INTERNATİONAL JOURNAL OF AGRİCULTURE & BİOLOGY
1560–8530/2005/07–6–875–878
http://www.ijab.org

Effect of Pre-Sowing Seed Treatment with Zinc and Foliar Spray of Humic Acids on Yield of Common Bean (*Phaseolus vulgaris* L.)

MUHARREM KAYA, MEHMET ATAK†, KHALID MAHMOOD KHAWAR‡[1], CEMALETTIN Y. ÇIFTÇI‡ AND SEBAHATTIN ÖZCAN‡
Department of Field Crops, Faculty of Agriculture, Süleyman Demirel Üniversity of Çünür, Isparta, Turkey
†*Department of Field Crops, Faculty of Agriculture, Mustafa Kemal University, Hatay , Turkey*
‡*Institute of Biotechnology, Department of Field Crops, Faculty of Agriculture, University of Ankara. Diskapi, Ankara, Turkey*
[1]Corresponding author's e-mail: kmkhawar@gmail.com

ABSTRACT

The effect of pre-sowing seed treatment with zinc and foliar sprays of humic acid on yield and protein contents of common bean (*Phaseolus vulgaris* L.) were investigated under semi arid conditions of Central Anatolia. It was observed that seed pretreatment with Zn or pre treatment of seeds + foliar spray of humic acid substances at three to six leaf stage significantly increased yield and yield components in common bean. Correlation coefficients of various agronomic characteristics showed that a unit increase in common bean seed yield was positively associated with number of seeds per plant (r = 0.797), number of pods per plant (r = 0.778), seed weight per plant (r=0.771), plant height (r=0.654), harvest index (0.945) and raw protein yield (0.961).

Key Words: Zinc deficiency; Humic acid substances; Foliar sprays; Common bean

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important source of energy. It is rich in protein, dietary fibers, minerals (Ca, P, Fe, K, Mg & Mn) and vitamins (A, B1, B2 & C) with 22-37 % protein and high amino acids (Şehirali, 1988; Ciftci & Unver, 1995). It is an important rotation crop in irrigated and rain fed areas of Turkey. Several studies carried out during past few years showed that soils of Central Anatolia are highly deficient in micro nutrient element zinc (Çakmak *et al.,* 1996; Ekiz *et al.,* 1998). Deficiency of zinc not only results in restricting effects on yield, plant growth and protein contents of common bean but also affects its availability in human food resulting in various diseases due to its deficiency (Lindsey, 1972; Graham & Welch, 1994). These factors make it necessary to put essential measures on research and development of common bean, which is third important crop in terms of production and consumption among seed legumes of Turkey. Humic acid is considered to increase the permeability of plant membranes and enhance the uptake of nutrients. Moreover, it (humic acid) is also considered to improve soil nitrogen uptake and encourage the uptake of potassium, calcium, magnesium and phosphorus, making these more mobile and available to plant root system (Singer *et al.,* 1998; Piccolo *et al.,* 1997; Pascual *et al.,* 1999). Similarly, Zn is more efficiently used by plant if applied before and during leaf growth stage before the appearance of inflorescence (Brohi *et al.,* 2000). This not only results in higher yield but also effects quality of the product. Obviously, micro elements are added to macro nutrient fertilizers during the manufacture. However microelements as Fe, Zn, Mn and Cu are also added to foliar fertilizers used throughout the world as effective, preventive and curative measure to compensate their deficiency. This has special importance in arid and semi arid regions where osmotic pressure promotes the absorption and activity of these elements influenced by the behavior of plant and the timing of foliar application (Marschner, 1995; Akaraz-Lopez *et al.,* 2003; Chapagain & Wiesman, 2004). The objective of this study was to find the influence of seed treatment with Zn and foliar spray of humic acid and their combined effect on agronomic performance and protein content of popularly grown Turkish common bean cultivar Karacaşehir-90.

MATERIALS AND METHODS

The experiment was conducted on Agricultural Research Station Haymana, University of Ankara, Turkey during 1999 using common bean cultivar Karacaşehir-90 in randomized complete block design with three replications and four treatments (unsprayed control, pre sowing treatment with liquid Zn, humic acid foliar spray and pre sowing seed treatment with liquid Zn + humic acid foliar spray). Plots were prepared using standard agronomic practices and each of the plots had eight 20 m long rows with row to row distance of 0.60 m. Sowing was done with machines. Care was taken to add phosphorus fertilizer (4 kg da^{-1}) before sowing. Commercial herbicide, Raptor (Cynamid) containing imazomux was sprayed at the rate of 300 ml ha-^{1} when the weeds were at 2-3 leaf stage. Commercial fertilizers, Teprosyn containing Zn at the rate of 600 g L^{-1} for seed pretreatment and Poliverim Extra containing polymetric polyhydoxy acids, humic acid, fulvic acid and micronutrients - Fe, Zn, K, Mn, Cu, hereafter called humic acid substances (HA) at the rate of 2000 ml ha^{-1} (PHOSYN, Pocklington, U.K) for foliar spray were used during the study. Soil moisture was kept at adequate levels to prevent water deficit and wilting. Foliar spray was done at third to sixth leaf stage in the evening, when the relative humidity was high; as day temperature exceeds 30 ^{o}C during the month of May and plants transpire rather than absorbing the nutrients. To target the stomatas, nozzle on the boom spray was tipped back about 90 degrees to encourage the spray to roll under the leaves. Phosphoric acid was used at the rate of 1 L per 200 L of solution ha^{-1}. The spray meter was adjusted to conductivity of 2000 at the time of spray to achieve maximum response.

Plant height (cm), number of pods per plant, number of seeds per plant and seed weight per plant (g) were determined from randomly selected ten plants from inter rows of each plot. 100 seed weight (g) and seed yield were determined from inter two rows of each plot by harvesting, weighing and counting. Then, seed yield were converted to the kg/ha. Harvest index (Şehirali, 1988) was determined using the formula

$$\text{Harvest index (\%)} = \frac{\text{Seed weight of selected 10 plants from each plot}}{\text{Plants weight of ten plants from each plot}} \times 100$$

Crude or raw protein was determined by Kjeldahl method (Markham, 1942). Row protein yield (kg/ha) was determined from seed yield (kg/ha) multiplied by the percentage (%) of raw protein.

All data were subjected to analysis of variance (ANOVA) and the differences between the means were compared by Duncan's multiple range test using MSTAT-C computer program (Freed & Scott, 1989). Moreover, correlation coefficient of all the variables was determined separately.

RESULTS AND DISCUSSION

Soil analysis report of the experimental area indicated extreme deficiency of Zn with range of 0.28 to 0.6 mg Zn kg^{-1} of soil (Annual report, Faculty of Agriculture, University of Ankara, 1998) which is lower than the appropriate amount required for satisfactory crop production. It appears that absorption efficiency of the humic acid substances (HA) through young leaves via stomata was higher, and no accumulation on the surface of the leaf was observed and thus no scorching and burning of the leaf noted.

Plant height was significantly enhanced by all treatments over control (Table I). Maximum plant height (77.86 cm) was recorded where pre-sowing seed treatment with zinc was accompanied with foliar application of HA. However, plant height did not vary significantly when both of these treatments were applied alone. Pre-sowing seed treatment with zinc and foliar application of HA when used alone did not influence number of pods per plant over control. However when these were used together, the same was increased significantly over control. Similar trend was observed for number of seeds per plant and seed weight per plant (Table I). None of the treatments in this experiment had any significant effect on 100 seed weight of common bean. Pre-sowing seed treatment with zinc did not influence seed yield as compared with control (Table I). HA foliar spray or pre sowing seed treatment with liquid Zn+ HA foliar spray gave statistically similar but the highest yield of 2250 kg/ha and 2398 kg/ha respectively; which was statistically different and higher than the control. Harvest index revealed a similar trend as was observed for seed yield. Singer *et al.* (1998) found that application of Delta mix (a fertilizer containing humic acid substances with micronutrients B, Zn, S, Mn, Fe & Cu) enhanced the growth with pod quality of common bean cv. Giza-3 under calcareous soil conditions. Sharif *et al.* (2002) found that addition of 0.5-1.0 kg ha^{-1} humic acid resulted in increased wheat grains yield by 25-69% over control. They further suggested that addition of humic acid with half dose of NPK produced significant and economical wheat yield with maximum nutrient accumulation and increased crop productivity by increased nutrient uptake. However, HA was better when applied singly. Similarly Khan and Mir (2002) emphasize significant effect of lignite humic acid on yield and yield components of wheat using different phosphate fertilizers.

The activation of many processes accompanied emergence of primary root and the emergence of shoot. Metabolic changes due to permeability of humic acid into leaf cells and effect of Zn pre treatment to embryonic cells might be the causative agent of variations in results and improved effects as are clearly demonstrated in the yield and yield components except protein which showed reduction in quality. The stimulating effect of humic acid and Zn pretreatment has been related in part to enhanced uptake of mineral nutrients. Many authors (Rauthan & Schnitzer, 1981; Chen & Aviad, 1990; Fagbenro & Agboole, 1993) have reported increased uptake of macro and microelements influenced by humic acid substances. When applying humic acid substances to young plants some of the humic acid also got sprayed on to the soil. It is assumed that, this also created a synergetic effect during uptake of nutrients by plants from soil (Lee & Bartlett, 1976; Vaughan & Malcom, 1985; David *et al*., 1994) suggesting existence of synergetic effect of combined applications of mineral nutrients and humic acid substances. Another explanation could be the effect of humic acid

Table I. Effect of pre sowing seed treatment with Zn pretreatment and foliar spray of humic acid on yield a bean under semi arid conditions of Central Anatolia

Treatments	Plant height (cm)[1]	Number of pods per plant	Number of seeds per plant	Seed weight per plant (g)	100 seed weight (g)	Seed yield (kg/ha)	Harvest index (%)	Raw protein contents (%)	Raw protein yield (kg/ha)
Unsprayed control	53.99 c	20.93 b	89.60 b	16.90 b	18.97 ns	1609.3 b	38.91 b	30.40 a	489.1 b
Pre sowing seed treatment with liquid Zn	68.63 b	22.73 b	95.07 b	17.39 b	18.22	1659.2 b	40.72 b	27.04 bc	448.5 b
HA foliar spray	64.96 b	23.93 b	99.20 b	18.41 b	18.60	2250.0 a	49.78 a	27.25 b	613.1 a
Pre sowing seed treatment with liquid Zn+HA foliar spray	75.86 a	27.00 a	114.80 a	21.76 a	18.95	2398.1 a	48.83 a	25.82 c	619.2 a
LSD value at 0.01	9.07	4.48	11.93	2.33	-	728	5.54	1.34	115

Values within a column followed by different letters are significantly different at 0.01 level of significance; ns= non significant

Table II. Correlation coefficients of various agronomic characteristics of common bean (*Phaseolus vulgaris* L.) expressed under semi arid conditions of Central Anatolia

	Plant height	Number of pods per plant	Number of seeds per plant	Seed weight/ plant (g)	100 seed weight (g)	Seed yield (kg/ha)	Harvest index (%)	Percentage (%) of raw protein	Raw protein yield (kg/ha)
Plant height	1.000								
Number of pods per plant	0.816**	1.000							
Number of seeds per plant	0.804**	0.824**	1.000						
Seed weight/plant	0.746**	0.841**	0.882**	1.000					
100 seed weight	-0.074 ns	0.319 ns	0.122	0.241 ns	1.000				
Seed yield	0.654*	0.778**	0.797*	0.771**	0.057 ns	1.000			
Harvest index	0.625*	0.748**	0.731**	0.676*	0.084 ns	0.945**	1.000		
Percentage of raw protein	-0.936**	-0.791**	0.791**	-0.665*	0.207 ns	-0.708**	-0.680*	1.000	
Raw protein yield	0.438 ns	0.641*	0.641*	0.673*	0.132 ns	0.961**	0.912**	-0.490 ns	1.000

**Significant difference at the 0.01 level ; *Significant difference at the 0.05 level ; ns non significant

substances in manner similar to plant growth substances (O'Donnel 1973; Casenave de Sanfilippo *et al.*, 1990).

Results for percentage of raw protein and raw protein per unit area were not consistent. Highest percentage of raw protein (30.40%) was obtained from unsprayed control and the combined treatments appeared to have negative effect on raw protein yield. Any treatment to seeds or plants resulted in reduction of the protein percentage or availability of raw protein. However, maximum raw protein per unit area (619.2 kg/ha) was achieved from combined treatments.

Correlation coefficients (Table II) showed that the plant height had positive and significant correlation with number of pods per plant (r=0.816), number of seeds per plant (r=0.804), seed weight (r=0.746), seed yield per unit area (r=0.654), harvest index (r=0.625); whereas, raw protein per unit area (r=0.438) had statistically non significant correlation with plant height. Correlation of plant height with 100 seed weight was negative and non significant (r=-0.074) and percentage of raw protein (r= -0.936) was negative and significant.

Number of pods per plant showed positive and significant correlation with number of seed per plant (r=0.824), seed weight (r=0.841), seed yield per unit area (r=0.778), harvest index (r=0.748) and protein yield per unit area (r=0.641). Correlation between number of pods per plant and 100 seed weight (r= 0.319) was positive and non significant. However, a negative and significant correlation (r=-0.791) was found between number of pods per plant and raw protein per unit area. Similarly, seed yield per unit area had positive significant correlation with plant height (0.654), number of pods per plant (0.778), number of seeds per plant (0.797), seed weight per plant (0.771), harvest index (0.945), and raw protein yield (0.961). Seed yield per unit area was positively correlated with 100 seed weight (0.057). Percentage of raw protein had negative and significant correlation with seed yield (-0.708). It may concluded that correlation coefficient values of coomon bean should be determined for observing the degree of relationship of traits with yield to ensure that these parameters significantly contribute or not and positive contributing traits should be treated to contain high yield.

REFERENCES

Akaraz–Lopez, C., B. Maria, C.F. Akakaz and F. Riquelme, 2003. Effects of foliar sprays containing calcium magnesium and titanium on plum (*Prunus domestica* L.) fruit quality. *J Pl. Physiol.,* 160: 1441–6.

Annual Report of Faculty of Agriculture, 1998. University of Ankara, 06110, Diskapi, Ankara, Turkey. (In Turkish).

Brohi, A.R., H. Karaata, S. Özcan and M. Demir, 2000. Topraktan ve yapraktan çinko uygulamasının ekmeklik buğday bitkisinin verim ve bazı besin maddesi alımına etkisi. (Factors effecting yield and nutrition of Zinc fertilization through soil and foliar sprays). *J. Fac. Agric.,* Univ of GOP, Tokat, Turkey, 17: 123–8

Çakmak, I., A. Yilmaz, M. Kalayci, H. Ekiz, B. Turun, B. Erenoğlu and H.J. Braun, 1996. Zinc deficiency as a critical problem in wheat production in Central Anatolia. *Pl. Soil*, 180: 165–72

Casenave de, Sanfillipo, E., J.A. Arguello, G. Abdala and G.A. Orioli, 1990. Content of auxin–inhibitor and gibberellin like substances in humic acids. *Biol. Pl.*, 32: 346–51

Chapagain, B.P. and Z. Wiesman, 2004. Effect of Nutri–Vant–PeaK foliar spray on plant development, yield, and fruit quality in greenhouse tomatoes. *Scientia Horticulturae,* 102: 177–88

Chen, Y. and T. Aviad, 1990. Effect of humic substances on plant growth. *In* : Humic substances in soil and crop science. *American Soc. of Agron. and Soil Sci. Soc. of America*., Madison, pp. 161–86

Ciftci, C.Y. and S. Unver, 1995. Yemeklik tane baklagillerin tarımımızdaki önemi (Importance of seed legumes in Agriculture). Karınca Kooperatif Postası Dergisi *Karinca J. Cooperative Posts*, 7035: 49–52

David, P.P., P.V. Nelson and D.C. Sanders, 1994. A humic acid improves growth of tomato seedling in solution culture. *J. Pl. Nutr*., 17:173–84

Ekiz, H., S.A. Bağcı, A.S. Kıral, S. Eker, I. Gültekin, A. Aklan and I. Cakmak, 1998. Effects of zinc fertilization and irrigation on grain yield and zinc concentration of various cereals grown in zinc–deficient calcareous soils. *J. Pl. Nutr*., 21: 2245–56

Fagbenro, J.A. and A.A. Agboole, 1993. Effect of different levels of humic acid on the growth and nutrition uptake of teak seedlings. *J. Pl. Nutr*., 17: 173–84

Freed, R.D. and E. Scott, 1989. *MSTAT–C; A Software Package for the Design, Management, and Analysis of Agronomic Experiments.* Michigan State University, USA.

Graham, R.D. and R.M. Welch, 1996. Breeding for stable–food crops with high micronutrient density. Working Papers on Agricultural Strategies for Micronutrients, No. 3. *Int. Food Policy Inst.*, Washington, DC.

Khan, A.R. and S. Mir, 2002. Plant growth stimulation of lignite humic acid part II. Effect of lignite derived ammonium humate on wheat (*Triticum aestivum* V) crop using different levels of phosphate fertilizer. *Pakistan J. Sci Indust. Res*., 45: 273–6

Lee, Y.S. and R.J. Bartlett, 1976. Stimulation of plant growth by humic substances. *Soil Sci. Soc. Am. J.*, 40: 876–9

Lindsey, W.L., 1972. Inorganic phase equilibria of micronutrients in soils. Micronutrients in Agriculture, *Soil Sci. Soc. America.* pp. 41–57. Inc. Madison USA

Markham, R, 1942. A steam distillation apparatus suitable for micro–Kjeldahi analysis. *Biochem. J*., 36: 170

Marschner, H., 1995. *Mineral Nutrition of Higher Plants.* 2nd Ed. Academic Press. San Diego.

O'Donnel, R.W., 1973. The auxin like effects of humic preparations from leonardite. *Soil Sci*., 116: 106–12

Pascual, J.A., C. Garcia and T, Hernandez, 1999. Comparison of fresh and composted organic waste in their efficacy for the improvement of arid soil quality. *Bioresources Technol.*, 68: 255–64

Piccolo, A., G. Pietramellara and J.S.C. Mbagwu, 1997. Reduction in soil loss from erosion–susceptible soils amended with humic substances from oxidized coal. *Soil Technol*., 10: 235–45

Rauthan, B.S. and M. Schnitzer, 1981. Effect of a soil fulvic acid on the growth and nutrient content of cucumber (*Cucumis sativus*) plants. *Pl. Soil,* 63: 491–5

Şehirali, S., 1988. *Yemeklik tane baklagiller Ders Kitabı. (Seed Legumes, Lecture Notes),* Publication No. 1089 pp. 314–35 Faculty of Agriculture, University of Ankara, Turkey (In Turkish).

Sharif, M., R.A. Khattak and M.S. Sarir, 2002. Wheat and nutrients accumulation as affected by humic acid and chemical fertilizers. *Sarhad J. Agric.,* 18: 323–9

Singer, S.M., O.M. Sawan, M.M. AbdelMouty and S.R. Salman, 1998. Study of the effects of the Delta mix™ and organic matter on growth and productivity of bean plants grown under calcareous soil conditions. *Egyptian J of Hortic.,* 25: 335–47

Vaughan, D. and R.E. Malcom, 1985. Influence of humic substances on growth and physiological processes. *In:* Vaughan, D. and R.E. Malcom (eds.), *Soil Organic Matter and Biological Activity.* pp. 37–75. Martinus, Nijhoff/Dr W Junk Publ. Dodrecht

(Received 20 April 2005; Accepted 03 October 2005)