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Effect of Bio- and Chemical Fertilization on Growth of Sunflower (*Helianthus annuus* L.) at South Valley Area

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Abstract: Effect of bio- and chemical fertilizers, separately and in different combinations, on the growth of sunflower (*Helianthus annuus* L.) was studied to reduce the chemical fertilizers used, maximizing their use efficiency and to obtain highest growth and productive parameters. The biofertilizers used as inoculums for seeds treatment of sunflower cv. Geza 1 were *Azospirillum* (nitrogen fixing bacteria, N.F.B.) and *Bacillus polymyxa* (phosphate dissolving bacteria, P.D.B.) and their mixture. Both bacterial inoculants and their mixture show an increase in growth parameters, nutrients content and yield when compared to the control (full dose of NPK chemical fertilization). The result reveals that biofertilization treatments of *Azospirillum* + *Bacillus* plus 100% chemical fertilizers produced the highest values in all growth and yield parameters compared with the control (full dose of chemical fertilization alone). The results also indicated that biofertilization, beside its ability to improve the nutrient supply in the soil, also increases the efficiency of added chemical fertilization.

Key words: *Helianthus*, ornamental, flowering date, *Azospirillum*, *Bacillus*, yield parameters

INTRODUCTION

Nitrogen and Phosphorus are two of the major essential elements for growth and development of ornamental crops, they are provided to plants in the form of chemical fertilizer. Such kind of fertilizers poses a health hazard and microbial population problem in soil besides the high cost of their application. In addition, soluble phosphorus under Egyptian soil condition converts to unavailable form because of phosphorus fixation. In such a situation the biofertilizers play a major role (Tiwary *et al*., 1998; Mahfouz and Sharaf-Eldin, 2007; Hasaneen *et al*., 2009).

Biofertilizers are the formulation of living microorganisms, which are able to fix atmospheric nitrogen and convert insoluble phosphorus to available one for the use of plants. Biofertilizers are inputs containing microorganisms which are capable of mobilizing nutritive elements from non-usable to usable form through biological processes (Subba Rao, 1993).

The beneficial effects induced by the inoculated bacteria on plant growth and yield were attributed to one or more of the following factors:

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- Production of growth promoting substances such as gibberellines, cytokinins, IAA and other auxins that specially stimulate the root system and may also alter the endogeneus plant phytohormone balance (Dobbelaere *et al*., 1999)
- Improvement of water and nutrients uptake, especially those of limited availability in soil such as P, N and micronutrients (Bashan *et al*., 1990; Kilian *et al*., 2000)
- Fixation of molecular nitrogen in rhizosphere that becomes available to inoculated plant (Skvortsova *et al*., 1998)
- Production of antibiotic metabolites effective against soil borne pathogens (Kraus and Loper, 1990; Pietr *et al*., 1990; Thomashow, 1990)
- Production of B-group vitamins that promote rooting capacity and affect the population of microbial community (Rodelas *et al*., 1993; Revillas *et al*., 2000)

Many researchers have reported the beneficial effects of inoculated rhizobacteria and free living diazotrophs on plant growth and yield (Okon and Labandera-Gonzalez, 1994).

Biofertilizers are considered as an important part of environment friendly sustainable agricultural practices (Shehata and El-Khawas, 2003).

The genus *Helianthus* includes about 70-80 species, some of them are perennials, many are popular garden ornamentals. *Helianthus tuberosus* L. is grown throughout the world for its edible tubers; beach sunflower (*Helianthus debilis* Nutt.) is used as a border plant and ground cover. *Helianthus annuus* L. is one of the most important species and belongs to the family Asteraceae (Compositae), popularly known as sunflower. All sunflowers are good as ornamental plants and long lasting as cut flowers. Its seed is commonly used for medicinal scope and to get a vegetable oil in many parts of the world including Egypt. Seeds are also used to feed the birds (Gvozdenovic *et al*., 2009).

In the present study, the effects of biofertilizers with nitrogen fixers, phosphate dissolving bacteria and chemical fertilizers, separately and in different combinations on growth of sunflower *Helianthus annuus* under field conditions of qena area were studied aiming to reduce the chemical fertilizers used, maximizing their use efficiency and to obtain highest growth and productive parameters.

MATERIALS AND METHODS

The field experiment was conducted at the Experimental Farm of Ornamental Plants Department, Faculty of Agriculture, South Valley University, Qena (Egypt) located at latitude 26°11' 25 N" and longitude 32° 44' 42" E, during the two successive seasons of 2008 and 2009. The soil of the experimental field was a salty loam and its characteristics are presented in Table 1.

Plant Materials and Biofertilizers

Seeds of sunflowers (*Helianthus annuus* L.) cv. Geza 1 were used. The biofertilizers used as inoculums for seed treatment were *Azospirillum* (nitrogen fixing bacteria, N.F.B.) and *Bacillus polymyxa* (phosphate dissolving bacteria, P.D.B.) and their mixture. All strains were locally isolated from soil rhizosphere of different plants during a previous study (Badawy *et al*., 2003). Seeds were coated with 10% gum Arabic as an adhesive and rolled into the biofertilizers treatments. Then seeds were sown directly on 31 May 2008 and 2 June 2009 for first and second seasons, respectively.

Table 1: Some physical and chemical characteristics of the used soil

Characteristics	Value
Physical properties	
Sand (%)	64.91
Silt (%)	13.49
Clay (%)	21.6
Soil texture	Sandy clay loam
SP (%)	32.0
Chemical properties	
pH	8.05
Ec_e (dS m^{-1})	3.68
CaCO (%)	6.9
Ca^{++} (meq L^{-1})	13.76
Mg^{++} (meq L^{-1})	11.79
K^{+} (meq L^{-1})	0.37
Na^{+} (meq L^{-1})	12.97
$CO_3^{-2} + HCO_3^{-}$ (meq L^{-1})	13.76
$SO4^{=}$ (meq L^{-1})	7.71
Cl^{-} (meq L^{-1})	15.26

SP (%): Saturation percentage

Table 2: Details of recommended doses of nitrogen and phosphorus chemical fertilization at various levels

Treatments	Recommendation
Control (T1)	Full recommended dose of phosphorus, nitrogen and potassium
P.D.B (T2)	Inoculation with phosphate dissolving bacteria (*Bacillus polymyxa*) without chemical fertilization
P.D.B + 25% (T3)	P.D.B + 25% of recommended dose of phosphorus and nitrogen
P.D.B + 50% (T4)	P.D.B + 50% of recommended dose of phosphorus and nitrogen
P.D.B +100% (T5)	P.D.B + 100% of recommended dose of phosphorus and nitrogen
N.F.B (T6)	Inoculation with Nitrogen fixing bacteria (*Azospirillum lipoferum*)
N.F.B + 25% (T7)	N.F.B + 25% of recommended dose of phosphorus and nitrogen
N.F.B + 50% (T8)	N.F.B + 50% of recommended dose of phosphorus and nitrogen
N.F.B +100% (T9)	N.F.B + 100% of recommended dose of phosphorus and nitrogen
N.F.B + P.D.B (T10)	Inoculation with mixture of Nitrogen fixing bacteria and phosphate dissolving bacteria
N.F.B + P.D.B + 25% (T11)	(N.F.B +P.D.B)+25% of recommended dose of phosphorus and nitrogen
N.F.B + P.D.B + 50% (T12)	(N.F.B +P.D.B)+50% of recommended dose of phosphorus and nitrogen
N.F.B + P.D.B + 100% (T13)	(N.F.B +P.D.B)+100% of recommended dose of phosphorus and nitrogen

P.D.B: Phosphate dissolving bacteria and N.F.B: Nitrogen fixing bacteria

Experimental Design

The experiment was carried out in a complete randomized blocks design with thirteen treatments and three replications. Each treatment had 16 plants/replicate, so reaching a total of 624 plants by whom (13×3×16). There were four ridges for each treatment/replicate and four holes in each row with distance 30 cm between them, four seeds were placed in each hole and thinning was made after two weeks to maintain one plant $hole^{-1}$.

Control treatments were supplied with the full recommended dose of phosphorus (100 kg fed^{-1} of supper phosphate 15.5% P_2O_5), nitrogen (100 kg fed^{-1} of ammonium nitrate 33% N) and full dose of potassium fertilization (50 kg of potassium sulphate 48% K_2O) which was applied for all experiment treatments.

The doses of recommended nitrogen and phosphorus chemical fertilization were manipulated at various levels (0, 25, 50 and 100%) in different combinations with PGPR as shown in Table 2.

Phosphorus fertilizer was added before planting during the soil preparation. The amount of nitrogen chemical fertilization was added at two equal batches, the first one was added after thinning with all amount of potassium and after two weeks from the first addition, the second amount of nitrogen was added.

Morphological Estimation

The morphological characters such as number of leaves, leaf area of the eight leaf node, plant fresh and dry weight and roots dry weight were estimated after 70 days from sowing. Number of ray florets, flowering date as days from sowing to full opening, inflorescence diameter, seed weight head^{-1} and total yield plant^{-1} were also estimated.

Biochemical Estimation

The biochemical constituents such as total chlorophyll content (SPAD unit) was determined with SPAD meter apparatus as described by Yadava (1986). Leaf nitrogen percentage was determined by the micro-Kjeldahl method as recommended by Bremner and Mulvany (1982). Total contents of nitrogen and phosphors were calculated by multiplying the nitrogen or phosphor percentage with dry weight of vegetative growth. Available phosphorus was determined by the chlorostannous phosphomolybdic acid method (Jackson, 1973). Experiments for the determination of above mentioned cellular constituents were carried out using dried leaves except for chlorophyll content where, fresh leaves were used.

Statistical Analysis

All obtained data were subjected to the analysis of variance according to the procedure outlined by Steel and Torrie (1982). The differences between means of the different treatments were compared using the least significant difference (LSD) at 5%.

RESULTS AND DISCUSSION

Seeds subjected to all biofertilization treatments with 100% chemical fertilization significantly recorded highest leaves number as shown in Table 3. The plants treated with biofertilization plus 100% chemical fertilization were significantly higher by 9.84, 8.9 and 10.16% as a result of inoculation with P.D.B alone, N.F.B alone and their mixture, respectively, than that of the 100% chemical fertilization alone (control). The same trends were also evidenced in the second season when the increase reached to 9.67, 14.0 and 16.0% for the previous treatments, respectively.

Higher leaf area was observed, during the two seasons, only in the plot treated with mixture of (N.F.B) and (P.D.B) plus 100% chemical fertilization compared to 100% chemical fertilization and other treatments (Table 2). The percentages of increase were 16.98 and 9.22% for the first and the second season, respectively.

These results are in agreement with those of Chandrasekar *et al*. (2005) who reported that the maximal plant height, number of leaves, leaf area and leaf length were observed in the plot treated with *Azospirillum* and 100% urea (N2), followed by *Azotobacter* and 100% urea.

Gholami *et al.* (2009) observed that Maize leaf number, shoot dry weight and leaf surface area were increased significantly by bacterial inoculation in both sterile and non sterile soil. In addition, these results were in agreement with those obtained by Ismail and Hasabo (2000), using commercial Egyptian biofertilizers (one of them was microbial containing nitrogen fixing bacteria and phosphate dissolving bacteria), found that all treatments significantly increased plant growth parameters compared with untreated plants., Similar trends were also observed by Galal *et al.* (2000) on wheat.

The stimulatory effects might be attributed to the activation of the growth of microflora including many plant growth stimulators (Fisinin *et al*., 1999).

Table 3: Effect of bio and chemical fertilizers on No. of leaves, leaf area, plant fresh and dry weight and roots dry weight of *Helianthus annuus* plants

	No. of leaves		Leaf area (cm^2)		Plant fresh weight (g $plant^{-1}$)		Plant dry weight (g $plant^{-1}$)		Roots dry weight (g $plant^{-1}$)	
Treatments	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	31.5	30.0	159.6	168.1	166.1	175.1	38.5	37.11	5.04	6.23
P.D.B	27.0	29.5	53.5	48.7	109.7	96.6	23.1	25.41	3.26	3.18
P.D.B + 25%	29.8	30.5	53.8	57.3	114.6	104.8	23.8	28.00	5.90	4.54
P.D.B + 50%	33.0	32.1	108.4	99.7	180.2	184.4	42.2	37.33	5.40	5.43
P.D.B + 100%	34.6	32.9	165.5	166.5	210.0	209.6	47.2	45.10	7.23	6.90
NFB	30.0	30.8	61.5	67.4	122.6	105.8	24.7	26.60	5.50	6.21
NFB + 25%	32.0	31.0	81.5	76.0	119.5	113.3	27.1	29.00	8.80	6.32
NFB + 50%	33.6	31.7	92.6	97.4	180.6	185.3	42.2	35.50	9.10	6.52
NFB + 100%	34.3	34.2	162.8	174.3	224.5	219.5	52.5	53.20	10.00	6.59
NFB + P.D.B	30.6	27.9	79.5	67.9	122.0	109.8	24.9	24.30	5.70	6.29
NFB + P.D.B + 25%	32.8	25.5	112.3	88.3	127.4	115.6	24.9	25.10	8.30	6.35
NFB + P.D.B + 50%	33.0	30.0	168.1	147.5	182.1	185.7	42.5	35.83	8.36	6.50
NFB + P.D.B + 100%	34.7	34.8	186.7	183.6	235.8	242.7	53.2	55.10	10.00	8.06
LSD 0.05	2.6**	2.6**	20.7**	20.1**	20.3**	12.5**	4.7**	37.11**	1.24**	1.52**

**Significant at p = 0.01

Highest plant fresh weight was recorded in the case of inoculation treatment with the mixture of nitrogen fixing bacteria and phosphate dissolving bacteria plus 100% chemical fertilizers as compared with 100% chemical fertilizers alone, followed by inoculation treatment with N.F.B and P.D.B singularly plus 100% chemical fertilizers dose Table 3. The increases in plant fresh weight in the first season were 41.96, 35.16 and 26.43% for the previous treatments, respectively. Similar trends were also seen in the second season, where the increases reached 38.61, 25.36 and 19.70% for the same treatments, respectively.

Data in Table 3 showed that the maximal plant dry weights were observed in the plot treated with mixture of N.F.B and P.D.B with 100% chemical fertilizers dose which recording highly significant increases reached 38.2% as compared with 100% chemical fertilizers only. Also, highly significant increases were recorded in case of the same chemical fertilizers dose and biofertilization treatments with P.D.B (22.6%) or N.F.B. (36.4%). The same trend was obtained in the second season. These results confirmed the outcomes of Shaukat *et al.* (2006), who stated that in *Helianthus annuus* var. sf-187, all the bacterial strains significantly stimulated the growth and yield parameters under field conditions, when compared with that of the control, associated with increase in auxin and protein contents as well as peroxidase and acid phosphate activities. Also, Ribaudo *et al.* (1998) reported that inoculation of maize plants with *Azospirillum* bacteria showed an appreciable increase in the mean of dry weight of shoot and roots.

Highest roots dry weights were obtained, during the first season, with treatments of biofertilizers with NFB plus 100% chemical fertilizers dose and mixture of NFB + PDB plus 100% chemical fertilizers (Table 3). The increase reached the 98.41% for both treatments. In the second season, the application of the mixture of NFB + PDB plus 100% chemical fertilizers was the treatment which recorded the highly significant increase in root dry weight (29.37%) compared with 100% chemical fertilizers dose.

The stimulatory effect of both biofertilizers used on growth parameters of sunflower are in accordance with the results obtained by Mahmoud and Amara (2000), Shehata and El-Khawas (2003), Shaukat *et al.* (2006) and Yasari and Patwardhan (2007).

The flowering date was significantly shortened in plants treated with different biofertilizers plus all rates of chemical fertilization compared with the control in both seasons

Table 4: Effect of bio- and chemical fertilizers on flowering date, No. of ray florets, inflorescence diameter, seeds weight head^{-1}, No. of seeds head^{-1}, 100 seeds weight and yield of *Helianthus annuus* plants

Treatments	Flowering date (days)		No. of ray florets		Inflorescence diameter (cm)		Seeds weight head^{-1} (g)		Yield (kg fed^{-1})	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	76.3	82.5	40.6	36.1	14.43	15.60	40.03	46.33	660.20	759.23
P.D.B	74.0	75.0	39.6	32.1	11.43	12.40	18.16	20.93	299.96	345.06
P.D.B + 25%	72.7	71.3	41.0	35.1	11.66	12.60	30.03	34.53	595.60	569.96
P.D.B + 50%	72.9	72.0	42.3	36.1	13.70	14.83	35.20	40.50	581.23	668.40
P.D.B + 100%	71.6	72.1	46.3	40.6	16.00	17.26	44.33	51.03	731.73	841.50
NB	74.0	75.2	30.3	36.4	8.36	9.03	13.30	15.26	219.03	251.90
NFB + 25%	73.2	70.8	39.3	34.2	10.10	10.90	16.03	18.40	264.50	304.13
NFB + 50%	73.2	71.0	42.0	37.0	12.70	13.66	32.53	37.46	537.27	617.90
NFB + 100%	72.6	71.6	45.6	37.2	16.60	17.96	46.13	53.00	751.00	875.17
NFB + P.D.B	73.0	76.0	35.6	33.7	10.10	10.93	16.06	18.50	265.03	304.18
NFB + P.D.B + 25%	72.1	72.9	36.0	32.2	13.40	14.53	21.33	24.50	351.90	404.66
NFB + P.D.B + 50%	71.6	72.0	39.6	35.7	14.23	15.40	36.66	42.10	604..50	695.20
NFB + P.D.B + 100%	70.6	69.0	45.0	35.9	18.10	19.56	50.16	57.70	827.87	952.07
LSD 0.05	2.5**	2.2**	3.5**	2.9**	3.38**	3.11**	6.97**	8.16**	128.90**	134.8**

**Significant at p = 0.01

as shown in Table 4. This result might be attributed to the stimulative effect of biofertilizers on vegetative growth as reported by Mahfouz and Sharaf-Eldin (2007).

Also, the number of ray florets was increased significantly over control with the application of different biofertilizer treatments plus 100% chemical fertilization as shown in Table 4, the increases reached 14.04, 12.31 and 10.84% as a result of applying both P.D.B, N.F.B and their mixture, respectively. In the second season, application of P.D.B plus 100% chemical fertilization was the only treatment which recorded significant increase (12.47%) over the control treatment.

With regard to inflorescence diameter, there was no significant increase over control treatment except for biofertilization treatments with mixture of phosphate dissolving bacteria and nitrogen fixing bacteria plus 100% chemical fertilizer in both seasons (Table 4). These increases reached 25.43 and 25.38% in the first and second seasons, respectively.

As regards yield parameters, the only highly significant increase on seeds weight head^{-1} over control was found with the biofertilization treatments with mixture of PDB and NFB plus 100% chemical fertilizer which recorded increases reaching 25.31 and 24.54% for the first and second seasons, respectively.

With regard to total seed yield, the highest value was recorded with mixture of phosphate dissolving bacteria, nitrogen fixing bacteria plus 100% chemical fertilizer (Table 4). The increase reached 25.39% in both seasons.

Previous studies indicate that the growth and yield attributes exhibited maximum values in treatments of bacteria inoculums and seedling treatments in combination with 75% and 100% nitrogen application (Hedge *et al.*, 1999; Selvakumari *et al.*, 2000).

Chandrasekar *et al.* (2005) reported that both morphological and yield parameters showed a better results through the combination of biofertilizers and chemical fertilizers than using either method alone. They also reported that the addition of *Azospirillum* with 100% urea produced the highest yield compared with 100% chemical fertilizer alone.

The total chlorophyll content of sunflower leaves in the first season increased by the application of biofertilizers as shown in Table 5. The highest significant increases in chlorophyll were obtained from treatments with the application of the biofertilizers plus 100% chemical fertilization dose. These increases reached 35.29, 34.67 and 31.27% for phosphate

Table 5: Effect of bio- and chemical fertilizers on Chlorophyll content (SPAD unit), nitrogen percentage (N%), total nitrogen content, phosphor percentage (P%) and total phosphor content of *Helianthus annuus* plants

Treatments	Chlorophyll (SPAD unit) 1st season	Chlorophyll (SPAD unit) 2nd season	N% 1st season	N% 2nd season	Total N content ($mg\ plant^{-1}$) 1st season	Total N content ($mg\ plant^{-1}$) 2nd season	P% 1st season	P% 2nd season	Total P content ($mg\ plant^{-1}$) 1st season	Total P content ($mg\ plant^{-1}$) 2nd season
Control	32.3	34.4	0.43	0.40	165.83	146.09	0.07	0.06	27.76	27.76
P.D.B	24.9	26.2	0.19	0.20	43.66	49.90	0.02	0.02	5.51	5.51
P.D.B + 25%	31.5	32.5	0.30	0.28	71.20	77.14	0.03	0.02	7.12	7.12
P.D.B + 50%	32.8	33.0	0.42	0.40	176.96	146.70	0.06	0.05	25.28	25.28
P.D.B + 100%	43.7	36.0	0.59	0.60	281.55	265.69	0.07	0.07	35.45	35.45
NFB	26.9	24.1	0.29	0.32	71.67	83.75	0.03	0.03	8.65	8.65
NFB + 25%	32.9	31.6	0.38	0.40	103.23	114.26	0.04	0.03	10.86	10.86
NFB + 50%	40.5	34.1	0.44	0.48	185.38	167.80	0.05	0.04	23.17	23.17
NFB + 100%	43.5	36.8	0.59	0.62	310.54	324.65	0.07	0.07	40.52	40.53
NFB + P.D.B	27.9	28.0	0.30	0.32	74.60	76.35	0.03	0.02	7.46	7.46
NFB + P.D.B + 25%	36.7	34.0	0.41	0.40	102.22	98.79	0.03	0.02	8.97	8.97
NFB + P.D.B + 50%	37.5	35.2	0.50	0.48	212.50	168.84	0.05	0.04	22.10	22.10
NFB + P.D.B + 100%	42.4	37.4	0.64	0.60	340.62	324.89	0.08	0.08	45.17	45.17
LSD 0.05	2.9*	2.7*	0.09**	0.10**	60.53**	20.91**	0.04^{NS}	0.03^{NS}	4.17**	3.76**

^{NS}, * and **Not significant and significant at $p = 0.05$ and 0.01, respectively

dissolving bacteria, nitrogen fixing bacteria and their mixture compared with 100% chemical fertilizers alone, respectively.

The beneficial effect of bacterial inoculation on increasing chlorophyll content might be due to the supply of higher amount of nitrogen to growing tissue and organs supplied by N2 fixing *Azospirllum.* Rukmani (1990) demonstrated the effect of *Azospirillum* on various growth and yield characters in Okra where, the treatment with *Azospirillum* resulted in significant increase in total chlorophyll content.

Moreover, the results of the second season showed that the application of the mixture of PDB and NFB plus 100% chemical fertilizer was the only treatment recorded significant increase in chlorophyll compared with the control treatment.

The results shown in Table 5 showed highly significant increases in the nitrogen percentage and total content only with inoculation treatments with PDB, NFB and their mixture plus 100% chemical fertilizer. The increase in nitrogen percentage reached 37.21, 37.21 and 48.84% for the three previously cited treatments, respectively in compared with control. Also, total content increased significantly by 69.78, 87.26 and 105.40% for the same treatments compared with control. The results of the second season showed the same trend obtained in first one (Table 4).

The effect of biofertilizers on phosphorus percentage showed that no differences was found between the control and the same treatment plus biofertilizers, while highly significant increase in total P content of sunflower plants was recorded with all biofertilizers treatments plus 100% chemical fertilizer (Table 5). The highest significant increases were obtained by biofertilizers mixture of PDB and NFB which reached 62.71%. Data of the second season showed the same trend of the first one.

The biofertilizers application had stimulated nutrients accumulation and plant growth comparable to the control with full inorgamic fertilization (Amir *et al.*, 2003). The application of biofertilization technology to coarse-textured soil with low fertility had a positive effect on plant growth, N-gained from air and enhancement of fertilizer uptake (Galal *et al.*, 2000).

It was found by Sarawgi *et al.* (1999) that uptake of N and P increased with application of phosphate-solubilizing bacteria.

In addition, Goel *et al.* (1999) reported that the inoculation with certain Plant Growth-Promoting Rhizobacteria (PGPR) may enhance crop productivity either by making the other nutrients available or protecting plant from pathogenic microorganisms.

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