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The effect of different Values of Nitrogen, Potassium and Zinc Fertilizers on Physiological characteristics of Green Bean (*Phaseolous vulgaris* gen. Sunray) in Iran

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ABSTRACT: The experiment was conducted with complete randomized block experimental design with split-plot arrangement with three replications in Varamin university field research in 2011. The main plots consisted of nitrogen levels from urea including 250 Kg ha^{-1} (N1), 300 Kg ha^{-1} (N2) and 350 Kg ha^{-1} (N3). And sub plots with different fertilizer were three treatments of Potassium (K) 120 Kg ha^{-1} and Zn-foliar application 6 per 1000 (Zn) and photash 120 Kg ha^{-1} with 6 per 1000 Zn-foliar application together (N, K*Zn) that sprayed by the results of soil analysis. The results showed that nitrogen uptake increased green bean yield. The interaction between (N level) and (K and Zn applications) was observed that the highest Nitrate uptake was obtained that N3K*Z treatment. The highest radiation use efficiency (RUE) and chlorophyll were obtained from 350 Kg.ha^{-1} Urea with application of (K* Zn fertilizers) treatment. The highest pod yield (4306.2 Kg.ha^{-1}) was presented by application of 250 Kg.ha^{-1} Urea with K and Zn fertilizers, however the lowest pod yield (2298 kg.ha^{-1}) was observed at 350 Kg.ha^{-1} Urea with K. The results of this experiment showed that the Zn-foliar application increased all features in bean and also, reduced N fertilizer rate without reduction in more plant characteristics.

Keywords: green bean, nitrate, radiation use efficiency (RUE) and growth index.

INTRODUCTION

Nitrogen is one of the fundamental compounds in nutrition of the plants. If there are enough water and food for plant, then the light is the only factor that effects on qualitative and quantitative properties of the crop (Zimdahl, 2006). One of the objectives of agricultural management is maximum utilization of solar energy by the canopy. It was evidenced that the application of N-fertilizer affects chlorophyll content and then it cause an increase in RUE (Pilbram *et al*, 2009). On the other hand, the maximum energy from light absorption and RUE cause the highest photosynthesis, and therefore biomass production and yield increase by penetrating light into different canopy layers (Haverkort, 2007). Majd Nasiri & Ahmadi (2005) showed that plants produce dry matter by sunlight absorption and store it in themselves during their vegetative stages. Lecocu & Ney (2008) proved that there was a linear relationship between the total dry matter production and photosynthetic active radiation (PAR) that the slope of that line was defined RUE. There are many factors affected RUE including nitrogen uptake, nitrate accumulation in the plant tissues, the amount of chlorophyll, leaf area index (LAI), crop growth rate. The application of plant nutrients especially Nitrogen, Potassium and Zinc affect the plants shoot characteristics such as leaf size, leaves direction and the ageing process of lower leaves that they cause an increase the light absorption by plants (Weiner *et al*, 2008). High application of N-fertilizer (especially in winter cultivation) increase nitrate accumulation in plant tissues (Mare, 2001). It announced that there was a positive relationship between LAI and RUE. An increase in LAI per area unit causes a decrease in the rate of extinction coefficient but an increase in RUE (Richards, 2004). It reported that the RUE for navy bean is between 0/8-1/05 g.Mj^{-1} (Morrison *et al*, 2009) and it is between 0.8 - 0.96 g.Mj^{-1} for vetch (Ramberg *et al*, 2008). Honeycutt *et al*. (2006) showed that yield of wax bean were strongly influenced by the nitrogen application. It is reported that plants accumulate nitrate in their tissues when Sulfur deficiency is suffering plants or available nitrogen increase (Santamaria *et al*, 1999). Nitrate accumulation in tissues of green bean occurs by absorption and transfer of this component. In the young plant, nitrate accumulates in stems and petioles (Caslo, 2003). This study has been conducted to increase radiation use efficiency (RUE) with optimization the application of nitrogen fertilizer in green bean and decrease nitrate accumulation in crop. Therefore the results of this study can provide a safe agricultural yield and prevent environment from nitrate pollution.

MATERIAL AND METHODS

Field experiment was conducted as split-plot in randomized complete block design in three replications, in Varamin university field research in farming year 2011. The experiment location is located in geographic coordinates of 39 and 51 degree along east and 19 and 35 degree north latitude at an altitude of 898 meters above sea level. The main plot included urea fertilizer at 3 levels (N1 = 250, N2 = 300, N3 = 350 $kg.ha^{-1}$). The sub plots were potassium (K) and zinc (Zn) from potassium sulfate and zinc sulfate fertilizers, respectively; including K1 = 120 kg $K.ha^{-1}$, Z1= foliar application of zinc solution with concentration of 6 $g.l^{-1}$ and K1Z1 = 120 kg $K.ha^{-1}$ with zinc foliar application with concentration of 6 $g.l^{-1}$ at 3 levels. The plot area was 15 m^2 including 6 ridges, each with 5 meters long and 3 meters width and plants were 20 × 50 cm^2 apart. All potassium and phosphor fertilizers and 1/3 nitrogen fertilizer was applied during planting and the remaining nitrogen fertilizer was applied two times as excess based on phonological stages of the plant. The distribution time of excessive fertilizer was after observing fifth leaf on the stem. According to the results of soil analysis, foliar application of micronutrients was done at vegetative growth and early flowering stages. The effect of treatments on following parameters was studied: plant height, chlorophyll content, LAI, extinction coefficient, RUE, fresh pod yield, nitrate in pod and the crop growth rate. Shoot height from soil surface was measured. Five samples of green leaves from each treatment were collected for Chlorophyll measurement.

The extinction coefficient (k) was calculated by the received daily light by canopy surface (lo) and latitude according to Nassiri, Kropft (1997). The extinction coefficient (K) was calculated by the following equation Based on Floyed *et al*. (2007).

Ii/Io=e-kl

Where (Io): active photosynthetic radiation in upper part of the plant, (Ii): active photosynthetic radiation in "i" layer of leaves, K: extinction coefficient or reduction of radiation, e: base of natural logarithm that is equal to 2/71827 and L: leaf area index in "i" layer. Dry matter (DM) and the accumulative radiation ($PAR_{adsorbed}$) were used to measure the radiation use efficiency (RUE) by following formula: (Monteith, 1997).

$DM = RUE^* \; PAR_{adsorbed}$

The leaf area was measured immediately in the samples by using the portable LAI meter, model IM-300.

The data was analyzed by MSTAT software. The differences between averages of treatments were examed by Duncan test at 5% significance level.

RESULTS AND DISCUSSION

A significant interaction between the application of nitrogen, potassium and zinc with together was observed on measured parameters except extinction coefficient at 5% level (Table 1).

Table 1. Analysis of variance Plant height Extinction coefficient Radiation use efficiency Wet Pod Yield, N in Pod and Chlorophyll under treatment N, K, ZN.

		(MS)					
S.O.V	**df**	**Wet Pod Yield**	**Radiation use efficiency**	**Extinction coefficient**	**Chlorophyll**	**Plant height**	**N in Pod**
Block	2	3499.864^{*}	0.00026^{ns}	0.00001^{ns}	0.0008^{ns}	825.86^{*}	0.00007^{ns}
FactorA	2	23452.845^{**}	0.00121^{ns}	0.00031^{*}	0.0092^{ns}	449.92^{ns}	0.00281^{*}
Error A	6	1085.266	0.00078	0.00002^{ns}	0.0041	332.87	0.00047
FactorB	2	11489.206^{*}	0.00334^{ns}	0.00303^{*}	0.0122^{ns}	3070.18^{*}	0.00321^{*}
A*B	4	86029.146^{**}	0.0245*	0.00048^{ns}	0.0687^{*}	3842.2^{*}	0.00993^{**}
Error B,	18	1459071	0.00103	0.00025	0.0097	555.45	0.00098
Cv		%15.56	%9.87	%12.07	%10.87	%14.49	%9.83

*,** , means significant in 0.05 and 0.01 level of probability respectively. ns : Non significant

The highest plant height was recorded 55 cm at N3K1Z1. It had 30.9 percent more than that the lowest plant height by 38 cm at N3K1 treatment (Table 2). The plants which were treated by N3K1Z1 had more new leaves on the top of stem than other treatments. It caused an increase in plant height and light efficiency. The results showed that the radiation absorption decreased when there were coating agents of nitrogen and potassium and zinc. The photosynthetic efficiency coefficient of these treatments showed a strong reduction and the vegetative growth and then rates of plant height decreased. On the other hand, Leaf Area Index (LAI) increased at N3K1Z1 treatment and then the receiving of the light source caused etiolating of the stem. It means that the solar radiation cannot be adsorbed enough because of the leaves shadow on older leaves then it causes a decrease in the photosynthesis efficiency. Finally these caused a decrease in yield and RUE.

Chlorophyll content: The results of variance analysis showed that the simple effects of treatments on chlorophyll content were not significant at 5% level but an interaction between different levels of nitrogen treatments and of K and Zn foliar spray treatments was observed (Table 1). That showed that the chlorophyll content decreased from 45.3 $mg.g^{-1}$ by N3*K1Z1 treatment to 42.2 $mg.g^{-1}$ by N1K1 treatment for fresh leaves (Table 2).

Table 2: Means comparison extraction as effected by elements N,K,ZN on Plant height Extinction coefficient Radiation use efficiency , Wet Pod Yield, N in Pod and Chlorophyll

Treatment	Wet Pod Yield ($Kg.ha^{-1}$)	Radiation use efficiency ($gr.mj.m^2$)	Extinction coefficient	Chlorophyll content ($\mu g.gr^{-1}$)	Plant height (cm)	N in Pod ($mg.Kg^{-1}$)
N1*K 250 Kg.ha	3716.70^{bc}	1.9269^{b}	0.5501^{a}	42.2^{b}	40^{b}	104.3^{c}
N1*Zn	4027.60^{ab}	1.9311^{b}	0.5489^{a}	43.2^{b}	46^{b}	99.7^{cd}
N1*(K+Zn)	4306.20^{a}	1.9468^{b}	0.5368^{a}	43.6^{ab}	52^{ab}	76.4^{d}
N2*K	2796.30^{d}	1.9342^{b}	0.5462^{a}	43.2^{b}	42^{b}	139.7^{bc}
N2*Zn	3461.80^{c}	1.9582^{b}	0.5396^{a}	43.7^{ab}	53^{ab}	121.3^{c}
N2*(K+Zn)	3794.20^{b}	2.0521^{ab}	0.5301^{a}	44.1^{ab}	54^{ab}	100.8^{cd}
N3*K	2298.60^{e}	1.9642^{b}	0.5411^{a}	43.9^{ab}	38^{b}	185.4^{a}
N3*Zn	2438.30^{e}	2.0967^{ab}	0.5389^{a}	44.6^{ab}	54^{ab}	157.9^{b}
N3*(K+Zn)	2827.11^{d}	2.2111^{a}	0.5296^{a}	45.3^{a}	55^{a}	135.6^{bc}

Means with the same letter in each column have not statistically significant difference

The changes in chlorophyll concentration are due to the ability of plants to maintain the source of power in environmental conditions; it was observed that Chlorophyll content is one of the key factors in determining the rate of photosynthesis and production of dry matter (Ghosh *et al*, 2004). The results of researches of Honeycutt *et al*. (2006) indicated that the wax bean yield is strongly affected by nitrogen nutrition. Also, by this study, the application of nitrogen associated with potassium and zinc foliar use cause an increase in chlorophyll content. The main factors which affected chlorophyll content are nitrogen and iron concentrations; chlorophyll content is being made in enough nitrogen concentration. The plant photosynthesis is increased by an increase in Chlorophyll content and it will lead to increase in yield. The highest level of chlorophyll was obtained by N3*K1Z1 treatment because of the important role of nitrogen in production and activity of vegetative pigments, especially chlorophyll, and direct effects of zinc and potassium on the production of chlorophyll.

Extinction coefficient: The results showed a significant simple effect of different levels of nitrogen and treatment of using potassium and Zn foliar spray on extinction coefficient. There were no significant interaction effects between the measured factors (Table 1). It showed that the treatment of 250 kg Urea.ha^{-1} (N1) had the highest extinction coefficient of 0.55 but the treatment of 350 kg Urea.ha^{-1} (N3) had the lowest rate of 0.5365. K-application treatment with 0.55 obtained the highest rate of extinction coefficient and (K + Zn) treatment with 0.5321 obtained the lowest rate of extinction coefficient. Plants can reach their maximum photosynthetic capacity with the whole environmental light. If plants are in the shadows, their growth rate decreases (Radosevich, 1997). The upper leaves of canopy receive the light more than utilization capacity. On the other hand, the light is prevented to the lower strata of canopy. Thus the rate of extinction coefficient increases (Floyed, *et al*, 2007). It showed that extinction coefficient decreased when LAI and the use efficiency of N-fertilizer increased. The biomass increased at N3 treatment because the light within the canopy was distributed more than another treatment and then it caused a reduction of extinction coefficient in this treatment. It announced a negative relationship between two factors of LAI and extinction coefficient. (Majd nasiri & Ahmadi, 2005). This is consistent with the results of this study.

Radiation use efficiency (RUE): RUE is the slope of the curve line of dry matter and accumulative radiation. The interaction between the measured factors showed a significant differences at the 5% level (Table 1). The results showed that the highest and the lowest RUE (2.2111 and 1.9269 g MJ m^2, respectively) was obtained by N3*K1Zn1 and N1K1 treatments, respectively.

One of the objectives of agricultural management is maximum utilization of solar energy by the canopy (Haverkort, 2007). The results showed that, LAI increased in N1K1Z1and this led to increase in the light absorption and then an increase was obtained in dry matter production per unit area. The positive effect of nitrogen on chlorophyll content was recorded by Pilbram *et al*., 2009. The effect of nitrogen and micronutrients on the canopy structure has been proved by changing the shoot arrangement such as leaf size, leaf orientation and aging of the lower leaves of biomass and then they caused an increase of the canopy light absorption (Weiner *et al*,2008, Bange and Sinclair, 2007). Leaves shadow limits the optical processes therefore the available carbon dioxide is decreased and electron transfer is reduced as a result of carbon dioxide limitations. and it is limited power of assimilation, in this case RUE is reduced as is evident in some treatments of this study.

Fresh pod yield: the maximum and minimum fresh pod yield was observed in N1*K1Zn1 and N3K1 treatments, respectively (Table 2). There was no significant effect of Zn on fresh pod yield in comparison to K treatment. The first condition for achieving a high yield per unit area is high dry matter production because about 90 percent of the dry weight of plants results from CO_2 assimilation during photosynthesis.

The results showed Nitrogen treatments had the lowest fresh pod yield because the number of flowers formed in the treatments of high nitrogen and without zinc or potassium application had significant decrease therefore the process of flowering and pod was reduced in these treatments. N1*K1Zn1 treatment with suitable environmental conditions lead to increase fresh pod yield by sugar production which resulted from photosynthesis and transferring them to seeds and pod, although the treated plants by N2*K1Zn1 had the suitable environmental conditions at flowering and graining stage. Therefore the produced assimilates was used for vegetative growth and thus the production of assimilation and fresh pod yield was reduced, so it was classified in to the second group. Nitrate in the pod: The results showed that the simple effects of nitrogen and use of potassium and Zn foliar spray and the interaction between the measured factors on the amount of nitrate had an impact on green bean pods and the statistical differences were significant at 1% level (Table 1). Nitrate levels were recorded from1854.4 mg.kg^{-1} to 76.4 mg.kg^{-1} at N3K1 and N1*K1Zn1 treatments, respectively (Table 2). The most obvious factor which it affected to accumulate nitrate in plants is known nitrogen fertilizers. The results of this study indicated. That the nitrate levels increased by higher use of nitrogen (N3*K1) and it significantly decreased by potassium and zinc used with nitrogen (N3*K1Zn1). Many scientists reported the effect of sources and amounts of nitrogen fertilizer and micronutrients in the reduction of nitrate accumulation in the plant (Krauss, 1999; Wei Hong. 2003. Martin & Anac. 2006). The reduction of nitrate to nitrite and ultimately hydroxyl amine is affected nitrate and nitrite reeducates enzymes when these processes are activated effectively by micronutrients such as zinc, iron, sulfur and copper. The effect of these components on decrease of nitrate accumulation in vegetations has been reported by Kheir *et al* (1999). Also the period of vegetative growth has become longer in N3K1 treatment with higher nitrogen application, and the plant has entered its reproductive phase later and thus its flowering and pod has coincided with hot season, function of enzymes was disrupted by environmental temperature and thus nitrate in the treatment above-mentioned increased significantly.

LAI: LAI indicates the amount of photosynthesis. When LAI decreases according to various factors, assimilate rate is reduced, so the amount of yield decreases and in this study it was evident that it corresponds to result of researches' Chaillou *et al* (2003).

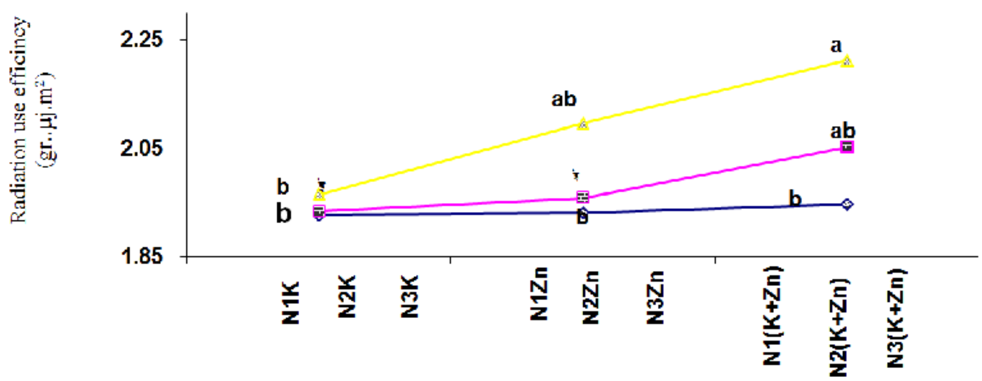

Fig. 1. Interaction effects of N, K and Zn application on Radiation use efficiency on Bean.

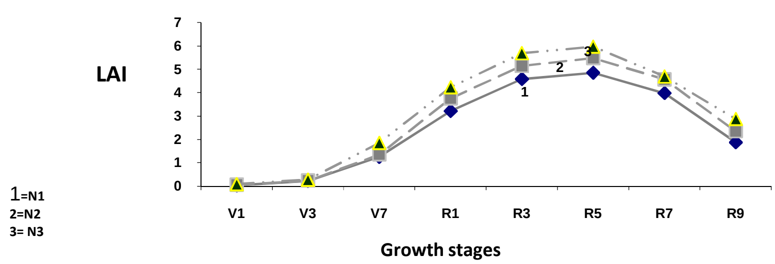

Fig. 2. Changing of LAI in different level of N.

Figure 2 showed a trend of changes in LAI at different levels of nitrogen application. The maximum LAI was at N3 treatment. The treated plant root by 350 kg $N.ha^{-1}$ could be developed better than other treatments, therefore the LAI showed an increase at this treatment. Of course, there was a slightly decrease in RUE; it might be due to the leaves shadow on lower one. Fig. 3 showed a trend of changes in LAI when the plants were treated by potassium and zinc application. The maximum LAI was found in the flowering stage therefore it caused to increase photosynthetic materials. Furthermore the leave development was stopped at flowering stage and the old leaves gradually became yellow and then LAI was decreased. In the beginning of the growth stage, LAI of green beans was approximately measured up to the third leaflet of plant (V4 of growth stage) and after that the plant were exposed to potassium and zinc treatment.

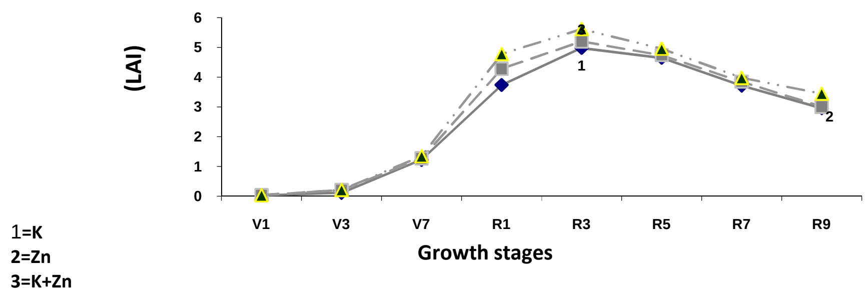

Fig. 3. Changes in LAI in treatments of potassium and zinc application.

These treatments were applied at reproductive stage of green bean plants. Therefore these treatments affected the photosynthetic materials and then it increased filling the grains in pod. It can be concluded that green bean was treated by potassium and zinc had the maximum LAI and the highest yield compared to the untreated plant by potassium and zinc

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