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EFFECT OF FOLIAR SPRAY WITH B -VITAMINS (B_1, B_6 AND B_{12}) ON GROWTH AND SEED YIELD OF COWPEA PLANTS

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ABSTRACT: Two field as well as pots experiments were carried out during two consecutive summer growing seasons of 1996 and 1997 at private Experimental Farm, Diarb Negm, Sharkia Governorate, to study the effect of spray with B- vitamins (B_1, B_6, B_{12}) and their combinations at two levels; i.e., 50 and 100 ppm, each on growth and seed yield of cowpea plants.

Foliar spray of cowpea plants with vitamin B_1, B_6 and B_{12} as single or in combinations at 50 and 100 ppm, each, of them, except Vit. B_{12} at 100 ppm singly, significantly enhanced root and nodules characters; i.e., total root length, number of branches/root, both nodules numbers and weight/plant and nitrogen fixation activity expressed as acetylene reduction.

Application of B-vitamins (B_1, B_6 and B_{12}) at the abovementioned concentrations to cowpea plants significantly increased dry weight of different plant organs, leaf pigments (chlorophyll a, b and carotenoids), number of pods/plant and dry seed yield per plant as well as per feddan compared to check.

Key words: Cowpea, B-vitamins (B_1, B_6 and B_{12}), dry seed yield, nitrogen fixation, nodulation.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) walp.) is a summer legume vegetable crop which is widely used as a source of protein and for its high nutritive value for human nutrition in Egypt.

Vitamins can be supplied to plant as seed soaking or as foliar spray to improve their growth and productivity. For legumes, these vitamins could be add practical significance as growth stimulation of roots. So, they would not only result in higher water and nutrients

uptake but also provide larger surface area for *Rhizobium* infection leading to increase in root nodules formation. These effects would be ultimately manifested in better growth and higher productivity coupled with considerable fertilizers economy (Samihullah and Afridi, 1988). The stimulative effect of Vit. B_1 on dry weight of pea plants is quite expected, due to its role in activation of plant metabolism, which in turn positively reflected on plant growth and dry matter accumulation.

Various responses of legume crops to application of vitamin B_1 were reported by Alsadon *et al.* (1995) on yield and its components of cowpea, El-Beheidi *et al.* (1995) on leaf chlorophyll content of pea, and Arisha (2000) on growth, leaf pigments content as well as yield and its components of pea.

Foliar spray of cowpea with Vit. B_1 at 50 ppm significantly increased leaves and branches dry weight / plant , number of pods/ plant and seed yield per feddan, whereas it did not significantly affect number of seeds/ pod and 100 dry seed weight, (El-Mansi *et al.*, 1994). All B group vitamins may be related to chlorophyll synthesis (Gopala Rao and Sastry, 1972).

Application of Vit. B_6 enhanced plant height, leaf number, fresh and dry weight of pea and broad bean (Barbieri, 1959). Also, Samihullah and Afridi (1988) mentioned that pre-sowing seed treatment of moong bean with Vit. B_6 (pyridoxine) increased pod number/plant, seed number/pod, 1000 seed weight, seed yield/ hectar.

Regarding the effect of vitamins B_{12} on vegetable crops, Filimonov (1967) found that soaking field bean in Vit. B_{12} solution before sowing enhanced seed yield. Also, soaking common bean seeds in Vit. B_{12} solution at 2.5 or 5 ppm significantly increased stem length, both number of leaves and branches/ plant, dry weight of different plant organs, number of pods/ plant, yield/ plant as well as per feddan (Abd El-Fattah and Arisha, 2000).

Spraying common bean plants with vitamin Bs (B_1+B_6+B_{12}) at 50 ppm, each of them, improved the growth characters, dry weight, chlorophyll content, both the number and weight of pods/ plant over those of the control (Fathy and Farid 1996).

Therefore, the objective of this work was to study the effect of foliar spray of cowpea plants with some B-vitamins (B_1, B_6, B_{12}) as

single or in combination on growth, leaf pigments, seed yield and its components and nitrogen fixation.

MATERIALS AND METHODS

Two field as well as pots experiments were carried out during two consecutive summer growing seasons of 1996 and 1997 at private Experimental Farm, Diarb Negm, Sharkia Governorate, to study the effect of some B-vitamins (B_1, B_6, B_{12}) and their combinations on growth and yield of cowpea plants.

The soil used was clay in texture with pH 7.51 and 7.58 and EC. 2.08 and 2.13 in the first and second seasons, respectively.

In both field and pots experiments the treatments were nine as follows:

Untreated (control), Vit. B_1 at 50 ppm, Vit. B_1 at 100 ppm, Vit. B_6 at 50 ppm, Vit. B_6 at 100 ppm, Vit. B_{12} at 50 ppm, Vit. B_{12} at 100 ppm, Vits. $B_1+B_6+B_{12}$ at 50 ppm, each and Vits. $B_1+B_6+B_{12}$ at 100 ppm, each. These treatments were randomly arranged in a randomized complete block design with three replications.

Plot area was 10.8 m^2. It contains six rows of 3 m long and 0.6 m wide. Two rows (3.6 m^2) were used, to take the samples, for evaluating vegetative growth parameters and the other four rows (7.2 m^2) were used for yield determination. In addition, one row was left between every two experimental units as a guard row.

Cowpea *cv* Cream 7 was used. The seeds were sown on April 20th in the two growing seasons. Before sowing, seeds were successively washed and inoculated with root nodule bacteria (*Bradyrhizobium sp)* with a dose of 5 gm/ kg seeds. The adhesive agent used was Arabic gum 20% concentration. The inoculated seeds were left in a shaded place for one hour before sowing for air-drying in all experiments.

In field experiments, the seeds were spaced in hills at 30 cm apart on one side of the row. After emergency, seedlings were thinned leaving two seedlings per hill.

Cowpea plants were sprayed two times, 25 and 35 days after sowing. Each experimental unit received two liters solutions from each concentration using spreading agent, while the control was sprayed with tap water and spreading agent (Super film one ml / liter).

All experimental units received 50 kg ammonium

sulphate (20.5%N),150 kg calcium superphosphate (15.5% P_2O_5) and 50 kg potassium sulphate (48-52% K_2O) per feddan. One third of these fertilizers was added at soil preparation. The other two thirds were added after 20 and 50 days from complete emergence.

In pots experiment, two seeds/ pot (30 cm diameter filled with clay and sand at 1 : 1 ratio V/V) were sown and thinned to leave one plant/pot. The normal agricultural treatments (irrigation, fertilization, and pest control), applied in the field experiment, were also applied in the pot experiment according to pot/size using four pots each treatment.

Data recorded

A. Pots Experiment

I. Root system parameters

One plant from each pot 50 days from sowing was carefully separated by washing with tap water for measuring the root parameters; i.e., number and fresh weight of nodules/ root, root dry weight and both total root length and number of branches/ root according to Helal and Sauerbeck (1986).

II. Nitrogenase activity

Nitrogenase activity as an indicator for N_2 fixation activity in root nodules was assayed after 55 days from sowing according to Hardy *et al.* (1973) using gas chromatography system.

B. Field Experiment

1. Plant growth

A random sample of five plants from each plot were randomly taken 55 days after sowing and different plant parts; i.e., roots, stems and leaves were dried at 70°C till constant weight. Dry weight of branches, leaves and whole plant were recorded.

2. Leaf pigments

Ten discs samples from the fourth upper leaf of the plant tip from every experimental unit were randomly taken, after 55days from sowing, to determine both chlorophyll a and b as well as carotenoids, according to the method described by Wettestein (1957).

3. Yield and its components

Dry pods of each plot were harvested at maturity stage, counted and weighed in each harvest and the following parameters were determined: pod number/ plant, seed number/pod, weight of 100 seeds (gm), and total dry seeds/ plot which converted to per feddan and calculated to per plant.

Statistical Analysis: Otained data were subjected to statistical analysis of variance according to Snedecor and Cochran (1980), and means separation was done according to Duncan (1958).

RESULTS AND DISCUSSION

Root and Nodule Characteristics

Data in Tables 1 and 2 show that spraying cowpea plants with some B- vitamins (B_1, B_6, B_{12}) and their combinations at 50 or 100 ppm, each of them, significantly increased root and nodules characteristics; i.e., total root length, number of branches/ root, root dry weight, number and fresh weight of nodules / plant as well as nitrogen fixation activity, expressed as acetylene reduction, except Vit. B_{12} at 100 ppm.

It is, also, clear from the same data that all studied B- vitamins as single treatments alone or in combinations recorded the uppermost values of root and nodules characters with no significant differences among them with one exception which was Vit.B_{12} at 100 ppm.

On the other hand, foliar application of Vit. B_{12} at 100 ppm did not significantly differ from control treatment, in all studied root and nodule characters, except number of branches / root.

The favorable effect of vitamin B with the suitable concentration might be attributed to the stimultave effect of B-vitamins on root growth and this in turn provide larger root length and root surface area for *Rhizobium* infection leading to increase in root nodules formation and consequently higher water and nutrients uptake, which increased growth and yield of cowpea.

Obtained results are in a good line with those reported by Oertli (1987) and Abd El-Fattah and Arisha (2000) on pea.

Moreover, data in Tables 1 and 2 indicate that application of used B- vitamins (B_1, B_6, B_{12}) at 50 or 100 ppm (except, Vit. B_{12} at 100 ppm as single) gave the same values of root and nodules characters. This means that foliar application of Vit. B_1, B_6 and B_{12} as single were economic treatments for cowpea plants

Plant Growth

Data presented in Tables 3 and 4 indicate that foliar spray of cowpea with vitamin B_1, B_6, B_{12} each alone or in combined together

Table 1 : Effect of foliar spray with vitamin B_1, B_6 and B_{12} and their combination on root and nodule characteristics and nitrogenase activity of cowpea plant in 1996 season

Treatments	Total root length (cm)	No./root		Noudles fresh weight /plant (gm)	Root Dry weight /plant(gm)	Nitrogenase µM /gm D.W. nodule/ h.
		Branches	Nodules			
Untreated (control)	151.3	31.3	15.3	0.164	1.39	63
Vit. B_1 at 50 ppm	256.1	46.0	26.6	0.293	2.06	116
Vit. B_1 at 100 ppm	284.4	39.1	27.1	0.356	2.34	118
Vit. B_6 at 50 ppm	236.0	40.2	23.0	0.341	1.88	113
Vit. B_6 at 100 ppm	243.1	42.7	19.5	0.286	1.96	108
Vit. B_{12} at 50 ppm	254.5	47.0	23.4	0.294	2.38	112
Vit. B_{12} at 100 ppm	181.3	48.5	19.8	0.228	1.68	91
Vit. B_1+B_6+B_{12} at 50 ppm	232.6	50.6	21.2	0.253	1.88	110
Vit. B_1+B_6+B_{12} at 100 ppm	230.7	41.3	19.8	0.251	1.86	108
LSD at 0.05 level	71.1	5.0	4.2	0.073	0.43	---

Vit. : vitamin , Vits. : vitamins

Table 2 : Effect of foliar spray with vitamin B_1, B_6 and B_{12} and their combination on root and nodule characteristics of cowpea plant in 1997 season

Treatments	Total root length (cm)	No. / root		Dry weight (gm)	
		Branches	Nodules	Nodules	Root
Untreated (control)	194.3	31.6	13.6	0.203	1.21
Vit. B_1 at 50 ppm	284.2	41.1	28.1	0.312	1.79
Vit. B_1 at 100 ppm	276.9	37.0	24.0	0.281	1.89
Vit. B_6 at 50 ppm	250.4	40.8	21.3	0.302	1.76
Vit. B_6 at 100 ppm	268.1	43.5	26.3	0.320	1.65
Vit. B_{12} at 50 ppm	295.5	36.3	31.4	0.254	1.84
Vit. B_{12} at 100 ppm	239.7	51.7	19.2	0.233	1.32
Vits. B_1+B_6+B_{12} at 50 ppm	268.0	57.2	22.8	0306	1.77
Vits. B_1+B_6+B_{12} at 100 ppm	311.1	51.6	19.9	0.289	1.78
LSD at 0.05 level	68.7	5.3	5.2	0.046	0.36

Vit. : vitamin , Vits. : vitamins

Table 3 : Effect of foliar spray with vitamin B_1, B_6 and B_{12} and their combination on the dry weight of cowpea plant in 1996 season

Treatments	Dry weight (gm/plant)		
	Leaves	Shoots	Total
		1996 season	
Untreated (control)	19.86	8.80	28.66
Vit. B_1 at 50 ppm	23.92	11.73	35.65
Vit. B_1 at 100 ppm	25.77	9.59	35.36
Vit. B_6 at 50 ppm	26.95	10.86	37.82
Vit. B_6 at 100 ppm	25.87	10.52	36.39
Vit. B_{12} at 50 ppm	26.35	10.12	36.47
Vit. B_{12} at 100 ppm	23.74	9.77	33.52
Vits. B_1+B_6+B_{12} at 50 ppm	27.68	10.02	37.71
Vits. B_1+B_6+B_{12} at 100 ppm	26.29	10.68	34.98
LSD at 0.05 level	4.14	1.67	5.43

Vit. : vitamin , Vits. : vitamins

Table 4 : Effect of foliar spray with vitamin B_1, B_6 and B_{12} and their combination on the dry weight of cowpea plant in 1997 season

Treatments	Dry weight (gm/plant)		
	Leaves	Shoots	Total
Untreated (control)	22.47	6.59	28.07
Vit. B_1 at 50 ppm	26.41	8.30	34.71
Vit. B_1 at 100 ppm	26.16	8.27	34.44
Vit. B_6 at 50 ppm	26.73	8.45	35.18
Vit. B_6 at 100 ppm	25.89	8.55	34.44
Vit. B_{12} at 50 ppm	26.60	10.05	36.66
Vit. B_{12} at 100 ppm	25.63	7.65	33.28
Vits. B_1+B_6+B_{12} at 50 ppm	27.22	8.42	35.64
Vits. B_1+B_6+B_{12} at 100 ppm	26.13	8.15	35.29
LSD at 0.05 level	3.90	1.87	5.35

Vit. : vitamin , Vits. : vitamins

at different concentrations significantly increased dry weight of leaves, branches and total dry weight / plant in comparison with unsprayed plants in the two seasons, except Vit. B_{12} at 100 ppm. It is also clear from the same data that cowpea leaves, branches and total dry weight/plant significantly increased by application of B- vitamins; i.e., B_1, B_6 and B_{12} concentrations up to 100, 100 and 50 ppm, respectively with no significant differences between the two tested concentrations either added alone singly or in combinations in this respect.

The stimulative effect of Vit B_1 on dry weight of cowpea plants is quite expected, due to its role in activation of plant metabolism, which in turn reflected positively on plant growth and dry matter accumulation. These results are in agreement with those reported by Gopala and Kodandaramaiah (1982) on green gram, Samihullah and Afridi (1988) on pea, El-Mansi *et al.* (1994) on cowpea, Fathy and Farid (1996) on common bean and Arisha (2000) on pea.

The stimulative effect of Vit B_6 on dry weight of plants was reported by Barbieri (1959) who found that application of Vit. B_6 enhanced fresh and dry weight of pea and broad bean plants. With respect to Vit. B_{12} application, Abd El-Fattah and Arisha (2000) found that soaking pea seeds in vitamin B_{12} solution significantly increased dry weight of different plant organs. In addition, Fathy and Farid (1996) reported that spraying common bean plants with vitamin Bs (B_1+B_6+B_{12}) at 50 ppm, each of them, improved the dry weight of common bean plants.

Leaf Pigments

Data presented in Table 5 cleared that spraying cowpea plants with different B- vitamins; i.e., B_1, B_6, B_{12} and their combination (combined together) significantly increased chlorophyll a, b and total chlorophyll (a+ b) as well as carotenoids in cowpea leaves tissues in comparison with untreated plants. The best vitamin treatments for increasing the photosynthetic pigments in the leaf tissues of cowpea plants were application of Vit. B_1+B_6 +B_{12} each at 50 or at 100 ppm, each of them, with no significant differences between them in the two seasons of study.

B-vitamins (B_1, B_6 and B_{12}) enhanced the formation of

Table 5: Effect of foliar spray with vitamin B_1, B_6 and B_{12} and their combination on the leaf pigments of cowpea plant in 1996 season

Treatments	Leaf pigments (mg/gm dry weight)			
	Chlorophyll			Carotenoids
	a	b	(a+b)	
Untreated (control)	1.18	1.13	2.31	2.98
Vit. B_1 at 50 ppm	1.91	1.48	3.39	3.50
Vit. B_1 at 100 ppm	1.70	1.72	3.42	3.54
Vit. B_6 at 50 ppm	1.93	1.57	3.50	3.70
Vit. B_6 at 100 ppm	1.87	1.50	3.37	3.57
Vit. B_{12} at 50 ppm	1.89	1.64	3.53	3.76
Vit. B_{12} at 100 ppm	1.91	1.75	3.66	3.74
Vits. B_1+B_6+B_{12} at 50 ppm	2.02	1.77	3.79	3.51
Vits. B_1+B_6+B_{12} at 100 ppm	1.87	1.83	3.70	3.70
LSD at 0.05 level	0.45	0.31	0.76	0.51

Vit. : vitamin , Vits. : vitamins

chlorophylls and delayed the degradation and senescence of chlorophyll of leaves. This favorable effect of B- vitamins on the formation of chlorophyll might be due to their important roles in the synthesis of succinyl-Co A and glycin amino acid and for the reaction of them to form α – aminolevulinic acid (Strove ,1989) the main intermediate compound for formation of protoporphyrin the precursor of chlorophyll (Hess, 1981). Obtained results are in agreement with those reported by El-Beheidi *et al.* (1995) and Arisha (2000) on pea.

All the B group vitamins may be related to chlorophyll synthesis in groundnut (Gopala Rao and Sastry 1972). B_{12} noted stimulate development of chloroplast (Easley, 1969).

Seed Yield and its Components

Results in Tables 6 and 7 indicate that spraying cowpea plants with vitamin B_1, B_6, B_{12} either singly or combined together at 50 or 100 ppm, each of them, had significant effect on number of pods/plant, 100-seed weight, number of seeds / pod, seed yield / plant and total seed yield / feddan, except number of seeds per pod, in the first season, and 100 dry seed weight in the second season. These results are true in the two growing seasons.

It is noticed also that foliar application of vitamin B_1, B_6, B_{12} each at 50 or at 100 ppm each singly or in combination stimulated the abovementioned seed yield and its components, except Vit. B_{12} at 100 ppm which did not show any stimulative effect on dry seed yield and its components.

The favorable effect of thiamine (Vit. B_1) application on yield was reported by Gutmanis (1976) on pea and Oertli (1987) on *Phaseolus mungo,* Alsadon *et al.* (1995) on cowpea and Similar conclusion was reported with Vit. B_{12} on seed yield (Filimonov (1967) on field beans and Kulieva *et al.* (1976), and Abdel Fattah and Arisha (2000) on pea. In addition, Samihullah and Afridi (1988) found that treating summer moong bean *cvs* by Vit. B_6 increased total seed yield, 1000- seed weight, seed number/pod and pod number/ plant.

The superiority of total seed yield/ feddan and yield components after spraying cowpea plants with Vit. B showed similar trend to that of plant growth. Therefore, obtained results might be attributed to the increase in root

Table 6 : Effect of foliar spray with vitamin B_1, B_6 and B_{12} and their combination on yield and its components of cowpea plant in 1996 season

Treatments	Yield and its components				
	Pods No/ plant	Seed No./ pod	Weight of 100 seeds (gm)	Seed yield / plant (gm)	Dry seed yield / (Kg/ *fed*)
Untreated (control)	18.15	12.20	13.41	25.40	918.55
Vit. B_1 at 50 ppm	22.11	13.30	14.05	31.23	1147.99
Vit. B_1 at 100 ppm	20.70	12.53	13.85	31.36	1088.33
Vit. B_6 at 50 ppm	20.00	12.55	13.38	32.10	1123.88
Vit. B_6 at 100 ppm	21.12	11.52	13.14	31.50	1139.44
Vit. B_{12} at 50 ppm	20.63	13.03	13.51	29.28	1076.05
Vit. B_{12} at 100 ppm	18.58	12.75	13.93	26.74	982.72
Vits. B_1+B_6+B_{12} at 50 ppm	20.91	12.58	14.00	28.54	1036.66
Vits. B_1+B_6+B_{12} at 100 ppm	20.86	12.30	13.93	28.46	1027.22
LSD at 0.05 level	1.77	NS	0.50	2.67	106.32

Vit. : vitamin , Vits. : vitamins

Table 7 : Effect of foliar spray with vitamin B_1, B_6 and B_{12} and their combination on yield and its components of cowpea plant in 1997 season

Treatments	Yield and its components				
	Pods No/ plant	Seed No./ pod	Weight of 100 seeds (gm)	Seed yield / plant (gm)	Dry seed yield/ (Kg/ *fed*)
Untreated (control)	16.77	11.62	15.22	23.61	867.84
Vit. B_1 at 50 ppm	20.62	11.77	15.24	31.16	1108.95
Vit. B_1 at 100 ppm	21.28	11.55	15.30	35.40	1240.91
Vit. B_6 at 50 ppm	20.51	11.20	14.32	29.30	1059.63
Vit. B_6 at 100 ppm	21.14	12.08	14.56	34.07	1192.56
Vit. B_{12} at 50 ppm	19.38	11.07	15.06	27.74	997.73
Vit. B_{12} at 100 ppm	17.44	10.83	15.67	21.40	761.94
Vits. B_1+B_6+B_{12} at 50 ppm	19.80	11.33	14.38	30.22	1028.39
Vits. B_1+B_6+B_{12} at 100 ppm	19.26	11.52	13.84	30.21	1075.25
LSD at 0.05 level	2.12	1.09	NS	2.77	128.45

Vit. Vitamin , vits : vitamin

surface area/plant, total root length and this in turn increase the amount of mineral and water absorption by roots and increasing plant growth and seed yield.

Generally, it could be conclude that application of vitamin B_1 and B_6 each alone at 50 or 100 ppm and Vit. B_{12} at 50 ppm singly as foliar application significantly gave the uppermost values of cowpea dry weight and seed yield. Also, application of Vit. $B_1+B_6+B_{12}$ in combinations each at 50 or at 100 ppm each recorded the same trend of that vitamins alone. Increasing the concentration of Vit. B_{12} up to 100 ppm did not show any effect compared to control. This means that foliar application of vitamin B_1, B_6 and B_{12} singly at the suitable concentration; i.e., 50 ppm each are the economic and beneficial treatment for cowpea plantation for increasing seed yield.

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تأثير الرش بفيتامين ب١ ، و ب٦ ، و ب١٢ على النمو والمحصول البذرى للوبيا

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أجريت تجربتان حقليتان وأصص خلال صيف موسمين متعاقبين ١٩٩٦، ١٩٩٧ فى مزرعة خاصة بد يرب نجم - محافظة الشرقية لدراسة تأثير الرش بفيتامين ب١، وب٦، و ب١٢ كل منهم على حده أو مجتمعه معاً بتركيز ٥٠، ١٠٠ جزء فى المليون من كل منهم على النمو والمحصول البذ رى لنباتات اللوبيا.

أدى رش نباتات اللوبيا بفيتامين ب١، وب٦ ، وب١٢ منفردا أو مجتمعة معا بتركيزى ٥٠، ١٠٠ جزء فى المليون من كل منهم ، باستثناء فيتامين ب١٢ منفردا بتركيز ١٠٠ جزء فى المليون الى تحسين صفات الجذر والعقد البكتيرية متمثلة فى الطول الكلى للجذور، وعدد تفرعات الجذور، وعدد العقد على الجذر، ووزن العقد على الجذر ، ونشاط تثبيت الازوت معبرا عنه باختزال الاستيلين.

أدت إضافة الفيتامينات (ب١، وب٦، وب١٢) بنفس التركيزات السابق ذكرها إلى زيادة معنوية فى الوزن الجاف لمختلف أعضاء النبات، وصبغات الأوراق (كلوروفيل ا، ب، والكاروتينويدات)، وكذلك عدد القرون على النبات، والمحصول البذرى للنبات وللفدان مقارنة بالنباتات غير المعاملة.