

Response of Snap Bean (*Phaseolus vulgaris* L) Plants to Nitrogen Fertilizer and Foliar Application with Methionine and Tryptophan

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Abstract: Two field experiments were carried out in two successive seasons of 2008 and 2009 at the Agricultural Experimental Station of the National Research Centre, EL-Nubaria, Elbehira Governorate, Egypt, to study the effect of different combinations of three levels of nitrogen fertilizer (100%, 65% and 35% of the recommended dose) with two levels of foliar spray methionine and tryptophan (100 and 200mgL⁻¹) on growth, pod yield, quality and some chemical constituents of snap bean plants. Results showed that fertigated snap bean plants with the highest nitrogen dose increased the vegetative growth, yield and quality. Tryptophan (100mgL⁻¹) improved vegetative growth, yield and quality. Foliar application of tryptophan at both concentrations increased free amino acids content and phenolics content in the leaves. In addition, both concentrations of methionine increased free amino acid, protein percentage and nitrogen percentage in pod. It can be concluded that nitrogen fertilizer can be reduced to 65% with sprayed tryptophan amino acid (100mgL⁻¹) to obtain the highest vegetative growth, yield and quality of snap bean plants.

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1. Introduction

Snap bean or 'French bean' (also referred to as green beans or string beans) is a strain of common bean, (*Phaseolus vulgaris* L), which is grown as a cash crop at large scale and smallholder farmers. So, snap bean is an important vegetable crop for local consumption and export.

Mineral fertilizers application is essential for plant growth, development and productivity of snap bean plants. With fertilizers, farmers can produce more food and cash crops of better quality, especially in the low soil fertility which has been over-exploited. Nitrogen is one of the 17 chemical elements required for plant growth and reproduction. On the other hand, frequent or excessive amounts of nitrogen fertilizer would lead to un-favorite effect on the growth and yield of snap bean plants and will lead to increase the losses of nitrogen fertilizer. So, the adequate amounts of nitrogen fertilization led to improve growth, yield and quality of pods.

Nitrogen is of vital importance for plant growth due to being a part of amino acid, protein, enzymes and chlorophyll molecule (Devlin and Witham, 1986). Many investigators reported that increasing NPK levels application improved the plant growth, yield and green pod quality of snap bean (Singer *et al.*, 2000, Saxena *et al.*, 2003, Hafez *et al.*, 2004, Abdel-Mawgoud *et al.*, 2005, Souza *et al.*, 2008, and El-Bassiony *et al.*, 2010). All vegetative growth parameters were gradually and significantly increased by increasing the level of nitrogen fertilizer

application (Asmaa *et al.*, 2010). Insufficient available N leads to reduced growth, reduced light interception, limited yield and early crop senescence. On the other hand, excessive available N can result in reduced and delayed yield and reduced dry matter content (Kleinkopf *et al.*, 1981).

El-Tohamy *et al.* (2009) reported the possibility of using slow release N fertilizer such as Ensyabine to maximize growth, yield and quality of bean plants grown under new-reclaimed sandy soils. This approach also provides an efficient way of applying nitrogen to such soils to increase the efficiency of N application and to minimize leaching as well as to prevent environmental pollution by the excess of nitrogen in the soil.

Bioregulator substances were shown to enhance the biosynthesis of certain chemical constituents in plants. In this respect the amino acids which have a high integrity with different metabolic pools in plants were used to promote plant growth (Coruzzi and Last, 2000). Maxwell and Kieber (2004) indicated the link of methionine to the biosynthesis of growth regulating substances, e.g. cytokinins, auxins and brassinosteroids in plants. Whereas the link of tryptophan to the biosynthesis of auxins, the phytoalexin camalexin, phenyl propanoids and other related natural products in plants was recently reported (Tao *et al.* 2008). Studies have proved that amino acids can directly or indirectly influence the physiological activities of plant growth and development. Many studies reported that the foliar

application of amino acids caused an enhancement in plant growth, fruit yield and its components (El-Shabasi *et al.*, 2005) on cucumber, and (Awad *et al.*, 2007) on garlic.

Thus, this study aimed to investigate the interactive effect of different nitrogen fertilizer levels in combination with foliar spray of methionine and tryptophan on the growth, productivity, quality and some chemical constituents of snap bean plant.

2. Materials and Methods:

A field experiment was carried out at the Agricultural Experimental Station of National Research Centre, Nubaria, Elbehira Governorate, Egypt, during two successive summer seasons of 2009 and 2010 to study the effect of the interaction between 3-rates of nitrogen fertilizers application (100%, 65% and 35% of the recommended dose i.e.

100 unit N/feddan 300Kg ammonium nitrate) and two concentrations of methionine and tryptophan (100 and 200mgL⁻¹) in addition to the control on the growth, yield and pods quality of snap bean (*Phaseolus vulgaris* L.cv. Paulesta). Seeds of snap bean were sown on the first week of April in the two seasons. Seeds were sown on two sides of the ridge 10 cm apart, ridge was 80 cm width and 4 m length and the plot area was 12.8 m². Nitrogen fertilizer was applied through fertigation three times per week. At 30 and 45 days after sowing methionine and tryptophan were sprayed on snap bean plants.

The experimental soil was sandy and the physical and chemical analyses are presented in Table (1). The normal agricultural practices required for snap bean production were applied as recommended.

Table (1): Physical and chemical properties of the experimental soil.

Physical properties		Chemical analysis	
Sand	90.08	Ca (Mg/L)	7.02
Clay	9.26	Mg (Mg/L)	0.527
Silt	0.66	Na (Mg/L)	0.982
Texture	Sandy	K (Mg/L)	0.31
F.C. %	16.57	HCO ₃ (Mg/L)	1.3
W. P. %	5.25	Cl (Mg/L)	0.566
E. C. (ds/m)	1.7		
PH	8.2		

Data recorded:

Vegetative growth: A sample of 5 plants from each plot was taken randomly at 45 days after sowing and the following characters were recorded: plant length, leaves number, branches number, fresh and dry weight of snap bean per plant.

Pods yield:

At harvest stage, the mature pods of bean for each experimental plot were collected along the harvesting season and the total pods yield was recorded as ton/fed (feddan=4200m²).

Pods quality:

Random sample of 50 pods from each plot was taken and average pod weight and pod length were recorded.

Chemical constituents:

Photosynthetic pigments (chlorophyll a, b, carotenoids and total photosynthetic pigments) content were estimated in snap bean leaves according to Wettstein (1957). Protein percentage was determined according to A.O.A.C. (1990). Total Free amino acids were determined using the ninhydrin colorimetric method defined by Plummer (1978).

Total phenolic compounds were estimated according to Snell and Snell (1952).

The treatments were arranged in a split plot design with four replicates where, nitrogen fertilizers rates were arranged in main plots, while methionine and tryptophan foliar application treatments were in addition the control distributed in the sub plots. The obtained data were statistically analyzed according to the method described by Gomez and Gomez (1984).

3. Results and Discussion:

Effect of nitrogen and amino acids on vegetative growth of snap bean plants:-

1- Effect of nitrogen level:-

Data in Table (2) cleared that the application of nitrogen fertilizer at recommended dose (100 %) increasing significantly the vegetative growth criteria (i.e. plant length, leaves number/plant, number of branches/plants and fresh and dry weight of leaves/plant) in the two seasons. While the lowest values of the vegetative growth criteria was obtained with the application of nitrogen at 35% of recommended dose. These results were true in the two seasons. This result may be due to nitrogen is vital importance for plant growth due to being a part of amino acid, protein, enzymes and chlorophyll

molecule (Devlin and Witham, 1986).

2- Effect of amino acids:-

Spraying snap bean plants with amino acid tryptophan at 100mgL^{-1} increased significantly the vegetative growth characters (number of leaves and shoots/ plant, number of branches /plants and fresh and dry weight of leaves) except for plant length in the first season and leaves dry weight in the second season, where no significant difference between both concentration of tryptophan (Table 2). These findings were true in both seasons. On the contrary, the lowest

values of number of leaves and shoots/plant, number of branches /plants and fresh and dry weight of leaves were recorded in the unsprayed plants. Amino acids can directly or indirectly influences the physiological activities of plant growth and development. El- Shabasi *et al.* (2005) on cucumber and Awad *et al.* (2007) on garlic reported that foliar application of amino acids caused an enhancement in plant growth, yield and its components. Amino acids which have a high integrity with different metabolic pools in plants were used to promote plant growth (Coruzzi and Last, 2000).

Table (2): Effect of nitrogen and amino acids on vegetative growth of snap bean plants in 2008 and 2009 seasons.

Treatments	2008 season					2009 season					
	Plant length (cm)	Leaf number /plant	Branch number /plant	Leaves fresh weight (g)	Leaves dry weight (g)	Plant length (cm)	Leaf number	Branch number	Leaves fresh weight (g)	Leaves dry weight (g)	
Effect of nitrogen fertilizer level											
35% N	36.2	11.8	6.8	19.1	3.6	39.8	13.4	4.6	34.8	8.8	
65% N	41.4	17.7	8.8	38.1	6.8	43.8	16.0	6.5	51.9	10.2	
100% N	43.1	23.4	9.2	57.7	8.9	47.2	21.2	7.0	65.9	11.9	
LSD at 5%	1.0	3.8	1.5	10.1	1.9	3.1	2.4	1.4	7.6	1.3	
Effect of methionine and tryptophan foliar application											
Control	37.3	15.2	7.5	29.5	5.2	38.0	14.0	5.0	32.4	7.4	
M1	41.1	16.7	8.2	33.4	5.8	43.2	16.7	6.5	41.9	8.7	
M2	40.0	18.2	8.2	40.8	6.3	44.0	17.3	5.8	53.6	10.4	
T1	40.7	21.3	9.5	48.5	8.2	47.3	18.3	6.8	68.1	12.6	
T2	42.0	16.8	8.0	39.3	6.6	45.7	18.0	6.0	58.4	12.6	
LSD at 5%	1.37	2.2	NS	3.5	1.0	2.3	1.97	NS	6.1	1.7	
Effect of interaction											
35% N	Control	32.0	9.0	6.5	13.3	2.1	33.0	10.5	4.0	29.6	5.4
	M1	37.0	11.5	7.0	14.2	2.6	38.0	13.5	4.5	30.2	8.3
	M2	35.0	11.5	6.5	16.3	3.5	36.0	13.5	4.5	33.9	9.8
	T1	40.0	15.0	7.0	26.6	5.5	47.0	14.0	5.5	41.1	9.6
	T2	37.0	12.0	7.0	25.5	4.4	45.0	15.5	4.5	39.1	11.0
65% N	Cont.	36.0	15.0	7.5	33.27	5.24	37.00	12.00	4.50	27.86	7.13
	M1	44.0	15.5	8.5	33.29	5.91	45.00	15.50	7.50	48.04	8.17
	M2	41.0	18.0	8.5	34.83	5.87	43.00	17.00	6.50	57.14	9.67
	T1	42.0	22.5	11.0	48.71	9.57	49.00	18.00	7.50	63.34	12.34
	T2	44.0	17.5	8.5	40.56	7.22	45.00	17.50	6.50	63.01	13.59
100% N	Cont.	44.0	21.5	8.5	41.95	8.21	44.00	19.50	6.50	39.72	9.84
	M1	42.4	23.0	9.0	52.76	8.75	46.20	21.00	7.50	47.38	9.53
	M2	44.0	25.0	9.5	71.37	9.57	53.00	21.50	6.50	69.59	11.61
	T1	40.0	26.5	10.5	70.32	9.57	46.00	23.00	7.50	99.89	15.72
	T2	45.0	21.0	8.5	51.96	8.25	47.00	21.00	7.00	72.93	13.09
LSD at 5%	2.5	5.1	1.1	12.46	1.87	3.22	2.15	1.23	11.12	1.46	

M1= Methionine (100mgL^{-1})

M2= Methionine (200mgL^{-1})

T1= Tryptophan (100mgL^{-1}) T2=

Tryptophan (200mgL^{-1})

3- Effect of the interaction:-

The interaction between nitrogen level and amino acids had a significant effect on the vegetative growth characteristics (Table 2). However, the highest growth of snap bean plants was found with using 100 % N and foliar spray of tryptophan at 100mgL⁻¹ in the two seasons, except for plant length, branches number and fresh weight of leaves in the first season and plant length in the second one. On the other hand, the lowest values of growth criteria

were found in the control treatment. These results were true in both seasons (Table 2).

Effect nitrogen and amino acid on yield and quality of snap bean plants:-**1- Effect of nitrogen level:-**

Data in Table (3) cleared that the highest yield, pod length and pod weight were recorded by using 100 % N.

Table (3): Effect of nitrogen and amino acids on yield and quality of bean plants in 2008 and 2009 seasons.

Treatments	2008 season			2009 season			
	Pod length (cm)	Pod weight (g)	Total yield ton/fed.	Pod length (cm)	Pod weight (g)	Total yield ton/fed.	
Effect of nitrogen fertilizer level							
35% N	11.3	3.4	4.24	12.41	3.41	4.25	
65% N	12.6	3.9	4.36	12.88	3.87	4.56	
100% N	12.7	4.5	4.69	13.91	4.64	4.84	
LSD at 5%	0.9	0.1	0.16	NS	NS	0.12	
Effect of methionine and tryptophan foliar application							
Control	11.13	3.14	3.76	12.53	3.05	3.82	
M1	12.27	3.47	4.05	13.23	3.94	4.20	
M2	12.19	3.92	4.57	13.23	4.08	4.60	
T1	12.55	4.77	5.28	13.52	4.44	5.32	
T2	12.95	4.37	4.50	12.81	4.37	4.79	
LSD at 5%	NS	0.17	0.09	NS	NS	0.77	
Effect of interaction							
35% N	Control	10.60	2.62	3.43	11.20	2.92	3.60
	M1	11.50	2.94	3.61	13.30	3.23	3.76
	M2	11.20	3.52	4.37	12.30	3.52	4.59
	T1	11.60	4.35	5.56	13.11	3.53	4.94
	T2	11.75	3.74	4.23	12.13	3.84	4.34
65% N	Cont.	11.50	2.84	3.46	12.50	2.74	3.76
	M1	12.50	3.51	3.81	12.50	3.87	4.36
	M2	12.60	3.87	4.37	13.10	3.76	4.33
	T1	12.74	4.63	5.49	13.50	4.59	5.44
	T2	13.60	4.55	4.69	12.80	4.38	4.90
100% N	Cont.	11.30	3.96	4.39	13.90	3.48	4.11
	M1	12.80	3.97	4.74	13.90	4.71	4.49
	M2	12.77	4.38	4.96	14.30	4.95	4.88
	T1	13.30	5.34	4.79	13.95	5.19	5.59
	T2	13.50	4.82	4.59	13.50	4.89	5.13
LSD at 5%	1.32	1.36	1.02	NS	1.24	1.04	

M1= Methionine (100mgL⁻¹) M2= Methionine (200 mgL⁻¹) T1= Tryptophan (100mgL⁻¹) T2= Tryptophan (200 mgL⁻¹)

On the contrary, the lowest total yield of snap bean and pod weight as well as pod length was found by using 35% N. These results were true in the two seasons. The results are in agreement with that obtained by Abdel-Mawgoud *et al.* (2005); Souza *et al.* (2008) and El-Bassiony *et al.* (2010).

The obtained results may be due to the vital importance of nitrogen for plant growth due to being a part of amino acids, protein, enzymes and chlorophyll molecule (Devlin and Witham, 1986).

2- Effect of amino acids foliar application:-

Data in Table (3) showed that, tryptophan at 100mgL^{-1} increased the total yield of snap bean and pod weight than the unsprayed plants in the two seasons, except for pod weight in the second season and pod length in the two seasons. Spraying snap bean plants with the two bioregulators enhancement the growth criteria (Table 2) and gave healthy plants with vigour growth which produced more pod weight and consequently resulted in more yield than that unsprayed plants (Table 3). Many studies reported that foliar application of amino acids caused an enhancement in plant growth, yield and its components (El- Shabasi *et al.* 2005) on cucumber and (Awad *et al.* 2007) on garlic.

3- Effect of the interaction:-

The interaction between nitrogen level and methionine and tryptophan foliar application had a significant effect on the total yield of snap bean and pod weight as well as pod length in both seasons, except for pod weight in the second season. However, the highest growth of snap bean plants was recorded by using 100 %N and foliar spray of tryptophan (100mgL^{-1}) in the two seasons, except for plant length, branch number and fresh weight of leaves in the first season and plant length in the second one. On the other hand, the lowest values of growth were found in the control treatment (Table 3).

Table (4): Effect of nitrogen and amino acids on photosynthetic pigments content (mg/g/fw) in leaves of snap bean plant in 2008 and 2009 seasons.

Treatments	2008 season				2009 season				
	Chl. a	Chl. b	Carotenoids	Total pigment	Chl. a	Chl b	Carotenoids	Total pigment	
Effect of nitrogen fertilizer level									
35% N	0.60	0.23	0.32	1.15	0.75	0.31	0.39	1.45	
65% N	0.54	0.20	0.29	1.03	0.70	0.29	0.38	1.37	
100% N	0.51	0.16	0.28	0.95	0.77	0.30	0.45	1.53	
LSD at 5%	0.04	0.03	NS	0.08	NS	NS	NS	NS	
Effect of methionine and tryptophan foliar application									
Control	0.46	0.13	0.27	0.86	0.68	0.26	0.35	1.29	
M1	0.57	0.22	0.30	1.09	0.70	0.29	0.39	1.38	
M2	0.65	0.22	0.31	1.19	0.84	0.34	0.49	1.67	
T1	0.42	0.16	0.24	0.82	0.64	0.27	0.34	1.25	
T2	0.65	0.24	0.37	1.26	0.85	0.35	0.46	1.66	
LSD at 5%	0.06	0.03	NS	0.14	NS	NS	NS	NS	
Effect of interaction									
35% N	Cont.	0.43	0.06	0.29	0.78	0.63	0.19	0.33	1.15
	M1	0.83	0.36	0.41	1.60	0.91	0.38	0.49	1.78
	M2	0.61	0.26	0.29	1.16	0.78	0.34	0.38	1.50
	T1	0.49	0.22	0.27	0.98	0.66	0.31	0.34	1.31
	T2	0.64	0.25	0.34	1.23	0.78	0.34	0.39	1.51
65% N	Cont.	0.47	0.18	0.24	0.89	0.53	0.22	0.27	1.02
	M1	0.49	0.19	0.26	0.94	0.51	0.21	0.31	1.03
	M2	0.66	0.26	0.35	1.27	1.02	0.44	0.52	1.98
	T1	0.38	0.14	0.22	0.74	0.56	0.23	0.29	1.08
	T2	0.68	0.24	0.37	1.29	0.89	0.35	0.51	1.75
100% N	Cont.	0.47	0.16	0.27	0.90	0.89	0.38	0.44	1.71
	M1	0.39	0.12	0.22	0.73	0.69	0.27	0.38	1.34
	M2	0.69	0.15	0.29	1.13	0.72	0.24	0.57	1.53
	T1	0.39	0.12	0.22	0.73	0.69	0.27	0.39	1.35
	T2	0.63	0.24	0.39	1.26	0.88	0.36	0.48	1.72
LSD at 5%	NS	NS	NS	NS	NS	NS	NS	NS	

M1= Methionine (100mgL^{-1}) M2= Methionine (200mgL^{-1}) T1= Tryptophan (100mgL^{-1}) T2= Tryptophan (200mgL^{-1})

Effect of nitrogen fertilizer level and amino acids on photosynthetic pigments content in leaves of snap bean plants:-

1- Effect of nitrogen level:-

Data in Table (4) show that, using 35 % N increasing chlorophyll a, b, carotenoids and total photosynthetic pigments content in the two seasons. Whereas, nitrogen levels had insignificant effect on photosynthetic pigments content in the second season and carotenoids in the first season. On the contrary, the lowest values of photosynthetic pigments content were recorded by 100 % N. These results were true and similar in the two seasons.

2- Effect of amino acid foliar application:-

Data in Table (5) showed that the highest values of chlorophyll a found with methionine (200mgL^{-1}) in the first season and by methionine (100mgL^{-1}) in the second season. While the highest values of chlorophyll b was found in control treatment in the first season and by methionine at low concentration (100mgL^{-1}) in the second one. Moreover, the highest values of carotenoids were found by foliar spray of methionine at 200mgL^{-1} in the two seasons. Spraying snap bean plants with methionine or tryptophan at 100mgL^{-1} recorded the highest value of total photosynthetic pigments (Table 4). The results which recorded by other investigator indicated that amino acids can directly or indirectly influence the physiological activities of the plant. Amino acids help to increase chlorophyll in the plant (Awad *et al.* 2007 and Al-Said *et al.* 2008).

3- Effect of the interaction:-

The interaction between nitrogen level and methionine and tryptophan foliar application had insignificant effect on chlorophyll a, b, carotenoids and total photosynthetic pigments in the two seasons of study (Table 4). This result show that each factor act independent.

Effect of nitrogen and amino acid on free amino acids, phenolic contents in leaves and free amino acids, protein, nitrogen in pod of snap bean plants:-

1- Effect of nitrogen level:-

The results in Table (5) show clearly that using 65 % or 100% N increasing free amino acids in both seasons. While fertigated bean plants with 35 % or 65% N increased the total phenolic compounds in leaves of bean plants in the two seasons. Whereas nitrogen levels had a significant effect on total free

amino acids, protein % and nitrogen percentage. The highest values of free amino acids were obtained when bean plants fertigated with 65% nitrogen. In addition, using 100% nitrogen gave the highest values of protein percentage and nitrogen percentage in the two seasons.

2- Effect of amino acids foliar spray:-

Data in Table (5) showed that, the two amino acids foliar spray had a significant effect on all measured characters. Tryptophan (200mgL^{-1}) gave the highest values of free amino acids and phenolic compounds in leaves of bean plants in both seasons. Whereas, using methionine (100mgL^{-1}) increased free amino acids, protein percentage and nitrogen percentage in pod of snap bean plants.

3- Effect of interaction:-

Concerning the interaction between nitrogen levels and amino acid treatments data in Table (6) indicated that using 100% nitrogen fertilizer with tryptophan (200mgL^{-1}) gave the highest values of free amino acids in leaves (36.25 and 38.48 mg g^{-1} dryweight) respectively. Moreover, using 35% nitrogen with tryptophan (200mgL^{-1}) increased the total phenolic in leaves of bean plants in both seasons. Fertigated bean plants with 35% nitrogen plus spraying with Methionine (100mgL^{-1}) increased the free amino acids in pods. Whereas, Methionine (100mgL^{-1}) foliar spray with 65% or 100 % nitrogen fertilizer gave the highest values of protein percentage and nitrogen percentage in pod of bean plants in the two seasons.

4. Conclusion

It can be concluded from the results of this study that using the bioregulator tryptophan (amino acids) as foliar spray at the concentration of 100mgL^{-1} under fertigated with 65% nitrogen fertilizer of snap bean plants resulted in improvement the plant growth, productivity and quality. Accordingly, we can minimize the negative impacts of excessive nitrogen fertilization on soil characteristics and the plants.

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Table (6): Effect nitrogen and amino acid on the total free amino acids (FAA), phenolic contents in leaves and free amino acid, protein, nitrogen percentage in pod of snap bean plants in 2008 and 2009 seasons.

Treatments	2008 season					2009 season					
	In leaves		In pod			In leaves		In pod			
	FAA	Phenolic	FAA	Protein%	N%	FAA	Phenolic	FAA	Protein%	N%	
Effect of nitrogen fertilizer level											
35% N	17.94	12.45	21.44	15.75	2.52	22.74	12.70	22.16	15.88	2.54	
65% N	23.43	13.22	27.06	19.25	3.08	25.62	13.41	27.97	18.88	3.02	
100% N	24.57	10.76	21.57	20.00	3.20	27.18	10.96	23.60	20.50	3.28	
LSD at 5%	1.35	1.16	2.35	1.15	0.18	2.05	1.13	1.22	1.17	0.19	
Effect of methionine and tryptophan foliar application											
Control	17.39	11.42	19.42	17.30	2.77	18.71	11.57	21.03	16.88	2.70	
M1	20.57	10.84	31.30	21.88	3.50	25.54	10.89	33.18	21.88	3.50	
M2	23.52	10.56	26.61	18.55	2.97	25.31	10.83	27.47	18.54	2.97	
T1	23.47	12.78	20.56	16.25	2.60	25.82	12.99	21.15	17.09	2.73	
T2	24.97	15.12	18.88	17.71	2.83	30.52	15.52	20.05	17.71	2.83	
LSD at 5%	1.35	1.23	3.67	1.09	0.17	2.34	1.10	2.14	1.37	0.22	
Effect of interaction											
35% N	Control	11.03	9.73	17.02	14.38	2.30	11.19	9.91	17.54	13.75	2.20
	M1	14.69	12.96	36.42	18.13	2.90	23.71	13.07	37.17	18.75	3.00
	M2	25.69	10.34	24.79	16.88	2.70	26.33	10.55	24.89	16.88	2.70
	T1	23.17	11.04	12.97	14.38	2.30	25.45	11.47	13.45	15.00	2.40
	T2	15.11	18.17	15.98	15.00	2.40	27.03	18.52	17.76	15.00	2.40
65% N	Cont.	19.66	15.29	22.82	18.13	2.90	22.00	15.56	23.24	17.50	2.80
	M1	29.11	10.93	35.69	25.00	4.00	31.08	10.94	35.78	23.75	3.80
	M2	21.38	10.06	31.37	19.38	3.10	24.34	10.19	32.83	18.75	3.00
	T1	23.48	16.04	17.46	13.75	2.20	24.65	16.04	18.73	14.38	2.30
	T2	23.54	13.80	27.98	20.00	3.20	26.05	14.34	29.27	20.00	3.20
100% N	Cont.	21.48	9.23	18.43	19.38	3.10	22.94	9.24	22.32	19.38	3.10
	M1	17.90	8.64	21.79	22.50	3.60	21.84	8.65	26.58	23.13	3.70
	M2	23.48	11.27	23.67	19.38	3.10	25.26	11.76	24.69	20.00	3.20
	T1	23.75	11.26	31.26	20.63	3.30	27.37	11.47	31.28	21.88	3.50
	T2	36.25	13.40	12.68	18.13	2.90	38.48	13.69	13.12	18.13	2.90
LSD at 5%	3.52	3.84	2.46	2.26	0.36	3.25	2.76	6.85	1.78	0.28	

M1= Methionine (100mgL⁻¹) M2= Methionine (200 mgL⁻¹) T1= Tryptophan (100mgL⁻¹) T2= Tryptophan (200 mgL⁻¹)

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