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EFFECT OF DUAL BIOFERTILIZATION, BIOGAS MANURE AND MINERAL FERTILIZER ON GUAR PLANTS *[Cyamopsis tetragonoloba* (L.) TAUB.]

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ABSTRACT

Two field experiments were conducted in June 1997 and 1998 to study the effect of dual inoculation with *Rhizobium* as symbiotic N_2-fixer and *Azospirillum lipoferum* as asymbiotic N_2-fixer, biogas manure and mineral fertilizer on nodulation, N_2-ase activity and yield of guar plants [*Cyamopsis tetragonoloba* (L.) Taub.]

Investigation results showed that, guar seeds application with dual inoculation of *Rhizobium* and *Azospirillum lipoferum* and provided with half dose of biogas manure (20 kg N/fed), gave the highest values of bacterial densities in soil rhizosphere, CO_2 evolution, N_2-ase activity, nodulation and guar seed contents of total carbohydrates and guar gum.

Dual inoculation combined with half dose of inorganic nitrogen or half dose of organic nitrogen (20 kg N/fed) increased all values than combination with full dose (40 kg N/fed) of each inorganic nitrogen or organic nitrogen fertilizers.

Also, individual *Rhizobium* inoculation supplied with 40 kg N/fed organic nitrogen or inorganic nitrogen increased the values of all treatments than individual inoculation with dual inoculation received the full dose of organic or inorganic nitrogen.

INTRODUCTION

Guar or cluster bean plant [*Cyamopsis tetragonoloba* (L.) Taub.] is a leguminous crop with good capabilities to fix atmospheric nitrogen. Guar seeds has a commercial importance due to the gum content (galactomanan) which used in food processing, pharmaceuticals, paper manufacturing and as an emulsifier in drilling mud's for the petroleum (Arayangkool *et al*., 1990). Also, seeds carbohydrate content has a great economical importance because of its capacity to give a high level of viscosity, which is useful in tobacco, mining, textile and cosmetics, (El Sheikh and Ibrahim, 1999). In general, guar seed is rich in protein content (42%) which could be used for poultry and animal nutrition.

Biofertilization by nitrogen fixers enhancing of nodulation, N_2-fixation and growth of leguminous crops. Rodelas *et al.* (1996) found that dual inoculation with *Rhizobium* and *Azospirillum* increased the total nitrogen content of leguminous plants. Tran *et al.* (1984) reported that the application of nitrogen fertilizers as N_2-fixers improved seed yield and agronomic characteristics of plants.

Biogas manure applications caused remarkable increase in nitrogen fixers. Monib *et al.* (1970) found that an application of organic matter increased the counts of non symbiotic nitrogen fixing bacteria and increased the efficiency of N_2-fixation correspondingly.

The present study was conducted to investigate the effect of dual inoculation *(Rhizobium + Azospirillum lipoferum)*, biogas manure and mineral fertilizer on nodulation, N_2-fixation and seed yield of *Cyamopsis tetragonoloba* (L.) Taub. (guar plant).

MATERIALS AND METHODS

The field experiment was conducted in June 1997 and 1998 at experimental farm Fac. Agric. Moshtohor, Zagazig University. Both chemical and mechanical analysis and biogas manure analyses were shown in Table (1) and (2). Mechanical analysis was estimated according to Jackson (1973), whereas, chemical analysis was carried according to Black *et al.* (1982).

Table(1): Chemical and mechanical analysis of experimental soil.

Chemical analysis					
Organic matter %	pH	T.N%	T.P%	E.Cm mhos/cm	Ca Co_3%
1.82	7.90	0.47	0.21	0.81	1.43
mechanical analysis					
Coarse Sand%	Fine Sand%	Silt%	Clay %	Textural class	
3.65	18.73	22.26	55.36	Clay	

T.N, total nitrogen **E.C, Electric conductivity**

Table (2): Analysis of biogas manure.

Organic matter %	Organic carbon%	Total nitrogen %	Total phosphorus %	Total potassium %	C:N ratio
61.20	33.60	1.84	0.94	1.42	18.28

The experimental soil was supplied with 40 kg N/fed biogas manure as a full dose before sowing. Also, 40 kg N/fed of (NH_4NO_3) as a full dose of nitrogen fertilizer were added at sowing, after 30 and 60 days of sowing. All treatments were supplemented with calcium superphosphate (15.5% P_2O_5) and potassium sulphate (48% K_2O) at rates of 30 and 48 kg P_2O_5 and K_2O/fed respectively in two equal doses after 15 and 45 days.

Bacterial cultures preparation and seed inoculation:

An active nitrogen fixer *Rhizobium* strain isolated from guar nodules (local) was obtained from Microbiological Unit, Desert Research Center, Mataria, Cairo, Egypt. For preparation of *Rhizobium* inoculum, yeast extract mannitol (Vincent, 1970) medium was inoculated with *Rhizobium* strain and incubated at 25°C for 6 days. Initial number of cells used for seed inoculation was about 10^8 cells/ml. Guar seeds surface were treated with16% gum Arabic (Dadarwal *et al.,* 1985) and soaked in inoculum for one hour. Gum Arabic was used as an adhesive agent for ensuring firm association of *Rhizobium* to the seeds.

Also, *Azospirillum lipoferum* strain was obtained from Microbiological Unit, Desert Research Center, Mataria, Cairo.

Semi-solid malate medium (Dobereiner, 1980) was inoculated with *Azospirillum lipoferum* and incubated at 30°C for 7 days as an inoculum. The number of viable cells at sowing time were 10^8 cells/ml.

Guar seeds were soaked in mixture of *Rhizobium* and *Azospirillum* for one hour and coated with gum arabic as adhesive agent (Mor, 1995) except seeds of control treatment were soaked in uninoculated media at the same time.

Treatments and cultivation process:

A split plot design with three replicates was used. Cultivation was performed by sowing inoculated or uninoculated guar seeds in hills at rows with 24 cm distance between hills and 50 cm between rows. The experiment included the following treatments:

1- Control
2- Nitrogen fertilizer (Full dose 40 kg N/fed).
3- Biogas manure (Full dose 40 kg N/fed).
4- *Azospirillum* + half dose nitrogen fertilizer (20 kg N/fed).
5- *Azospirillum* + half dose biogas manure (20 kg N/fed).
6- *Azospirillum* + full dose biogas manure.
7- *Azospirillum* + full dose nitrogen fertilizer.

All treatments (except control) were inoculated with *Rhizopium* as usual. The soil was immediately irrigated after sowing.

Determination and assessment of samples:

Five plants were taken to determine the acetylene reduction rates of each treatment at preflowering and flowering stages to estimate the N_2-ase activity. At preflowering and flowering and maturity representative rhizosphere soil samples were obtained and total microbial counts, azospirilla densities and Co_2 evaluation were determined.

Also, guar seeds were taken at harvest time for determining the N.P.K., total carbohydrates, total crude protein, guar gum percentage and seeds yield.

Microbiological determinations:

1- Acetylene reduction activity (ARA or N_2-ase activity) of intact root nodule was determined by GLC according to Hardy *et al.* (1973).
2- Carbon dioxide (CO_2) evoluted by soil microorganisms was estimated according to the method of Page *et al.* (1982).
3- Densities of azospirilla were determined on the semi-solid malate medium according to Dobereiner (1980) using the most probable densities technique. Also, total microbial count were carried out on the soil yeast extract agar medium according to Skinner *et al.* (1952).

Chemical determinations:

Seeds were cleaned then ground to pass through a 0.4 mm screen. Analysis method of A.O.A.C., (1980) was followed for the determination of total nitrogen (6.25x%T.N. = T.C. protein). Total phosphorus content was colourimetrically determined according to APHA, (1989). Total potassium content of the seeds were determined according to Dewis and Freitas (1970).

Total carbohydrates were determined in guar seeds spectrophotometrically according to Thomas (1977) method. Also, guar gum (Galactomanan) was estimated using the method described by Das *et al.* (1977).

Parameters and yield:

Ten plants were harvested randomly at preflowering and flowering stages to determine per treatment:

1- Dry weight of nodules mg/plant.
2- Number of nodules/plant.

Also, ten plants were harvested randomly from each treatment at harvest time for seeds yield estimation and weight was calculated per plant.

Statistical analysis:

Each sample was analyzed in triplicate and figures were then averaged. Data were assessed by analysis of variance (ANOVA) to determine the significant differences between treatments by L.S.D. according to Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Effect of dual inoculation , biogas manure and meniral N- fertilizer on microbial desnities in rhizosphere of [*Cyamopsis tetragonoloba* (L.) Taub.]

1. Changes in total microbial population in rhizosphere of guar plant.

Data presented in Table (3) show that the total microbial counts in rhizosphere soil increased in all soil application treatments compared with control. It was observed that the highest total microbial counts was in the treatment of guar seed inoculation with both of *Rhizobium* and *Azospirillum lipoferum* combained with half dose of biogas manure. On the other hand, dual inoculation combined with the full dose of nitrogen fertilization showed the lowest count of total microbial population. Also, it is noticed that the total microbial counts with treatments of azospirilla with half dose of biogas manure (20 kg/fed) or azospirilla with half dose of in organic nitrogen fertilization (20 kg/fed) were higher than treatments of biogas manure fertilization alone (40 kg N/fed.) or nitrogen fertilization alone (40 kg N/fed). Similar trends were noticed in the two growing seasons. These resultes are in agreement with Gowily and Abd El-Ghany (1993) who found that the microbial total counts increased with asymbiotic or symbiotic nitrogen fixers biofertilization. Data in Table (3) indicated that the total microbial counts in the rhizosphere of inoculated guar plant increased in all treatments comparing to control. The counts of microbes increased at flowering stage than vegetative and maturity stages, whereas increasing occured between perflowering and flowering period then decreased after flowering till maturity. This result may be due to the rich exudates of roots by carbohydrates and amino acids (Kloepper, 1990).

2. Changes in azospirilla densities:

Recorded data in Table (3) revealed that the azospirilla densities in rhizosphere soil of guar plant increased in all soil treatments compared to control. Inoculation of seeds by both of *Rhizobium* with *Azospirillum lipoferum* and half dose of biogas manure (20 kg N/fed) gave the highest densities of azospirilla. Also, inoculation by both of *Rhizobium* with *Azospirillum lipoferum* and half dose of nitrogen fertilization (20 kg N/fed) increased azospirilla densities compared to biofertilization and addition full dose of biogas manure or biofertilization with full dose of nitrogen fertilization. The lowest densities of azospirilla were observed in the treatment azospirilla with full dose of nitrogen fertilizatioin at different stages of growth. Biogas manure application + *Rhizobium* also affected the rate of azospirilla proliferation than seed inoculation with *Rhizobium* and *Azospirillum lipoferium* provided with full dose of inorganic nitrogen fertilization. (Hashem, 1992). Similar trends of results were noticed in the two growing seasons.

Table (3) showed that densities of azospirilla were increased at flowering stage than vegetative or maturity stages in all treatments. This result may attributed to microbial activity and production of biologically active substances in rhizosphere of guar plant. This result was in agreement with Rodelas *et al.* (1997). They reported that azospirilla live in close association with plant roots. This kind of plant-bacterial interactions often result in plant growth. Promotion by an enhancement of other beneficial associations such as rhizobia symbiosis and production of active substances such as phytohormones, amino acids and water-soluble vitamins.

Effect of different applications of guar plant on CO_2 evolution and N_2-ase activity:

It is obvious from data presented in Table (4) that CO_2 evoluation rates and N_2-ase activity as indications soil microorganisms activity and N_2-fixing activity in rhizosphere of guar plants. The highest values of evoluted CO_2 and N_2-ase activity were observed with seed inoculation by *Rhizobium* combined with *Azospirillum lipoferum* in the presence of half dose of biogas manure (20 kg N/fed).

Both of evoluted CO_2 and N_2-ase activity remarkably increased at flowering stage than preflowering or maturity stage in the two growing seasons. This result may attributed to increased counts of asymbiotic nitrogen fixing bacteria and increased the efficiency of nitrogen fixation correspondingly (Monib *et al.*, 1970).

The lowest values of CO_2 evolution and N_2-ase activity were resulted with seeds inoculated by *Rhizobium* combined with *Azospirillum lipoferum* and provided with the full dose of nitrogen fertilization (40 kg N/fed). Data in Table (4) showed that increasing the dose of nitrogen feritlization resulted in decreasing of N_2-ase activity whereby display a negative effect on N_2-fixation. Singh *et al.* (1984) mentioned that nitrogen fixation decreased with increasing N levels. Similar trends of results were observed in the two growing seasons and the values of evoluted CO_2 and N_2-ase activity were increased in all treatments compared to control. Data in Table (4) also clearly indicate that biofertiliztion by symbiotic with asymbiotic N_2-fixers and provided with low doses of N-fertilizer gave increases in soil microorganisms activity and rate of nitrogen fixation.

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Effect of different applications on nodulation and seed yield of the guar plant.

It is evident from data presented in Table (5) that *Rhizobim* inoculation in the presence of full dose of organic manure significantly increased nodules dry weight at flowering stage than *Rhizobium* inoculation with full dose of mineral nitrogen. Dual inoculation of *Rhizobium* + *Azospirillum* and half does of biogas manure significantly increased nodules dry weight. Nodules dry weight has been significantly differed between all treatments irrespectively control.

Table (5) : Effect of dual inoculation, organic manure and inorganic N-fertilizer on nodulation and seed yield in guar plant .

Treatments	Parameters *Rhizobium* inoculation	Dry weight of nodules (mg/plant) Flowering		Nodules number/ plant Flowering		Seeds yield g/plant	
		1997	1998	1997	1998	1997	1998
N. F. F.D	+	463.00	503.33	8.73	8.47	16.60	16.69
Biogas	+	610.00	640.00	9.60	9.63	17.14	16.67
Az+ half D.N.F.	+	696.67	726.67	9.87	9.83	17.82	17.41
Azo +haf. D. Bio	+	793.33	826.67	10.33	10.40	18.57	18.69
Azo + F.D. Bio	+	433.33	448.33	8.20	7.97	15.95	15.07
Azo +F.D. N.F.	+	398.33	413.33	7.27	7.43	15.79	15.40
Control	-	0.0	0.0	0.0	0.0	15.33	14.77
L.S.D at 5%		45.85		0.4023		0.7199	

The same footnotes of Table (3)

Data in Table (5) clearly indicate that a significant increase of nodules number was observed at flowering stage in dual inoculation with *Rhizobium* + *Azospirillum* and half dose of biogas manure.

The biogas fertilizer with *Rhizobium* inoculation gave significant increase in nodules number than mineral fertilizer. Singh and Singh (1989) mentioned that seed inoculation + 20 kg N/fed gave the maximum number of nodules. Data in Table (5) showed a significant difference between dual inoculation of *Rhizobium* + *Azospirillum* and received half dose of biogas manure and other treatments. Dual inoculation with *Rhizobium* + *Azospirillum* and full dose mineral nitrogen had no significant difference with uninoculated control.

Effect of biofertilizer, inorganic N- fertilizer and organic fertilizer on seeds NPK percentage and crude protein of guar plant.

Table (6) showed the percentage of NPK in guar seeds content and total crude protein. Recorded data revealed that the highest percentage of total nitrogen, total potassium and total phosphorus were obtained with seeds inoculated by *Rhizobium* combined with *Azospirillium lipoferum* and received half dose biogas manure (20 kg N/fed). Also, data showed increased total percentage of N.P and K with seeds inoculation by *Rhizobium* combined with *Azospirillum* and provided with 20 kg of mineral N/fed. These results were confirmed by Singh *et al.*(1990). They reported that *Cyamopsis tetragonoloba* (guar) seeds inoculated with *Rhizobium* + 20 kg N/fed was the most effective in increasing NPK and protein contents over uninoculated control and uptake in seed. This could probably be attributed to the increase in the nitrogen fixing efficiency of inoculated plants where more nitrogen is fixed and translocated to the seeds (El Sheikh and Ibrahim, 1999). The lowest treatment of NPK and protein content were observed with seeds inoculation by *Rhizobium* combined with *Azospirillum* and supplied with full dose of N-fertilizer (40 kg N/fed). Total nitrogen and total phosphorus percentages in guar seeds were higher in the 1st season than in the 2nd in all treatments, may be resulted from the climatic conditions difference.

Table (6) : Effect of dual inoculation, organic manure and inorganic N-fertilizer on seeds NPK percentage and crude protein of guar plant.

Treatments	determination *Rhizobium* Inoculation	N %		P %		K %		Crude protein %	
		1997	1998	1997	1998	1997	1998	1997	1998
N. F. F.D	+	2.91	2.88	0.69	0.66	2.01	1.97	18.19	18.00
Biogas	+	2.42	2.41	0.75	0.71	2.16	2.22	15.13	15.06
Az+ half D.N.F.	+	2.92	3.63	0.85	0.81	2.58	2.52	18.23	22.69
Azo +haf. D. Bio	+	3.90	3.72	0.95	0.93	2.96	2.88	24.40	23.25
Azo + F.D. Bio	+	2.41	2.37	0.63	0.66	1.79	1.76	15.06	14.81
Azo +F.D. N.F.	+	1.96	1.99	0.54	0.53	1.66	1.63	12.27	12.44
Control	-	1.99	2.37	0.31	0.32	1.43	1.52	12.46	14.81

The same footnotes of Table (3)

Effect of different applications on total carbohydrates and seed gum percentages in the seeds of guar.

Data presented in Table (7) reveal that the highest percentage of total carbohydrates was recorded with seeds inoculation by *Rhizobium* combined with *Azospirillum* and provided with half dose of biogas manure (20 kg N/fed). The lowest treatment under control was with *Rhizobium* combined with *Azospirillum* and received the full dose of N-fertilizer (40 kg N/fed). Similar trends of results were noticed in the two growing seasons.

Table (7) : Effect of dual inoculation, organic manure and inorganic N- fertilizer on total carbohydrates and guar seeds gum percentage of guar plant.

Treatments	determination *Rhizobium* inoculation	Total Carbohydrates %		Guar gum (guaran%)	
		1997	1998	1997	1998
N. F. F.D	+	32.89	30.58	31.54	32.17
Biogas	+	38.15	38.20	36.87	36.80
Az+ half D.N.F.	+	51.99	47.96	40.16	40.30
Azo +haf. D. Bio	+	63.00	59.82	42.57	45.23
Azo + F.D. Bio	+	29.53	28.33	28.60	27.99
Azo +F.D. N.F.	+	22.98	23.98	25.76	26.97
Control	-	26.15	23.74	22.77	22.22

The same footnotes of Table (3)

Data in Table (7) showed that guar gum percentage increased by inoculation by *Rhizobium* combined with *Azospirillum* in the presence of half dose of biogas manure (20 kg N/fed) giving the highest percentage of seed gum.

All treatments gave percentages over control and the same trends of results were observed in the two seasons. Baboo and Rana (1995) found that seed inoculation with *Rhizobium* combined with 20 kg N/fed increased the seed yield and seed gum content. The inoculation by *Rhizobium* combined with *Azospirillum* and application of 40 kg N/fed addition gave the lowest value of seed gum percentage irrespectively control treatment. Application of full dose of biogas manure with *Rhizobium* inoculation gave higher seed gum percentage than that obtained in the case of application with full dose of N-fertilizer.

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تأثير التلقيح الحيوى المزدوج والتسميد العضوى بسماد البيوجاز والتسميد المعدنى على نبات الجوار

طه عبده توفيق

قسم النبات الزراعى (ميكروبيولوجيا زراعية) - كلية الزراعة بمشتهر - فرع بنها جامعة الزقازيق - مصر

أجريت تجربتان حقليتان بمزرعة مركز البحوث والتجارب الزراعية بكلية الزراعة بمشتهر خلال شهرى يونيه لموسمى ١٩٩٧ و ١٩٩٨ وذلك لدراسة تأثير التلقيح المزدوج لبذرة نبات الجوار باستخدام بكتيريا الريزوبيوم المعزولة من العقد الجذرية لنبات الجوار كبكتيريا مثبتة للأزوت تكافليا مع بكتريا *Azospirillum lipoferum* كبكتريا مثبتة لأزوت الهواء الجوى وذلك فى وجود التسميد العضوى (سماد البيوجاز) أو التسميد المعدنى بجرعة كاملة (٤٠ كجم نتروجين /فدان) أو جرعة نصف كاملة (٢٠ كجم نتروجين / فدان) مضافة إلى التربة عند الزراعة وبعد ٣٠ و ٦٠ يوم من الزراعة.

وقد بينت الدراسة الآتى:

أدى تلقيح بذور نبات الجوار بالرايزوبيوم مع بكتريا *Azospirillum lipoferum* قبل الزراعة مع وجود جرعة نصف كاملة (٢٠ كجم نتروجين/ فدان) من سماد البيوجاز إلى اعلى معدل من زيادة أعداد ميكروبات التربة. وكذلك أعلى معدل من تصاعد ك أ٢ فى التربة وكذلك زيادة أعداد بكتريا الأزوسبيرلا فى التربة وكذلك أعطى أعلى معدل من نشاط إنزيم النتروجينيز فى العقد الجذرية وزيادة أعداد العقد الجذرية كذلك أعطى أعلى معدل من نسبة الكربوهيدرات الكلية ونسبة المادة الهلامية (الجوران) فى البذرة.

كما وجد أيضا أن استخدام التلقيح المزدوج فى وجود نصف جرعة (٢٠ كجم نتروجين/فدان) نتروجين عضوى أو نتروجين غير عضوى يؤدى إلى زيادة جميع المعاملات عن استخدام جرعة كاملة (٤٠ كجم نتروجين/فدان).

كما أدى استخدام التلقيح الفردى بالريزوبيوم فى وجود (٢٠ كجم نتروجين / فدان) سواء عضوى أو غير عضوى إلى زيادة نشاط إنزيم النتروجينيز ومعدل تصاعد ك أ٢ مع زيادة محتوى البذرة من الكربوهيدرات والمادة الهلامية (جوران) عنه فى حالة استخدام ٤٠ كجم نتروجين /فدان

Table (3) : Effect of dual inoculation, biogas manure and mineral N-fertilizer on total microbial counts and azospirilla densities in the rhizosophere of guar plant.

Treatments	determination / *Rhizobium* inoculation	Total microbial densities (x10^6 cells/g dry soil) 1997			Total microbial densities (x 10^6 cells/g dry soil) 1998			Azospirilla *d*ensities (x10^4 cells/g dry soil) 1997			*Azospirilla d*ensities (x10^4 cells/g dry soil) 1998		
		P.F.	F	M	P.F.	F	M.	P.F.	F	M	P.F.	F	M.
N. F. F.D	+	147	233	153	166	241	182	97.8	152.6	122.3	92.3	160.5	126.4
Biogas	+	164	270	144	173	266	186	129.6	266.8	132.7	131.4	188.8	135.6
Az+ half D.N.F.	+	198	325	283	186	332	237	154.4	282.8	198.7	159.8	215.6	173.5
Azo +haf. D. Bio	+	225	362	305	231	356	224	178.6	298.5	205.3	175.3	240.7	192.6
Azo + F.D. Bio	+	108	228	210	103	238	207	82.9	115.4	93.4	85.5	120.3	95.2
Azo +F.D. N.F.	+	102	220	151	109	228	98	66.5	92.2	79.5	63.7	97.5	65.4
Control	-	92	212	97	98	220	103	43.8	77.6	68.3	47.8	81.5	71.2

N.F.F.D = Nitrogen fertilization full dose (40 kg N/fed).
Azo = *Azospirillum lipoferum.*
half. D = Half dose (20 kg N/fed.)
F.D. = Full dose
Bio = Biogas manure Full does (40 kg N/fed)

PF= Preflowering
F = Flowering
M = Maturity

Table (4) : Effect of dual inoculation, biogas manure and mineral N-fertilizer on CO_2 evolution and N_2 - ase activity in the growth stages of guar azospirilla densities in the rhizosphere of guar plant.

Treatments	determination / *Rhizobium* inoculation	CO_2 mg/g dry soil / hr						µL C_2H_4/g dry nodule/ hr					
		1997			1998			1997			1998		
		P.F.	F	M	P.F.	F	M.	P.F	F	M	P.F	F	M
N. F. F.D	+	109.32	166.63	132.33	115.55	172.00	143.77	47.32	97.15	80.03	51.58	94.58	73.00
Biogas	+	112.76	178.51	172.71	110.15	181.30	176.18	68.18	130.46	99.00	61.70	130.78	95.00
Az+ half D.N.F.	+	123.55	201.55	187.66	120.00	210.12	185.66	110.8	156.45	133.00	108.08	159.02	130.00
Azo +haf. D. Bio	+	132.17	215.43	196.52	141.44	227.30	201.00	133.06	198.05	163.00	137.58	198.20	165.00
Azo + F.D. Bio	+	98.66	142.81	137.37	90.55	145.20	136.15	44.23	85.18	65.00	27.90	86.93	55.00
Azo +F.D. N.F.	+	92.41	140.15	122.33	88.55	144.00	115.30	25.58	70.52	45.00	50.56	65.33	56.00
Control	-	27.15	140.31	36.61	32.20	62.50	42.55	0.0	0.0	0.0	0.0	0.0	0.0

The same footnotes of Table (3)