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COMPARATIVE EFFECTS OF AMMONIUM NITRATE AND BLOOD MEAL ON PLANT MORPHOLOGY AND LEAF COLORING IN GREEN BEAN (*Phaseolus vulgaris* L.) SEEDLINGS UNDER SALT STRESS CONDITION

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ABSTRACT

This study was carried out in order to determine the comparative effects of ammonium nitrate and blood meal on plant morphology and leaf coloring in Green Bean (*Phaseolus vulgaris* L.) seedlings are grown under 100 mM salinity stress. The trial was applied in 2 (control and salt stress) x 2 (ammonium nitrate and blood meal doses) factorial experimental design with 5 replicates. In this trial, ammonium nitrate was used as nitrogen source to get 5 N doses (0, 50, 100, 200 and 300 ppm) and similarly, blood meal was used comparative N source to get the same respective N doses. Salt was applied to perlite in impermeable pots at 100 mM NaCl concentration. Results showed that both ammonium nitrate and blood meal treatments increased plant height, fresh and dry weight of shoot and root tissues compared to controls. According to color measurements on the leaves, although salt stress increased L, a and b values both ammonium nitrate and blood meal reduced these values. Especially, blood meal giving 200 ppm N per pot increased significantly the measured morphological parameters and color quality of green bean plants. To conclude, blood meal affected plant morphology and leaf coloring as ammonium nitrate on the basis of their equivalent N content, suggesting that blood meal can be used in fertilizing vegetables as alternative nitrogen source with taking precautions against possible contaminations.

Keywords: Salt stress, blood meal, nitrogen, green bean

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1. INTRODUCTION

One of the most important abiotic stress factors limiting vegetative production, especially in arid and semi-arid regions is salt stress. It is estimated that more than 6% of the world's terrestrial areas and approximately 20% of the irrigated area in the world are affected by salinity (Munns and Tester, 2008).

Salinity prevents plant growth by causing accumulation of toxic ions, reduction of water and nutrients available (Ashraf, 1994; Parida and Das, 2005). Accumulated in plants in excessive amounts under the influence of salt stress, Na prevents potassium uptake (Siegel et al. 1980) while Cl prevents NO_3 uptake in particular (Güneş et al. 1994) which may cause nutrient deficiency in plants and deterioration of ion balance (Hu and Schmidhalter, 2005).

Plants develop different physiological, biochemical and molecular responses to provide tolerance to osmotic and ionic stress caused by salinity. These are the accumulation or disposal of ions causing salt stress, the prevention of transmission of ions from root to shoot, the accumulation of ions in certain regions of the plant, the synthesis of osmotic regulators, antioxidant defense systems, signal transmission pathways and the activation or inactivation of various genes (Parida and Das, 2005). In order to reduce the negative effects of salt stress on plants, it is necessary to restore the distorted osmoregulation (Greenway and Munns, 1980). Plants accumulate some protective compounds in their tissues under the influence of salt stress for the purpose of reducing their osmotic potential lost (Hussein et al., 2008). These osmotic protectors are nitrogen-containing compounds such as sugars, organic acids, quaternary ammonium compounds, polyols, amino acids (proline) and amide, and soluble proteins (Rabe, 1990; Shannon, 1987).

For many plants, salinity and low amount of N are important factors that limit growth (Güneş et al., 2007). Nitrogen, which is in the structure of many organic compounds such as proteins, amino acids, nucleic acids, enzymes, chlorophyll, ATP and ADP, is a nutrient element that is effective not only in photosynthesis in plants, but also in the synthesis of hormones such as ethylene, abscisic acid, oxine and cytokinin (Aktaş, 1995).

There are many studies showing that nitrogen application is effective in increasing tolerance to salt stress (Arslan et al., 1997; Kaçar et al., 2004; Caporn et al., 1994). It is effective in defending themselves against free oxygen radicals which are increased under stress conditions that the plants increase the amount of proline, a nitrogen-containing compound, and accumulate polyamines in their tissues by nitrogen application for tolerance to stress (Ghoulam et al., 2002; Mittova, and Guy, 2002). It is reported that the nitrogen form used affects the plant's response to saltiness (Cerda and Martinez, 1988) and that it is more effective to the use NO_3 and NH_4

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together, namely in the form of ammonium nitrate (NH_4NO_3) than to use any of them separately (Drihem and Pilbeam, 2002; Nathawat et al. 2007).

One of the most important conditions of productive and quality cultivation is the proper and adequate nutrition of the plants. When applied in sufficient amount, fertilizers give positive results while they cause pollution by mixing with basin and surface waters by means of washing like nitrogenous fertilizers in particular and they can lead to the production of greenhouse gases with increased emission of nitrogen oxides when used excessively (Güler, 2004). It is a known fact that chemical fertilizers have adverse effects on health as well as environmental pollution. Excessive nitrogen fertilization reaches a level that threatens human health by causing accumulation of nitrate in the leaves of leaf-edible plants (Roorda van Eysinga, 1984). Nitrates can turn into harmful substances that may lead to cancer in humans and animals while fertilizers with organic origin do not change nitrate contents in plants significantly (Şensoy et al., 1996; Demir et al., 1996). Having the potential to provide the nutrients necessary for plants, the use of organic wastes as an alternative to chemical fertilizers is of great importance in terms of sustainability in agricultural production. Organic wastes such as blood meal can be used as a nutrient source in plant production by processing them for this purpose.

Blood meal is obtained by drying and grinding the precipitate derived from slaughtered animal blood by heating it until coagulation and separating its water (serum) from the other proteinaceous part (plasma) (Ciavatta et al., 1996). The amount of blood obtained from an animal is up to 4% of its live weight while about 20-25 kg of blood meal can be obtained from a hundred kilograms of liquid blood (Filstrup, 1976). Blood meal contains 8-14% nitrogen, 0.3-1.5% P_2O_5 and 0.5-0.8% K_2O (Reddy, 2005). Since the nitrogen contained in the blood meal is in organic form and especially because it is in the form of protein, it can be taken up by the plant in a short time when it is applied to the soil. Because of this feature, blood meal is better than other organic fertilizers.

The matters to be considered at blood meal use are its effect in increasing the soil acidity due to its low pH content and that it can show toxic effects especially at early stage due to its high nitrogen content and ammonia release when it is used at high concentration. In humid and hot conditions, blood proteins should be used under control since they are quickly converted to ammonia through bacteria. In addition to bringing nutrients such as nitrogen and iron to the soil which are absolutely necessary for plants, blood meal may increase the activities of useful microorganisms in the soil as well as it may put pressure on nematodes such as *Meloidogyne incognita, Tylenchus filiformis, Rotylenchus reniformis* and *Helicotylenchus indicus* (Alam, 1989; Muller and Gooch, 1982).

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In this study, some of the morphological and leaf color parameters of the bean plant were examined to determine the effects of different doses of ammonium nitrate and blood meal, which are used as a nitrogen source, on preventing salt stress.

2. MATERIAL AND METHOD

The study was carried out in the gothic greenhouse with polycarbonate cover material belonging to Ahi Evran University Faculty of Agriculture in April 11 -May 12, 2015 according to the factorial design pattern of 2 (Control and NaCl application) x 2 (Ammonium nitrate and blood meal doses) with 5 repetitions. As plant material, romana type, early dwarf Gina cultivar was used. 500 ml round pots with plastic bags inside for preventing drainage were used for seed sowing. The perlite used as a growing medium was first passed through tap water and then passed through pure distilled water and placed in a pot. The bean seeds were planted in each pot and the pots were irrigated with distilled water every day until the germination occurs.

After the plant germination occurred when it formed 1-2 true leaves, an application of 5 doses of ammonium nitrate (NH_4NO_3 - 33% N) including 0, 50, 100, 200 and 300 ppm nitrogen (N) and of blood meal which contains N equivalent to the nitrogen at these doses were carried out (Table 1). After 5 days from these applications, salt stress was applied gradually (first 50 mM then 100 mM NaCl) to avoid shock effects when plants grow 2-3 true leaves. The experiment was terminated 15 days after the application of salt stress.

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Table 1: Treatment subjects and dosages

Treatments	N (ppm)	NaCl (S) (mM)	N source and quantity (mg pot^{-1})
Control	0	0	-
N_1	50	0	75.75 mg pot^{-1} NH_4NO_3
N_2	100	0	151.51 mg pot^{-1} NH_4NO_3
N_3	200	0	227.27 mg pot^{-1} NH_4NO_3
N_4	300	0	454.54 mg pot^{-1} NH_4NO_3
BM_1	50	0	208.33 mg pot^{-1} Blood Meal
BM_2	100	0	416.66 mg pot^{-1} Blood Meal
BM_3	200	0	833.33 mg pot^{-1} Blood Meal
BM_4	300	0	1250 mg pot^{-1} Blood Meal
Control+S	0	100	-
N_1+S	50	100	75.75 mg pot^{-1} NH_4NO_3
N_2+S	100	100	151.51 mg pot^{-1} NH_4NO_3
N_3+S	200	100	227.27 mg pot^{-1} NH_4NO_3
N_4+S	300	100	454.54 mg pot^{-1} NH_4NO_3
BM_1+S	50	100	208.33 mg pot^{-1} Blood Meal
BM_2+S	100	100	416.66 mg pot^{-1} Blood Meal
BM_3+S	200	100	833.33 mg pot^{-1} Blood Meal
BM_4+S	300	100	1250 mg pot^{-1} Blood Meal

When the study is completed, the heights of the plants height, from the root to the growth end, are measured in cm measured with the help of a ruler. The samples of plant shoot and root weighed on a precision scale (Shimadzu AY220- at the precision of 0.0001g) fresh weights per gram were determined, then dry weight was recorded in grams after drying the same samples when they reached a constant weight at 65ºC in drying-oven (Koç, 2005). The color of the leaves was measured as L (whiteness, brightness / blackness), a (positive a is red, negative a is green) and b (positive b is yellow, negative b is blue) by using a color meter (Pantone Capsure) (Mc Guire, 1992).

The analysis of the data obtained as a result of the study was performed with the "SPSS 17 V" statistical program. In the analysis of variance, the Duncan test, one of the multiple comparison tests, was used in order to determine which group or groups the differences between the means were derived from (Düzgüneş et al., 1987).

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3. RESULTS AND DISCUSSION

Statistically significant reductions were found in all of the morphological parameters of plants under the influence of salt stress, which are investigated in the study compared to non-stressed plants (Table 2). The salt stress, which prevents plants from growing and developing, can stop the growth of plants and it can cause them to die as well. It has been reported by many researchers that the plants under salt stress exhibit responses such as, growth retardation, reduction in green component formation, reduction in plant height, leaf area and leave count (Termaat and Munns, 1986; Mer et al., 2000).

Although not statistically significant at many doses, the effect of applications on plant height increased in the plants not subjected to salt stress, which ammonium nitrate and blood meal applied as nitrogen source compared to control plants and the highest plant height was determined as 26.12 cm in BM_3 application. In plants subjected to salt stress, plant heights were higher in all doses of ammonium nitrate and blood meal than in control plants (P= 0.000) (Table 2). On the other hand, it has been reported by many researchers that the height of the plants decreased with the effect of salt stress (Colla et al.,2007; Yetişir and Uygur, 2009; Daşgan et al., 2002).

Among the morphological parameters examined in the plants not subjected to salt stress, the shoot fresh weight was the most significantly affected one by the increased ammonium nitrate and blood meal doses. The shoot fresh weight values determined at all doses were significantly higher than the control plants. The highest shoot fresh weight was determined as 4.296 g in BM_3 application. In plants subjected to salt stress, shoot fresh weights determined at all application doses were also higher than control plants and the highest shoot fresh weights were determined as 3.458, 3.696 and 3.482 g in N_3S, BM_2S and BM_3S applications, respectively (Table 2).

Smika et al. (1965) reported that there is a close relationship between the nitrogen uptake of the plants and the water uptake in the root zone and that water intake will positively affect the amount of nitrogen which is increased to an adequate level in conditions that are osmotically limited due to salt stress.

Similarly, in our study, the applications of ammonium nitrate and blood meal used as a nitrogen source have been found to decrease the negative effect of salt stress on growth.

Abdelgadir et al. (2005), in the study by using two paddy species, three different salt concentrations (0, 50 and 100 mM NaCl) and three nitrogen (N) levels (0, 7, 7, and 14 mM), found that N increased up to 7 mM was a significant positive contribution to plant stem development root development was less affected by nitrogen application, and the amount of NO_3

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in the stem increased while the content of Cl decreased at 0 and 50 mM salt levels.

It is known that the amount of photosynthesis increases with the increase of N content in plants (Muchow and Sinclair, 1997). According to our findings, it is thought that the increase in fresh weight, a sign that the plant is less affected by stress, may be due to the increase in the amount of photosynthesis. While all doses of ammonium nitrate and blood meal applications increase the dry weight of shoot under stress-free conditions, the highest values were found as 0.528 and 0.524 g in N_3 and in BM_4 applications, respectively. Although shoot dry weights determined in all applications are higher than control plants in plants under salt stress, the highest value was found to be 0.482 g in BM_2S application (Table 2).

Table 2: Some Morphological Parameters Determined in Bean Plants.

TREATMENTS		N	Plant height	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight
- NaCl (-S)	**CONTROL**	5	22.62^{abcd}	2.914^{ef}	0.426^{abcd}	3.398^{abcde}	0.2038^{abcdef}
	N1	5	23.00^{abcd}	3.644^{abcde}	0.472^{abc}	3.396^{abcde}	0.2296^{abcd}
	N2	5	24.76^{abc}	4.052^{ab}	0.478^{abc}	3.618^{abcd}	0.2002^{abcdef}
	N3	5	23.38^{abcd}	3.952^{abc}	0.528^{a}	3.836^{ab}	0.2000^{abcdef}
	N4	5	19.78^{de}	3.828^{abcd}	0.486^{abc}	3.372^{abcde}	0.2044^{abcdef}
	BM1	5	21.32^{bcd}	3.338^{bcde}	0.514^{ab}	4.184^{a}	0.2500^{a}
	BM2	5	21.42^{bcd}	3.442^{bcde}	0.508^{abc}	3.770^{abc}	0.2240^{abcde}
	BM3	5	26.12^{a}	4.296^{a}	0.516^{ab}	3.856^{ab}	0.2440^{ab}
	BM4	5	24.94^{ab}	3.858^{abcd}	0.524^{a}	3.630^{abcd}	0.2412^{abc}
+NaCl (+S)	**CONTROL**	5	17.08^{e}	2.534^{f}	0.327^{d}	2.446^{e}	$0.1440^{hı}$
	N1	5	20.86^{cde}	3.158^{cdef}	0.408^{cd}	3.856^{ab}	0.1750^{efgh}
	N2	5	20.30^{de}	3.362^{bcde}	0.428^{abcd}	3.424^{abcde}	$0.1640^{fghı}$
	N3	5	23.26^{abcd}	3.458^{bcde}	0.416^{bcd}	3.324^{abcde}	$0.1480^{ghı}$
	N4	5	20.82^{cde}	3.102^{def}	0.342^{d}	2.720^{de}	$0.1244^{ı}$
	BM1	5	23.48^{abcd}	3.316^{bcdef}	0.428^{abcd}	2.594^{de}	$0.1664^{fghı}$
	BM2	5	22.96^{abcd}	3.696^{abcde}	0.482^{abc}	2.648^{de}	0.1850^{defgh}
	BM3	5	21.02^{bcd}	3.482^{bcde}	0.414^{bcd}	2.794^{cde}	0.1966^{bcdefg}
	BM4	5	20.06^{de}	3.210^{cdef}	0.406^{cd}	2.938^{bcde}	0.1900^{cdefgh}
	P value		0.000*	0.001*	0.000*	0.001*	0.000*
Mean	- **NaCl**	45	23.04	3.70	0.495	3.67	0.22
Mean	+ **NaCl**	45	21.09	3.26	0.406	2.97	0.17
General Mean		90	22.06	3.480	0.450	3.322	0.1939

*; significant at P< 0.001 levels. The differences between mean values indicated by different letters are significant.

Salinity causes a decrease in the amount of NO_3, especially in the leaves of plants while NO_3 application increases the stress tolerance by reducing the Cl intake (Hu and Schmidhalter, 2005).

Root fresh weights were less affected by ammonium nitrate and blood meal applications compared to shoot fresh weights in untreated plants; however, BM_1S, BM_3S and N_3 applications

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were found statistically higher than control plants at a significant level. In plants under salt stress, the doses of ammonium nitrate were more effective than the doses of blood meal and provided higher root fresh weight values (Table 2).

All doses of blood meal application on root dry weights of plants without salt stress were more effective than ammonium nitrate application. The highest root dry weights were determined among all applications as 0.25 g and 0.244 g in BM_1 and BM_3 applications, respectively. Blood meal applications were also more effective in root dry weights of plants subjected to salt stress and the highest values were found in BM_3S and BM_4S applications (Table 2). Similar to our findings, many researchers have reported that under the influence of salt stress, significant reductions occur in fresh and dry weights of the shoots and roots of plants (Irshad et al., 2002).

Many researchers have stated that nitrogen applied at adequate levels increases yield under saline conditions Selassie and Wagenct, 1981; Akram et al., 2010) and that this is because applied nitrogen increases salt tolerance. Cordovilla et al. (1995) reported that NO_3 application in the bean plant improved plant growth by alleviating the effect of saltiness.

In their studies investigating the effect of chemical fertilizers used in traditional production on the mineral matter content of tomatoes, with different organic fertilizers, which they used blood meal as a nitrogen source, Demir et al. (2003) suggested that there was no significant difference between organic fertilization and chemical fertilization in terms of mineral content of the plant. This result is important since it shows that organic fertilizers are as effective and sufficient as chemical fertilizers in plant nutrition.

Polat et al. (2001), in their study investigating the effect of different organic fertilizer applications on yield and quality of lettuce, yield in all organic fertilizer applications were found to increase at rates ranging from 56% to 212% compared to control plants. While 75 kg/da blood meal and 300 kg da^{-1} liquid chicken fertilizer application yielded 1698.3 kg da^{-1}, they obtained 1049.3 kg da^{-1} yield from the application of chemical fertilizer (ammonium nitrate). In the study, they found an increase in head height, root neck diameter and head weight of the plants in which blood meal applied as the organic fertilizer compared to control plants. In addition, the effect of organic fertilizer application on the amount of plant nutrients removed from the soil (N, P, K, Ca, Mg, Fe, Mn, Zn and Cu) was also found statistically significant. In Table 3, the effect of ammonium nitrate and blood meal on the L value ($P \leq 0.05$), a and b values ($P \leq 0.001$) were found to be significant at different doses in young bean leaves.

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Table 3: L a b Values Determined in Bean Leaves

Treatments		N	L	a	b
- NaCl (- S)	Control	5	40.41^{abc}	-10.42^{bc}	20.80^{abcd}
	N1	5	39.43^{abc}	-10.56^{bc}	19.05^{bcdef}
	N2	5	37.57^{bcd}	-11.58^{bc}	15.35^{fg}
	N3	5	34.47^{d}	-12.30^{cd}	13.99^{g}
	N4	5	39.82^{abc}	-10.91^{bc}	19.54^{abcde}
	BM1	5	38.31^{abcd}	-11.44^{bc}	20.13^{abcd}
	BM2	5	36.05^{cd}	-11.87^{bcd}	15.64^{efg}
	BM3	5	38.03^{bcd}	-13.88^{d}	16.65^{defg}
	BM4	5	37.33^{bcd}	-11.09^{bc}	17.58^{cdefg}
+NaCl (+S)	Control	5	42.55^{a}	-7.38^{a}	23.73^{a}
	N1	5	40.53^{ab}	-9.75^{b}	20.44^{abcd}
	N2	5	39.58^{abc}	-10.51^{bc}	21.80^{abc}
	N3	5	38.71^{abcd}	-10.74^{bc}	21.38^{abc}
	N4	5	40.97^{ab}	-10.49^{bc}	22.05^{ab}
	BM1	5	39.20^{abc}	-10.49^{bc}	20.32^{abcd}
	BM2	5	39.23^{abc}	-10.90^{bc}	19.05^{bcdef}
	BM3	5	39.66^{abc}	-10.02^{bc}	19.24^{bcdef}
	BM4	5	40.60^{ab}	-9.53^{b}	20.43^{abcd}
	P value		0.03	0.001*	0.000*
Mean -	NaCl	45	37.94	-11.56	17.64
Mean +	NaCl	45	40.11	-9.98	20.94
General Mean		90	39.03	-10.77	19.29

*; significant at P< 0.001 levels. The differences between mean values indicated by different letters are significant.

It was determined that the values of L, a and b increased in all applications under the influence in salt stress. It is foreseen that this situation may have been caused by increased L-value due to leaf color fading under the influence of saltiness, by increased a-value due to the decrease in the amount of chlorophyll, namely green color and by increased b-value due to the increase of chlorosis namely turning yellow. Even though there was a slight decrease in L-value compared to the control plants, both in subjected to and not subjected to salt stress plants, statistically significant reductions were obtained in N_3 dose which was not subjected to stress.

The highest green color tone in plants not subjected to salt stress in a-value representing the green and red color tone was determined -13.88 in BM_3 dose while the a-value of plants under salt stress was lower than that of control plants at all doses. This situation is interpreted as the application of nitrogen in the form of ammonium nitrate and blood meal mitigates the loss of chlorophyll under the influence of salt stress. The b-value, which is interpreted as the increase in leaf yellowing namely chlorosis, were determined as 13.99 at the lowest level in the N_3 dose in plants not subjected to stress while the lowest values were found to be 19.05 and 19.24 in the

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BM_2S and BM_3S plants, respectively in plants subjected to stress (Table 3).

The decrease in green color tone on bean leaves due to salt stress and increase of yellow color tone can be caused by a decrease in chlorophyll synthesis or by decomposition of chlorophyll pigments due to stress. However, salinity reduces chlorophyll synthesis by disrupting the molecular structure of chlorophylls (Ashraf, 2004) or by increasing the activity of chlorophylase enzyme (Yıldız et al., 2010).

Doğan (2012) reported that 1% nitrogen dose applied to the leaf increased enzyme activities (SOD, APX, CAT, GR), MDA and chlorophyll content in tolerant genotypes among 5 tomato genotypes which are subjected to 150 mM NaCl salt stress and that nitrogen application has a positive effect on the protection against salt stress. In another study, different salt concentrations (50, 100, 150, 200 mM NaCl) were applied and the physiological changes were recorded for 10 weeks and it has been found that increased salt concentrations reduce the relative water content (BSI) and chlorophyll concentration of the plant, in particular the damage at 150 mM is more pronounced (Najafi et al., 2006).

4. CONCLUSIONS AND RECOMMENDATIONS

When the results obtained are generally evaluated, nitrogen application in the form of ammonium nitrate and blood meal affects plant growth and leaf color values positively either under or not under salt stress conditions. The application of blood meal (BM_3) including N at a dose of 200 ppm, in particular, increased all measured morphological parameters and leaf color qualities considerably.

Utilizing organic wastes such as blood meal in agricultural production not only prevents environmental pollution, but also reduces the need for chemical fertilizers significantly by bringing necessary nutrients to the soil for plants. In addition, it is of great importance to be able to use a waste, which causes environmental pollution and health problems when released to the environment, through a simple process as a substitution of chemical fertilizers used in agricultural production which will also cause environmental pollution and health problems.

Athough its usage as an organic fertilizer is not a new phenomenon, blood meal is not widely used in agricultural production. In plant production in which organic fertilizer variety is limited, it is necessary to use it widespread especially in organic agriculture.

Since the blood meal affects the yield and quality more, especially for the plants of the Brassicaceae family, it will be beneficial to continue studies concerning the plants in this species in different dosage and application forms.

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Consequently, blood meal positively affected plant morphology and leaf color values as much as ammonium nitrate, which contains equivalent N. It is thought that blood meal can be used as an alternative nitrogen source in plant production, provided that measures are taken against possible contamination risk during its production.

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