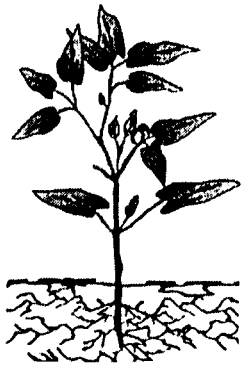

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STUDY THE INDUCTION EFFECT OF *AZOSPIRILLUM* INOCULATION ON THE FORMATION OF PARA-NODULES ON GRAMINEAE

[7]

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ABSTRACT

In order to study the effect of *Azospirillum brasilense* inoculation on graminae, two field experiment were conducted at Maruit experimental station, Alex., Desert Research Center. *Azospirillum brasilense* characterized by its ability to fix atmospheric nitrogen and production of plant hormones like indole- 3- acetic acid (IAA). Also, *Azospirillum brasilense* has the ability to transform tryptophan into IAA. *Azospirillum brasilense* was inoculated into wheat and maize to show its effect on growth and development of these plants. The achieved results could be summarized as follows

1- Addition of tryptophan in concentration up to 200 ppm to the growth media of *Azospirillum brasilense* lead to vigorous increase in the production of indole acetic acid (IAA) by the microorganism.
2- *Azospirillum brasilense* in presence of tryptophan up to 200 ppm had promoting effects on seedlings of wheat and maize compared to all other treatments, whereas treatment with tryptophan only in concentration of 500 ppm gave an inhibitory effect on treated seedlings, reaching a level lower than control.
3- Inoculation of wheat and maize seedlings with *Azospirillum brasilense* and tryptophan up to 200 ppm lead to formation of tumor like structures called paranodules on roots of treated seedlings, which enhanced the nitrogen-fixing activity, growth parameters and increased yield in two crops of maize and wheat.

INTRODUCTION

Soil microorganisms play an important role in releasing essential nutrients for plants. The free living nitrogen fixing rhizobacteria of the genus *Azospirillum* lives in close association with plants mostly of graminae. The importance of *Azospirillum* is due to its capacity to fix atmospheric nitrogen and suppling it to plants in addition to its ability to synthesize several phytohormons, enzymes, siderophores, polysaccharides and other useful substances. **Hegazi *et al* (1983)** found that *Azospirillum* inoculation of maize caused increase in plant dry weight, total nitrogen content. **Reynders and Vlassak (1979)** found that *Azospirillum* converted tryptophan into IAA in pure cultures and reported that auxins produced by *Azospirillum* can greatly affected growth of gramineous plants. Also **Malhotra, and Srivastava, (2006)** reported that IAA biosynthesis by a natural isolate of *Azospirillum brasilense* SM was studied and observed to be tryptophan-inducible and dependent in nature. **Jain and Patriquin (1984 a and b)** confirmed that the root deformation of wheat was caused by IAA (branching substances) produced by *Azospirillum*. They found a similar phenomena up on application of authentic IAA.It is demonstrated that gramineous plants are potentially capable of developing an endophytical diazotrophic symbiosis through paranodule formation when inoculated with diazotrophs (*Azospirillum spp.*, *Azorhizobium caulinodans, Rhizobium spp.*) which

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potentially inhabit tissues of both stem and root tumors with the central meristem as a major colonization niche. **(Christiansen- Weinger, 1998)**.

The aim of this work was therefore, to study the effect of inoculation of *Azospirillum brasilense* in the presence of tryptophan on the formation of paranodules in roots seedlings of graminie and thus on plant growth and yield.

MATERIALS AND METHODS

Bacterial preparation and Indole acetic acid (IAA) detection

The soil bacteria used in this study (*Azospirillum brasilense*) was isolated from different soil samples (geographically and climatically diverse locations) then purified and identified according to **(Tarrand *et al* 1994)**. For Indole acetic acid (IAA)detection, pure bacterial isolate was cultured in NFb medium according to **Doberiener, (1995)** to investigate the influence of tryptophan on IAA production, NFb medium was supplied by 0.1 % NH_4Cl and amended by various amounts(100–200–500 ppm)of L- tryptophan sterilized by filtration on 0.02 mm Milipore filter.

Determination of IAA in the culture medium

IAA was estimated in supernatant of the bacterial culture which was centrifugated at 1500 rpm for 15 min at 4°C then using High performance liquid chromatography method (HPLC), it was possible to determine IAA according to the method described by **Tien *et al* (1979)** and **Somers *et al* (2005)**.

Determination of bacterial growth

a- Most Probable Number method (MPN): This method was used to enumerate the bacteria in roots and rhizosphere of inoculated plants **(Dobereiner *et al* 1995)**..

b- Colorimetric method: The bacterial population was determined in the liquid culture by measuring the light absorption at 492nm using spectrophotometer (Perkin Elmer II).

Effect of inoculating *Azospirillum brasilense* and/or tryptophan on wheat and maize growth and paranodule formation.

a- Sterilization of wheat grains (*Triticum sativum* cv. Maruit 5) and maize grains (*Zea mays* cv. hybrid 310) according to the method described by **Christiansen–Weniger and Vanderleyden (1994)**. Maize and wheat grains were surface sterilized with 1.5% sodium hypochlorite solution for 15 min, washed 5 successive times in sterilized distilled water and germinated on nutrient agar plates for 3 days.

b- Medium used for seed germination and bacterial inoculation

According to **Barbieri *et al* (1986)**, NFb medium was prepared, dispensed in test tubes (20cm of length X 3 cm of diameter) , sealed with a cotton and autoclaved.

The prepared medium was divided into 4 groups; group 1 was control without amendments, group 2 was supplemented with filter sterilized L-tryptophan with different concentrations (100 – 200 – 500 ppm) in three treatments, group 3 was inoculated with *Azospirillum brasilense* only with concentration of 10^9 CFU/ml and group 4 was supplemented with filter sterilized L-tryptophan with different concentrations (100–200 –500 ppm) and inoculated with *Azospirillum brasilense* in three treatments. Sterilized wheat and maize seedlings were transplanted in every tube aseptically, the tubes were incubated for 3 weeks in the green house at light / dark cycles (12.5 h in the light at 25 °C and 11.5 h in the dark at 18 °C.

c- Measurements of growth parameters and para-nodules formation of transplanted wheat and maize

The dry weight of roots and stems was determined using digital electric balance; the root depth and stem height were measured by graduated ruler; surface area of the roots were estimated according to the gravimetric method described by **Carly and Watson, (1966)**

Para-nodule formation was described where root segments containing para-nodules were magnified and photographed.

Effect of inoculating *Azospirillum brasilense* and/or tryptophan on wheat and maize growth and paranodule formation in a field experiment

Two field experiments were conducted at Maruit experimental station. In two successive seasons of 2003/2004 and 2004/2005. (in winter season for wheat, in summer for maize).Soil used in this experiment was characterized by its clay loam texture and high content of calcium carbonate reaching 37%. Salinity (E.C 10.5 ds/m) and contained less than 1% of organic matter.

Eight treatments used (control, *Azospirillum brasilense*, three concentrations of tryptophan and *Azospirillum brasilense* with three concentrations of tryptophan).

Grains were inoculated with *Azospirillum brasilense* (fresh liquid culture medium of *A. brasilense* at 28 ± 2°C for 7 days) seeds before sowing (damped in the inoculum density ≈ 10^8 cfu /ml), *A. brasilense* used purified and identified according to **(Tarrand *et al* 1994),** the ability to fix nitrogen was tested by Modified Kjeldahl method (**Chapman and Pratt, 1961**).

Wheat (Maruit 5) and maize (triple cross 310) were the varieties of grains of the tested crops, wheat sown in November and maize in May each season.

The mineral fertilization was applied as a basal dose using single rates of 31 kg P_2O_5/fedan in calcium superphosphate form before sowing, N and K fertilizers were added at a rate of 60 kg N/fedan as NH_4NO_3 and 75 kg K_2O/fedan as K_2SO_4 into three equal doses applied at three stages of wheat and maize plant growth. Tryptophan added at three concentrations 100, 200, 500 ppm alone or with *A. brasilinse.* Sheep manure analysis recorded O.C (30.98 %), N(2.06%),C/N(15),pH(7.5) was added as organic material to the soil before sowing at the rate of 20 m^3/fedan for all treatments.

Rhizosphere soil samples and plant samples were taken at three stages. The soil samples were analyzed for total microbial counts on **Bunt and Rovira medium (1955)** using the decimal plate method technique, *Azospirillum* on Doberiner's medium (**Doberiner, 1978**), and plant samples were tested for measuring growth parameters.

RESULTS AND DISCUSSION

In the present study, the effect of adding different concentrations of tryptophan to the cultural medium on the bacterial activities was studied. The results in **Table (1)** revealed that, tryptophan at concentrations up to 200 ppm have stimulatory effects on growth (1.45 nm), nitrogen fixation (160.51) and IAA production (5.27) by tested microorganism. The stimulatory effects may be due to; it's utilization as precursor for IAA biosynthesis by bacteria and consequently higher growth rate. It may be consumed as N-source stimulates more cells multiplications, resulting in high growth rate and accordingly higher nitrogen fixation activity. Higher concentration of tryptophan (500 ppm) was inhibitory, repress most of the bacterial activities. These results agreed with that of **El-Desoky, (1999) and Malhotra, and Srivastava, (2006).** It has been assumed that bacterial IAA synthesis is a path for the detoxification of tryptophan, no other route exists in *A.brasilense* bacteria, through which tryptophan could be degraded, as pointed by **Lebuhan *et al* (1997). Iosipenko and Ignatov (1995)** Tryptophan supplementation resulted in 20-fold increase of the IAA levels (**Prinsen *et al* 1993**). **Dobbelaere *et al* (1999)** and **Jaeger *et al* (1999)** showed that IAA produced by *A. brasilense* is important for stimulating root development.

Results in **Table (2)** revealed that the overall effect of inoculation with *Azospirillum brasilense* at tryptophan addition up to 200 ppm gave higher promotion effect than inoculation with each of them separately. Addition of tryptophan (500 ppm) alone or with *Azospirillum brasilense* inoculation drastically inhibited the growth of seedlings. The deactivation effect was more powerful in case of treatment with 500 ppm tryptophan only than the treatment of 500 ppm of tryptophan with *A. brasilense* inoculation. The increase was 92.96%, 35.7%, 38.9%, 29% and 57.7% for root length, root fresh weight, root surface area, shoot length and shoot fresh weight of treated wheat seedlings treated with tryptophan 200 ppm and *A. brasilense* respectively. There was a noticeable decrease in all these parameter of wheat seedlings treated with tryptophan 500 ppm only compared with control. On other hand, the increase was 65.6%, 58.82%, 82.8%, 17.5% and 16% for root length, root fresh weight, root surface area, shoot length and shoot fresh weight of treated maize seedlings treated with tryptophan 200 ppm and *A. brasilense* respectively. Decreases were noticed for all these parameters of maize seedlings treated with tryptophan 500 ppm only compared with control. These findings were in agreement with those obtained by **(Salomone and Doberiener, 1996 and Kirchhof *et al* 1997).**

Paranodules formation: A new structures appeared when tryptophan was added with concentrations up to 200 ppm together with *Azospirillum brasilense* treatment. Small swollen structures or tumor like knots called paranodules were formed on the roots of wheat and maize seedlings by this treatment **(Fig. 1).** *Azospirillum brasilense* had the ability to induce plant seedlings to form paranodules via transforming tryptophan into auxins (inodole acetic acid).This observation in accordance with that of **Christiansen-Weniger, (1998)** and **Youssef *et al* (1998).** Several authors reported that paranodules formed under the influence of *Azospirillum brasilense* and

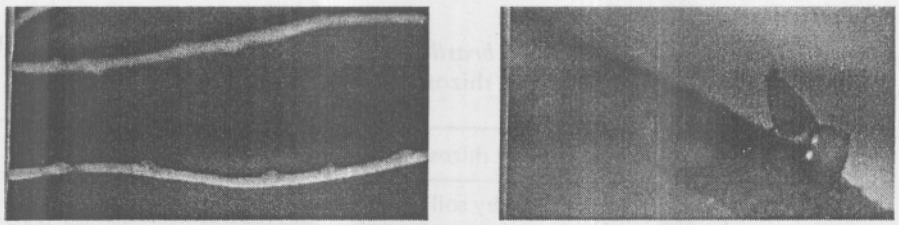
Table 1. Effect of tryptophan additions on total nitrogen, IAA, and pH of *Azospirillum brasilense* medium and its growth

Treatment	T.N ppm	IAA ppm	Growth (O.D) at 492 nm	pH
Control	17.3	-	-	9.87
Tryp. 100	39.8	-	-	9.73
Tryp. 200	41.23	-	-	9.67
Tryp. 500	46	-	-	9.23
Azosp	147.2	2.76	1.05	8.99
Az+ Tryp 100	157.45	5.2	1.365	8.81
Az+ Tryp 200	160.51	5.27	1.45	8.6
Az+ Tryp 500	156.86	3.69	1.339	8.54
LSD at 5%	0.37	0.13	0.036	0.19

T.N: total nitrogen, IAA: indole acetic acid, O.D: optical density, Tryp: Tryptophan, Az: ***Azospirillum brasilense***

Table 2. Effect of inoculation of *Azospirillum brasilense* and / or tryptophan additions on growth of wheat and maize seedlings

Wheat						
Treatment	Root L (cm)	Root F.W (gm)	Root Surface area (cm^2)	Shoot L (cm)	Shoot F.W (gm)	*Azospirillum* Count (MPN×10^4/ml)
Control	7.1	0.14	0.18	10.7	0.26	-
Tryp. 100	9.5	0.16	0.23	11.5	0.29	-
Tryp. 200	10.9	0.19	0.24	12.8	0.32	-
Tryp. 500	6.8	0.13	0.17	8.9	0.24	-
Azosp	10.6	0.16	0.21	12.7	0.29	59
Az+ Tryp 100	11.5	0.17	0.24	13.3	0.34	76
Az+ Tryp 200	13.7	0.19	0.25	13.8	0.41	92
Az+ Tryp 500	8.4	0.15	0.20	10.9	0.28	84
LSD at 5%	1.6	0.08	0.09	1.7	0.12	1.2
Maize						
Control	9.6	0.34	0.39	5.7	0.64	-
Tryp. 100	10.6	0.36	0.45	6.2	0.69	-
Tryp. 200	13.7	0.41	0.46	6.3	0.70	-
Tryp. 500	8.2	0.23	0.36	5.5	0.61	-
Azosp	11.6	0.39	0.44	6	0.66	60.7
Az+ Tryp 100	15.2	0.46	0.48	6.4	0.72	82.5
Az+ Tryp 200	15.9	0.54	0.526	6.7	0.74	98.4
Az+ Tryp 500	9.9	0.35	0.43	5.8	0.643	87.1
LSD at 5%	1.7	0.27	0.3	1.7	0.46	1.8



a- Paranodule on wheat root hairs b- Paranodule on maize root hairs

Figure 1. Paranodules on roots of wheat and maize plants in mixed treatment with *Azospirillum brasilense* and 200 ppm tryptophan

tryptophan on roots of wheat plants led to increase the nitrogen-fixing activity of the paranodulated plants two times higher than that of the inoculated plants lacking paranodules and five times higher than that of the control. Similarly the paranodulation, led to a 40% increase in the crop yield of rape plants and provided for a statistically significant increase in the total nitrogen as well as protein nitrogen contents of the plants as reported by **Sriskandarajah *et al* (1993) and Koval'skaya *et al* (2001)**. Thus paranodules represented one of the most important factors causing promotion effects as a response to treatment with *Azospirillum brasilense* and treptophan up to 200 ppm.

Effect of treatments on different microbial groups

This part of study was carried out to evaluate the effect of inoculation with *Azospirillum brasilense* and concentration of tryptophan on the persistence and interaction of different microbial group in rhizosphere of wheat and maize plants.

Data presented in **Table (3)** showed that the total microbial and *Azospirillum* counts were generally higher in inoculated treatments than uninoculated ones and in second stage of second season than in first one of wheat and maize plant growth. The highest total microbial counts and *Azospirillum* counts were observed in case of inoculation with *A. brasilense* + T 200 being 234 and 228 $\times 10^5$ /gm dry soil in the second stage of second season for wheat and maize respectively. Also, the highest *Azospirillum* counts were 492 and 448 X 10^4 /gm dry soil in the second stage of second season for wheat and maize respectively. On the other hand treatment with 500 ppm tryptophan only caused the lowest total microbial counts being 122 and 105 X10^5 /gm dry soil in the first stage of the first season for wheat and maize respectively.

The same treatment caused the lowest *Azospirillum* counts being 276 and 261 X10^4 /gm dry soil in the first stage of the first season for wheat and maize respectively compared with control. Thus higher concentrations of tryptophan were inhibitory, repress most of the bacterial activities. These results agreed with that of **El-Desoky (1999). Steenhoudt and Vanderleyden (2000) and Dobbelaere *et al* (2001).**

It could be concluded that there was a general increase in total microbial and in *Azospirillum* counts with increasing the tryptophan concentration up to 200 ppm. Further increase was observed with the treatment with *Azospirillum* either alone or in combination with tryptophan up to the same concentration (200 ppm). However, increasing the concentration of tryptophan to 500 ppm had an inhibitory effect on microbial and *Azospirillum* counts.

Plant measurements

It seemed probable to state that plant growth substances produced by *Azospirillum brasilense* improved plant growth by their direct effects on metabolic processes. However, since they induced proliferation of lateral roots and root hairs and thus increase nutrient absorbing surfaces, and greater rates of nutrient absorption. This in turn would be expected to increase plant growth. **(Tien *et al* 1979 and Costacurta and Vanderleyden, 1995).**

Data in **Table (4)** show that inoculation with *Azospirillum* +T 200 treatment in ascending order significantly increased plant height of wheat and maize plants. The highest significant increase being 59.4 and 253 cm comparing with control being 39.2 and 191 cm, for plant height of wheat and maize plants at third stage of second season, respectively. Treatment with 500 ppm tryptophan only recorded the lowest response being 25.5 and 53 cm for plant height of wheat and maize plant

Table 3. Effect of inoculation with *Azospirillum brasilense* and/ or tryptophan on total microbial and *Azospirillum* counts of wheat and maize rhizosphere

	First season (x 10^5 cfu/g dry soil)				Second season (x 10^5 cfu/g dry soil)			
	1st stage	2nd stage	3rd stage	Mean	1st stage	2nd stage	3rd stage	Mean
Total microbial Count wheat rhizosphere (x 10^5 cfu/g dry soil)								
Control	137	180	142	153	145	169	158	157.33
Tryp.100	158	174	162	164.67	167	182	170	173
Tryp.200	173	185	176	178	194	200	190	194.67
Tryp.500	122	138	129	129.67	131	157	141	143
Azo. brasilense	169	183	168	173.33	176	191	184	183.67
Azo. +Tryp.100	181	188	179	188.67	203	213	193	203
Azo. + Tryp.200	203	221	210	211.33	219	234	208	220.33
Azo. + Tryp.500	149	162	157	156	150	174	158	160.67
Mean	161.5	178.9	167.6		173.1	190	175.3	
LSDat 5%: Stage	1.67				1.65			
Treatment	2.87				2.69			
Azospirillum counts of wheat rhizosphere(MPN x 10^4 /g dry soil)								
Control	298	321	305	308	303	336	314	317.7
Tryp.100	312	329	319	320	318	353	325	332
Tryp.200	371	390	381	380.7	387	421	397	401.7
Tryp.500	276	309	300	295	294	320	308	307.3
Azo. brasilense	389	415	399	401	410	434	403	415.7
Azo. +Tryp.100	420	436	428	428	433	462	432	442.3
Azo. + Tryp.200	437	459	441	445.7	445	492	449	462
Azo. + Tryp.500	307	325	317	316.3	313	339	323	325
Mean	351.3	373	361.3		362.9	394.6	368.9	
LSDat 5%: Stage;	3.69				1.63			
Treatment	6.04				2.67			
Total microbial count maize rhizosphere (x 10^5 cfu/g dry soil)								
Control	131	146	139	138.67	137	152	147	145.33
Tryp.100	148	166	158	157.33	153	174	163	163.33
Tryp.200	151	178	162	163.67	159	194	175	176
Tryp.500	105	117	109	110.33	119	136	122	125.66
Azo. brasilense	149	173	160	160.67	157	189	168	171.33
Azo. +Tryp.100	164	186	171	173.67	173	205	182	186.67
Azo. + Tryp.200	189	213	195	199	197	228	198	207.67
Azo. + Tryp.500	138	152	141	143.67	146	168	159	157.67
Mean	146.9	166.4	154.4		155.1	180.8	164.3	
LSDat 5%: Stage;	1.49				1.59			
Treatment	2.43				2.6			
Azospirillum counts of maize rhizosphere(MPNx 10^4u/g dry soil)								
Control	289	325	310	308	304	342	316	320.7
Tryp.100	329	362	350	347	341	386	375	367.3
Tryp.200	354	390	375	373	369	419	402	396.7
Tryp.500	261	284	273	272.7	268	293	279	280
Azo. brasilense	360	395	380	378.3	375	422	415	404
Azo. +Tryp.100	385	423	403	405.3	403	436	421	420
Azo. + Tryp.200	413	442	435	430	435	448	439	440.7
Azo. + Tryp.500	311	337	320	322.7	320	368	361	349.7
Mean	337.75	369.75	355.75		351.88	389.25	370	
LSDat 5%: Stage;	7.35				7.01			
Treatment	12				11.45			

Table 4. Effect of inoculation with *Azospirillum brasilense* and/ or tryptophan on plant height of wheat and maize in two seasons (cm/plant)

Plant height of wheat in two seasons (cm/plant)								
	First season (cm/plant)				Second season (cm/plant)			
	1st stage	2nd stage	3rd stage	Mean	1st stage	2nd stage	3rd stage	Mean
Control	26.9	29.8	38.3	31.7	28.2	33.5	39.2	33.6
Tryp.100	28.69	34.7	39.1	34.2	30.6	37	43.5	37
Tryp.200	29.3	36.3	43	36.2	32.5	40.3	46.9	39.9
Tryp.500	25.5	28.3	29.6	27.8	27.1	30.5	32.8	30.1
Azo. brasilense	28.8	35	40.9	34.9	32.1	38.2	44.5	38.3
Azo. + Tryp.100	30.5	38.9	48.6	39.3	36	44.2	50.2	43.5
Azo. + Tryp.200	32.9	43.5	59	45.1	39.5	45.9	59.4	47.3
Azo. + Tryp.500	27.4	32.1	38.9	32.8	29.8	36.3	42.8	36.3
Mean	28.7	34.9	42.2		32	38.2	44.5	
LSD at 5% Stage : 0.19						0.15		
Treatment : 0.31						0.24		
Plant height of maize in two seasons (cm/plant)								
Control	59	146	186	130.3	63	158	191	137.3
Tryp.100	69	162	201	144	82	169	215	155.3
Tryp.200	72	186	209	155.7	93	195	231	167.3
Tryp.500	53	134	169	118.7	58	145	180	127.7
Azo. brasilense	70	171	205	148.7	90	184	210	160
Azo. + Tryp.100	75	184	212	157	86	189	223	173
Azo. + Tryp.200	78	197	229	168	95	204	253	184
Azo. + Tryp.500	68	151	197	138.7	76	165	203	148
Mean	68	166.4	201		80.4	176.1	213.3	
LSD at 5% Stage : 0.37						0.31		
Treatment : 0.61						0.51		

growth at first stage of first season, respectively compared with control. The inhibitory effect of high concentration of tryptophan generally caused a retardation in all plant growth parameters and microbial biochemical activities in plant rhizosphere as recorded by other investigators **(Bothe *et al* 1992; Sarwar and Frankenberger, 1994 and El-Desoky (1999)**

Data in **Table (5)** show that inoculation with *Azospirillum* + T200 treatment in ascending order significantly increased fresh and dry weights of wheat and maize plant. The highest significant increase recorded with *Azospirillum* +T200 treatments being 15.4, 7.64 and 693, 161 gm/plant for fresh and dry weights of wheat and maize at third stage of the second season respectively. The lowest response was recorded with the 500 ppm tryptophan only treatment being (2.7 and 1.02) gm fresh and dry weight for wheat and (91 and 26) gm fresh and dry weight of maize at first stage of the first season, respectively compared with control.

Chlorophyll content in fresh leaves was obtained at three stages in two seasons of wheat and maize plant growth **(Table 6).** The highest chlorophyll content was recorded 48.9 % for wheat in second stage of the second season and 51.3 % for maize in third stage of the second season in treatments amended with *Azospirillum* + 200 ppm tryptophan followed by Azo. + 100 ppm tryptophan being 47.8 % and 49.8 % comparing with control Treatment with 500 ppm tryptophan only recorded the lowest response being 30.5% for wheat in third stage of first season and 32.5% for maize in first stage of first season respectively.

Yield of wheat

Data in **Table (7)** showed that *Azospirillium* + 200 ppm tryptophan treatment gave highest number of tillers and weight of 100 grains. The promoting effect of this treatment extended to yield product and significantly increased grains, straw and biological yield. The highest significant

Table. 5 Effect of inoculation with *Azospirillum brasilense* and/ or tryptophan on fresh and dry weights of wheat and maize in two seasons (gm)

Fresh and dry weights of wheat in two seasons (gm/plant)																
	First season (gm)								Second season (gm)							
	1st stage		2nd stage		3rd stage		Mean		1st stage		2nd stage		3rd stage		Mean	
	F.W	D.W	F.W	D.W	F.W	D.W	F.W	D.W	F.W	D.W	F.W	D.W	F.W	D.W	F.W	D.W
Control	2.9	1.52	5.18	2.82	7.92	3.36	5.3	2.6	3.74	1.69	7.42	3.28	10.15	3.76	7.103	2.9
Tryp.100	3.97	1.93	6.48	3.4	9.46	3.92	6.6	4	4.58	1.95	8.63	3.86	10.69	4.09	7.967	3.3
Tryp.200	4.6	2.64	8.9	4.9	12.8	5.64	8.8	4.4	4.84	2.92	10.41	5.2	12.86	5.91	9.37	4.7
Tryp.500	2.7	1.02	4.63	1.42	7.35	2.76	4. 9	1.7	2.95	1.06	5.64	1.89	9.04	3.15	5.87	2
Azo. brasilense	3.99	2.51	6.91	4.11	11.3	4.63	7.4	3.8	5.21	2.75	10.12	4.48	11.84	5.13	9.057	4.1
Azo. + Tryp.100	5.21	3.19	9.57	5.68	13.54	6.81	9.4	5.2	5.34	3.61	11.94	6.21	14.3	7.35	10.527	5.7
Azo. + Tryp.200	5.9	3.6	11.8	6.23	14.69	7.01	10.8	5.6	6.1	4.2	12.82	6.53	15.4	7.64	11.44	6.1
Azo. + Tryp.500	3.53	1.74	6.53	3.18	9.8	3.84	6.6	2.9	4.07	1.81	7.96	3.96	10.53	3.98	7.52	3.3
Mean	4.1	2.27	7.5	3.97	10.86	4.75			4.6	2.5	9.4	4.5	11.9	5.2		
LSDat5% Stage :	F.W: 0.07				D.W.: 0.14						F.W: 0.06			D.W: 0.06		
Treatment :	F.W: 0.12				D.W : 0.23						F.W: 0.09			D.W: 0.1		
Fresh and dry weights of maize in two seasons (gm/plant)																
Control	127	31	252	69	391	79	256.7	59.7	215	341	284	83	418	92	305.7	67
Tryp.100	164	50	291	79	439	92	294.3	72.7	256	53	369	109	551	135	386.7	93.3
Tryp.200	178	56	314	86	465	121	355	83.7	281	62	435	131	592	159	430	113
Tryp.500	91	26	234	61	321	72	215.3	53	121	31	265	68	368	89	251.3	62.7
Azo. brasilense	169	47	309	80	428	95	305.7	75	248	51	381	103	543	126	396	99
Azo. + Tryp.100	184	52	342	83	539	113	355	86.7	263	56	461	127	640	156	460.7	117.7
Azo. + Tryp.200	226	59	385	94	618	137	409.7	96.7	284	73	519	141	693	161	498.7	125.7
Azo. + Tryp.500	160	42	284	74	414	86	286	67. 3	238	49	320	92	476	108	344.7	83
Mean	162.4	45.4	301.4	78.3	451.9	99.4			238.3	52.6	379.3	104.3	535.1	128.6		
LSDat5% Stage :	F.W: 1.56				D.W.: 0.74						F.W: 1.69			D.W: 0.94		
Treatment :	F.W: 2.54				D.W : 0.12						F.W : 2.83			D.W: 0.27		

Table 6. Effect of inoculation with *Azospirillum brasilense* and/ or tryptophan on Chlorophyll % of wheat and maize plants in two seasons

Chlorophyll % (Wheat)								
	First season (cm/plant)				Second season (cm/plant)			
	1st stage	2nd stage	3rd stage	Mean	1st stage	2nd stage	3rd stage	Mean
Control	33.7	35.9	32	33.87	34	38.8	36	36.37
Tryp.100	37	40.8	39	38.93	38.3	42	40.1	40.13
Tryp.200	40.3	44	41.2	41.83	42.5	46.5	42	43.67
Tryp.500	32.4	35.1	30.5	32.67	34.4	36.5	35	35.3
Azo. brasilense	37.5	41.2	38.5	39.07	38.2	43.2	40.01	40.47
Azo. + Tryp.100	41.4	46.5	42.3	43.4	43	47.8	44	44.93
Azo. + Tryp.200	42.04	47.1	43	44.05	43.4	48.9	45	45.77
Azo. + Tryp.500	34.2	36.8	34.1	35.03	35.2	39.4	37.2	37.27
Mean	37.32	40.93	37.58		38.63	42.89	39.99	
LSDat5% Stage : 0.46					0.18			
Treatment : 0.76					0.29			
Chlorophyll % (Maize)								
Control	33.4	35.6	37.7	35.6	33.7	36.2	38.4	36.1
Tryp.100	35.8	37.1	40.9	37.9	36.1	38.9	43.5	39.5
Tryp.200	37.6	39.5	44.3	40.5	38	41.7	48.4	42.7
Tryp.500	32.5	34.9	36	34.5	33.1	35.2	36.3	34.9
Azo. brasilense	36.2	37.2	41.6	38.3	36.5	39.1	45.6	40.4
Azo. + Tryp.100	39	40.8	45.4	41.7	39.4	42.9	49.8	44
Azo. + Tryp.200	40.5	43.9	48.7	44.3	41.2	46.4	51.3	46.3
Azo. + Tryp.500	35	36.5	39.5	37	35.4	37.3	41.2	38
Mean	36.25	38.19	41.76		36.68	39.71	44.31	
LSDat5% Stage : 0.13					0.13			
Treatment : 0.21					0.22			

increase recorded 1.84, 2.71 and 4.55 T/fed for grains, straw and biological yield in wheat respectively compared with the control in the second season. Treatment with 500 ppm tryptophan only reduced all yield parameters lower than the control during both seasons of the field experiment. It was explained that the gram-negative nitrogen-fixing rhizobacterium *A. brasilense* lives in close association with plant roots, where it has beneficial effects on plant growth and the yields of many crops of agronomic importance **(Okon & Labander-Gonzalez, 1994 and Okon & Vanderleyden, 1997).**

Yield of maize

The result obtained in **Table (8)** showed a positive response of maize growth to inoculation with *Azospirillium* and tryptophan at concentration of 200 ppm where yield product, oil %, weight of 100 grains, weight of kerneal and weight of stalks were significantly improved while treatment with 500 ppm tryptophan only showed inhibitory effect for all yield parameters during the two seasons of the field experiment. In table 8 it was clear that treatment with Azo. + Tryp.200 at second stage of the second season reached the maximum values for oil %, weight of 100 grains and weight of grains being 23.7 %, 59.9 gm and 3.5 T/fed compared with (16.2%, 43.9 gm and 1.5 T/fed) in the control. Treatment with 500 ppm tryptophan only caused the lowest response (13.5%, 41 gm and 0.95 T/fed) for the same parameters.

Conclusion: It can be concluded from the above-mentioned results that biofertilization with *Azospirillum brasilense* showed a powerful effects on plant growth and yield on gramineae(what-maize) and microbial community in rhizosphere of inoculated plant. Mixed application of *A.brasilense* and tryptophan gave positive results higher than *Azospirillum* inoculation alone or tryptophan alone, and also lead to formation of paranodules on root hairs of treated plant which increase the enhancement effect as well as reduce the dose of mineral nitrogen fertilizer applied.

Table 7. Effect of inoculation with *Azospirillum brasilense* and/ or tryptophan on yield product of wheat in two seasons

	First season					Second season				
	No. of tillers	Wt. of 100 grains (gm)	Grains (T /fed.)	Straw (T/fed.)	Biological yield (T/fed)	No. of tillers	Wt. of 100 grains(gm)	Grains (T /fed.)	Straw (T/fed.)	Biological Yield (T/fed)
Control	3	3.3	0.87	1.84	2.71	3	3.34	0.96	1.93	2.89
Tryp.100	4	3.46	0.95	2.16	3.11	5	3.5	1.21	2.23	3.31
Tryp.200	4	3.65	1.16	2.54	3.7	5	3.7	1.4	2.59	3.99
Tryp.500	3	3.22	0.83	1.79	2.62	3	3.31	0.75	1.76	2.71
Azo. brasilense	4	3.58	1.13	2.49	3.62	5	3.62	1.35	2.6	3.95
Azo. + Tryp.100	5	3.74	1.28	2.6	3.88	6	3.81	1.46	2.64	4.1
Azo. + Tryp.200	5	3.8	1.39	2.64	4.03	7	3.86	1.84	2.71	4.55
Azo. + Tryp.500	3	3.38	0.92	2.06	2.98	3	3.4	1.08	2.11	3.32
LSD at 5%	1.6	1.1	0.36	0.57	1.3	1.3	0.72	0.64	0.67	0.66

Tryp: tryptophan, Az: ***Azospirillum brasilense*** , wt. : weight , T: Ton , fed. : feddan

Table 8. Effect of inoculation with *Azospirillum brasilense* and/ or tryptophan on yield product of maize in two seasons

	First season					Second season				
	Oil %	Wt. of 100 grains (gm)	Wt. of Grains T/fed	Wt. of kerneals T/fed	Wt. of stalk T/fed	Oil %	Wt. of 100 grains gm	Wt. of Grains T/fed	Wt. of kerneals T/fed	Wt. of stalk T/fed
Control	15.2	42.1	1.4	0.27	3.24	16.2	43.9	1.5	0.28	3.26
Tryp.100	17.8	45.2	1.8	0.34	3.38	18.1	46.2	2.7	0.35	3.41
Tryp.200	19.5	48.5	2.6	0.46	3.69	19.8	48.4	3	0.48	3.95
Tryp.500	13.5	41	0.95	0.26	2.49	13.9	42.3	1.1	0.27	2.57
Azo. brasilense	18	46	1.9	0.38	3.5	18.3	46.9	2.8	0.39	3.43
Azo. + Tryp.100	19.8	49.4	2.8	0.49	3.87	20.4	50.2	3.1	0.49	4.08
Azo. + Tryp.200	23	54.3	3.2	0.54	4.3	23.7	59.9	3.5	0.56	4.8
Azo. + Tryp.500	17.5	44.6	1.6	0.29	3.27	17.8	45.8	1.72	0.29	3.31
LSD at 5%	0.7	1.95	0.68	0.22	0.67	1.2	1.13	1.04	0.19	0.74

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حوليات العلوم الزراعية
جامعة عين شمس ، القاهرة
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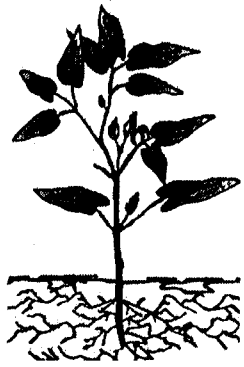

دراسة التأثير الحفزى للتلقيح بالازوسبيريللم على تكوين اشباه العقد فى النجيليات

[٧]

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اجريت تجربتين حقليتين لنباتى القمح والذرة موسمين متتاليين لكل نبات بمحطة بحوث مريوط التابعة لمركز بحوث الصحراء باستخدام التلقيح بـ *Azospirillum brasilense* والتربتوفان (بثلاث تركيزات ١٠٠-٢٠٠-٥٠٠ جزء فى المليون) فرادى ومجتمعين. وقد اوضحت النتائج التأثير الايجابى للتلقيح بالازوسبيريللم على النبات والمحصول والمحتوى الميكروبى فى القمح والذرة . وقد أعطت المعاملة بالازوسبيريللم مع التربتوفان تركيز ٢٠٠ جزء فى المليون اعلى استجابة مقارنة بباقى المعاملات. كما اظهرت المعاملة بالتربتوفان فقط بتركيز ٥٠٠ جزء فى المليون تثبيط واضح مقارنة بالكنترول. كما ادت المعاملة بالازوسبيريللم والتربتوفان الى ظهور اشباة عقد على الشعيرات الجذرية للنباتات المعاملة والتى ادت الى زيادة التأثير الايجابى على النباتات والمحصول.

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