640, and 524 showed that the nitroxin fertilizer increased shoot growth and thus increased the shoot dry weight (Azadbakht et al., 2008).

There was a significant correlation between most morphological characteristics and plant pigments. There was a significant relationship between fresh and dry weights of leaves, stems, and also between fresh and dry weights of leaves and stem height. The study by Aynehband et al. (2007) also showed that the dry weight of shoots significantly correlated with dry and fresh weight of leaf, stem and plant height.

On the other hand, there was a significant relationship between chlorophyll b and total carotenoids. The relation between pigments in C4 plants is important and therefore, in Amaranthus which is a C4 plant, the relationship between plant pigments impacts plant growth (Black Jr and Mayne, 1970).

Step-wise regression analysis showed that dry weight of stem, had positive and fresh weight of shoots reduced effect on dry weight of shoots. The study by Mottaghian et al (2009) also showed a regression relationship between the shoot fresh and dry weights in beans. The study by Ansari and Rosta (2008) also showed that the application of nitroxin biofertilizer in corn (C4 plant) increased plant height and shoot weight suggesting a regression relationship between plant height and shoot dry weight.

Nitroxin biological fertilizer has effect on the morphological and physiological traits of Amaranthus retroflexus. Also plant breeding should be amended with attention paid to wet and dry weight of leaves, stems, and shoots and then pigments.

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