



Effect of Phosphate Solubilizing Microorganisms and Phosphorus Levels on Growth Yield and Quality of Chickpea (*Cicer arietinum* L.) in Inceptisol

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A field study was conducted to know the "Effect of phosphate solubilizing microorganisms and phosphorus levels on growth, yield and quality of chickpea (*Cicer arietinum* L.) in inceptisol." The experiment was laid in Factorial Randomized Block Design (FRBD) with sixteen treatments, replicated thrice during *rabi* season of 2019-20 at the Department Research Farm of SSAC, College of Agriculture, Latur. The treatments comprises four main (absolute control, *Bacillus megaterium*, *Aspergillus niger* and *Aspergillus awamori* @ 10 ml kg⁻¹ seed treatment) and four sub treatments (0,45,60 and 75 P₂O₅ kg ha⁻¹). The results indicated that, the incorporation of phosphate solubilizing

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microorganisms viz. *Aspergillus awamori* @ 10 ml kg⁻¹ seed in combination with soil application of 75 P₂O₅ kg ha⁻¹ found to be effective in improving growth and yield attributing characters viz. number of root nodules, fresh and dry weight, chlorophyll content, grain yield and straw yield as compared to *Aspergillus niger* and *Bacillus megaterium* along with 60 P₂O₅ kg ha⁻¹ and over control. Further results revealed that test weight and protein percentage was significantly influenced with the seed treatment of *Aspergillus awamori* @ 10 ml kg⁻¹ seed in combination with application of 75 P₂O₅ kg ha⁻¹.

Keywords: Chickpea; microorganisms; phosphorus; quality; yield.

1. INTRODUCTION

Chickpea is the *rabi* pulse crop grown in country supplementing protein (17-25 per cent), amino acid, Vit. A, Vit. C, Vit. B, Vit. K, source of folic acid for demand of vegetarian diet, also it plays a significant role in improving soil fertility by fixing the atmospheric nitrogen. Chickpea leaves provide substantial amount of residual nitrogen for subsequent crops and add plenty of organic matter to maintain and improve soil health and fertility [1].

Phosphorus can be termed as 'life mineral' because of its crucial role in metabolic and energy transfer reactions in plant. Phosphorus is an essential element in DNA and RNA that contain the genetic code of the plant to produce protein and other compounds essential for plant structure, seed yield and also associated with increased root growth, chlorophyll content, and N₂-fixation in legumes. The phosphorus deficiency leads to stunted root and shoot growth, bluish green coloration of leaf, delayed maturity and poor grain development in cereals. Thus, phosphorus has become a major constrain in agricultural production mainly because of its fixation in soils involving both adsorption and precipitation reactions. The rate and magnitude of phosphate adsorption depends upon the properties of soils and phosphorus resources Barros et al. [2] Boparai and Sharma [3]. Use of phosphorous in soils Phosphorous Solubilizing Microorganisms (PSMs) and unlike bacteria, soil fungi also have ability to are capable to convert insoluble phosphorous to soluble forms can function as biofertilizers to increase the native phosphorous in soil [4]. Low fertility, particularly phosphorus deficiency is one of the major constrains to increase the chickpea productivity [5]. Use of biofertilizers is low-cost renewable source of plant nutrients, which supplement chemical fertilizer. PSB solubilize insoluble phosphorus compounds by exerting organic acids, which is the primary mechanism of

solubility of insoluble inorganic phosphates. Besides organic acids, production of chelating substances, mineral acids and proton extrusion also involved [6]. Mittal et al. [7] found that seed inoculation of chickpea with *Aspergillus awamori* increased shoot height by 7-12 per cent, a nearly three fold increase in seed weight as compared to un inoculated control. Seed inoculation with *Aspergillus awamori* increased the growth, total P content and biomass of mungbean [8].

2. MATERIALS AND METHODS

The field experiment was conducted during the *rabi* season of 2019-20 at research farm of SSAC, College of Agriculture, Latur using chickpea crop (Var. BDNG-797) to evaluate the interactive effect of phosphate solubilizing microorganisms and phosphorus levels on growth parameter, grain yield and seed quality of chickpea. After completion of preparatory tillage operations, the experiment was laid out in Factorial Randomized Block Design (FRBD) with sixteen treatments replicated thrice. Organic manures i.e. FYM was applied at the rate of 5 t ha⁻¹ prior to 15 days of sowing of chickpea crop and all the plots were fertilized with recommended dose of fertilizer NPK (25:50:00 kg ha⁻¹) was applied as a basal dose through urea, SSP treatment wise at the time of sowing. The treatments comprised a seed treatment T₀: Control, T₁: *Bacillus megaterium* @ 10 ml kg⁻¹ seed, T₂: *Aspergillus niger* @ 10 ml kg⁻¹ seed, T₃: *Aspergillus awamori* @ 10 ml kg⁻¹ seed as a main treatments and application P₀: 0 P kg ha⁻¹, P₁:45P kg ha⁻¹, P₂:60P kg ha⁻¹, P₃:75P kg ha⁻¹ as a sub main treatments.

Chickpea seed was sown on 09th October 2019 by dibbling method as per randomly replicated plot having size 3× 2 m² maintaining row to row spacing of 30 cm and plant to plant 10 cm and adopting a seed rate of 80 kg ha⁻¹. After sowing, seed was covered with soil. Sowing depth was kept approximately 5 cm. The crop was

harvested at maturity stage on 22 January 2020. The observation recorded viz., number of the nodules per plant was recorded at 45 and 60 DAS. The fresh weight and dry weight was also weighed in grams. The seed yield, fodder yield were recorded at harvest stage. Quality parameter like protein, and test weight value were recorded. The data collected from the above observation were analyzed statistically by the procedure prescribed by Panse and Sukhatme [9].

3. RESULTS AND DISCUSSION

3.1 Effect of Phosphate Solubilizing Microorganisms and Phosphorus Levels on Growth Parameters of Chickpea

3.1.1 Total number of root nodule

Total number of root nodule per plant expressed in table 1. It was observed that, among the different treatments, total number of root nodule per chickpea plant were recorded significantly maximum (15.83 and 18.50 per plant) with seed inoculation of *Aspergillus awamori*, followed by declined up to

13.33 to 16.00 with *Aspergillus niger* and 13.16 and 14.75 with *Bacillus megaterium* at 45 and 60 DAS, respectively. Whereas, the number of nodules per plant was recorded minimum in control at 45 and 60 DAS (i.e. 10.83 and 13.33 respectively). The data showed that maximum root nodules of chickpea (15.67 and 18.41) per plant was recorded with application @ 75 kg P₂O₅ ha⁻¹ increased at 45 and 60 DAS respectively. The number of root nodules per plant was further decreased to minimum (11.58 and 14.16) in control at 45 and 60 DAS of chickpea respectively. The interaction effect of phosphate solubilizing microorganisms and phosphorus levels was found significant at 45 and 60 DAS. The increase in the root nodules per plant of chickpea due to seed inoculation of *Aspergillus awamori* increased the availability of soluble phosphorus by production of organic acid, this acid solubilize unavailable phosphate to available phosphorus helps to enhance nitrogen fixation which leads to increase in the number of root nodules per plant of chickpea. Similarly, phosphorus plays an important role in nodule initiation and increase the root proliferation thereby increase in the root nodules [10]. Similar results also reported by Vidhyashree et al. [11].

Table 1. Nodules per plant of chickpea as influenced by phosphatesolubilizing microorganisms and phosphorus levels

PSM	Phosphorus levels (P ₂ O ₅ kg ha ⁻¹)				Mean
	P ₀	P ₄₅	P ₆₀	P ₇₅	
Number of nodules per plant at 45 DAS					
T ₀	10.00	10.33	10.33	12.67	10.83
T ₁	11.67	13.00	13.33	14.67	13.16
T ₂	11.67	12.67	13.00	16.00	13.33
T ₃	13.00	15.00	16.00	19.33	15.83
Mean	11.58	12.75	13.17	15.67	
	T		P		TXP
SE	0.20		0.20		0.40
C.D at 5%	0.57		0.57		1.15
Number of nodules per plant at 60 DAS					
T ₀	11.66	12.66	13.33	15.66	13.33
T ₁	13.33	14.00	14.66	17.00	14.75
T ₂	14.33	15.66	15.00	19.00	16.00
T ₃	17.33	15.66	19.00	22.00	18.50
Mean	14.16	14.50	15.50	18.41	
	T		P		TXP
SE	0.22		0.22		0.45
C.D at 5%	0.65		0.65		1.31

3.1.2 Fresh and dry weight of root nodules

Data in respect of fresh and dry weight of root nodules of chickpea presented in the Table 2. Among the phosphate solubilizing microorganisms seed inoculation of *Aspergillus awamori* recorded significant fresh and dry weight of root at 45 DAS (0.23 and 0.13 g) and at 60 DAS (0.54 and 0.33 g). While minimum fresh and dry weight of nodule was recorded in control (0.12 and 0.05 g). The similar pattern was observed in fresh and dry weight at 60 DAS. Among the different levels of phosphorus application at @ 75 kg P₂O₅ ha⁻¹ recorded maximum fresh (0.20 and 0.38 g) and dry weight (0.11 and 0.22 g) of root nodules at 45 and 60 DAS respectively, over rest of the phosphorus levels. While, minimum fresh (0.16 g) and dry weight (0.07 g) was recorded with no application of phosphorus at 45 DAS. Similar trend in fresh and dry weight of nodule per plant was also noticed at 60 DAS. The interaction effect of phosphate solubilizing microorganisms and phosphorus levels showed non-significant effect on fresh weight at 45 DAS and dry weight at 45 and 60 DAS. However, it was showed significant effect on fresh weight of root nodules at 60 DAS only. Improvement in nodulation by the application of phosphate solubilizing microorganisms could be attributed to a greater solubility and availability of phosphate, which is essential for nodule development [10]. Similar results were also reported by Kumawat et al. [12] and Singh et al. [13].

3.1.3 Chlorophyll content

The data furnished in Table 3 revealed that *Aspergillus awamori* recorded maximum chlorophyll a, chlorophyll b and total chlorophyll (2.06, 1.02 and 3.08 mg g⁻¹, respectively) at 45 DAS followed by *Aspergillus niger* (1.8, 0.92 and 2.70 mg g⁻¹ respectively) and *Bacillus megaterium* (1.52, 0.81 and 2.33 mg g⁻¹).

Whereas, lowest values of chlorophyll a, chlorophyll b and total chlorophyll content was found in control (1.03, 0.63 and 1.66 mg g⁻¹). The application of phosphorus @ 75 kg P₂O₅ ha⁻¹ was influenced significantly and recorded maximum values of chlorophyll a, chlorophyll b and total chlorophyll content (1.77, 0.93 and 2.70 mg g⁻¹ respectively) in chickpea table 3. While minimum values of chlorophyll a, chlorophyll b and total chlorophyll content was found in control (i.e. 1.45, 0.79 and 2.24 mg g⁻¹). The combined

effect of *Aspergillus awamori* and application of phosphorus @ 75 kg P₂O₅ ha⁻¹ showed highest chlorophyll a, chlorophyll b and total chlorophyll content. The application of higher dose of phosphorus in combination of phosphate solubilizing microorganisms increased the chlorophyll content in leaves of chickpea might be ascribed to increase the solubility of phosphorus in the root environment thereby more utilization of phosphorus by plant for their growth and metabolic activity [14]. These results were inconformity with the finding of Vidhyashree et al., [11] reported that the seed inoculation with PSB + *Aspergillus awamori* significantly increased the total chlorophyll content of mungbean (4.13 mg g⁻¹).

3.2 Effect of Phosphate Solubilizing Microorganisms and Phosphorus Levels on Yield and Yield Attributes of Chickpea

3.2.1 Seed yield

The seed yield of chickpea as influenced by phosphate solubilizing microorganism narrated in table 4. The seed inoculation with *Aspergillus awamori* produced higher seed yield (1502.68 kg ha⁻¹) as compared to application of *Aspergillus niger* (1406.6 kg ha⁻¹) and *Bacillus megaterium* (1344.53 kg ha⁻¹). Significant improvement in yield was noticed with the application different phosphorus levels. Among the different phosphorus levels application of phosphorus @ 75 kg P₂O₅ ha⁻¹ recorded higher seed yield (1451.0 kg ha⁻¹) as compare to application of phosphorus @ 60 kg P₂O₅ ha⁻¹ (1403.25 kg ha⁻¹) and 45 kg P₂O₅ ha⁻¹ (1346.18 kg ha⁻¹). While, minimum seed yield was recorded in control (1322.77 kg ha⁻¹). Interaction between phosphate solubilizing microorganisms and phosphorus levels was found to be significant. The seed inoculation with *Aspergillus awamori* and application of phosphorus @ 75 kg P₂O₅ ha⁻¹ recorded highest value of seed yield than rest of the interactions. This may attributed *Aspergillus awamori* increase more values of growth parameters at almost all growth stages and helped in reducing P fixation by its chelating effect and also solubilized the unavailable form of P leading to more uptake of nutrients resulted in better growth of the plant [15]. The increase in seed yield due to increase in P level may be attributed to increase in the availability of P in soil. Similar findings are noted by Nawange et al. [16].

Table 2. Fresh and dry weight of root nodule in chickpea as influenced by phosphate solubilizing microorganisms and phosphorus levels

PSM	Phosphorus levels (P ₂ O ₅ kg ha ⁻¹)				Mean
	P0	P45	P60	P75	
Fresh Weight of Root Nodules at 45 DAS (g)					
T0	0.10	0.12	0.13	0.14	0.12
T1	0.14	0.16	0.18	0.19	0.17
T2	0.20	0.20	0.21	0.22	0.21
T3	0.22	0.23	0.21	0.26	0.23
Mean	0.16	0.18	0.18	0.20	
	T		P		TXP
SE	0.002		0.002		0.005
C.D at 5%	0.008		0.008		NS
Dry Weight of Root Nodules at 45 DAS (g)					
T0	0.02	0.03	0.04	0.10	0.05
T1	0.06	0.08	0.09	0.10	0.08
T2	0.10	0.11	0.12	0.11	0.11
T3	0.13	0.15	0.12	0.11	0.13
Mean	0.07	0.09	0.09	0.11	
	T		P		TXP
SE	0.003		0.003		0.008
C.D at 5%	0.010		0.010		NS
Fresh Weight of Root Nodules at 60 DAS (g)					
T0	0.17	0.18	0.19	0.20	0.18
T1	0.26	0.27	0.29	0.32	0.28
T2	0.33	0.33	0.38	0.40	0.36
T3	0.47	0.52	0.56	0.62	0.54
Mean	0.31	0.32	0.35	0.38	
	T		P		TXP
SE	0.008		0.008		0.015
C.D at 5%	0.017		0.017		0.04
Dry Weight of Root Nodules at 60 DAS (g)					
T0	0.06	0.08	0.12	0.11	0.09
T1	0.17	0.15	0.16	0.14	0.15
T2	0.17	0.17	0.20	0.22	0.19
T3	0.26	0.30	0.35	0.42	0.33
Mean	0.16	0.17	0.20	0.22	NS
	T		P		TXP
SE	0.012		0.012		0.024
C.D at 5%	0.035		0.035		NS

3.2.2 Straw yield

It is evidenced from the data presented in Table 5. The straw yield of chickpea as significantly influenced by phosphate solubilizing microorganism, The seed inoculation with *Aspergillus awamori* produced maximum straw yield of chickpea (1803.2 kg ha⁻¹) as compared to application of *Aspergillus niger* (1688.0 kg ha⁻¹) and *Bacillus megaterium* (1613.4 kg ha⁻¹). Among the different phosphorus levels application of phosphorus @ 75 kg P₂O₅ ha⁻¹

recorded higher straw yield (1741.19 kg ha⁻¹) as compare to application of phosphorus @ 60 kg P₂O₅ ha⁻¹ (1683.90 kg ha⁻¹) and 45 kg P₂O₅ ha⁻¹ (1615.41 kg ha⁻¹). However, the minimum straw yield was recorded in control (1587.32 kg ha⁻¹). Interaction between phosphate solubilizing microorganisms and different phosphorus levels on straw yield was found to be significant. The seed inoculation with *Aspergillus awamori* and application of phosphorus @ 75 kg P₂O₅ ha⁻¹ recorded higher value of straw yield than rest of

Table 3. Chlorophyll content as influenced by phosphate solubilizing microorganisms and phosphate levels in chickpea

Phosphorus levels (P_2O_5 kg ha ⁻¹)					
PSM	P0	P45	P60	P75	Mean
Chlorophyll a (mg g⁻¹)					
T0	0.81	0.89	1.18	1.27	1.03
T1	1.33	1.46	1.60	1.71	1.52
T2	1.74	1.77	1.82	1.87	1.80
T3	1.93	1.92	2.15	2.24	2.06
Mean	1.45	1.52	1.69	1.77	
	T		P		TXP
SE	0.01		0.01		0.02
C.D at 5%	0.05		0.05		0.09
Chlorophyll b (mg g⁻¹)					
T0	0.55	0.58	0.69	0.72	0.63
T1	0.78	0.79	0.82	0.86	0.81
T2	0.87	0.90	0.91	0.93	0.90
T3	0.97	0.94	0.99	1.22	1.02
Mean	0.79	0.80	0.85	0.93	
	T		P		TXP
SE	0.009		0.009		0.01
C.D at 5%	0.02		0.02		0.05
Total chlorophyll (mg g⁻¹)					
Phosphorus levels (P_2O_5 kg ha ⁻¹)					
PSM	P0	P45	P60	P75	Mean
T0	1.36	1.47	1.87	1.99	1.67
T1	2.11	2.25	2.43	2.57	2.33
T2	2.61	2.72	2.80	2.90	2.70
T3	2.86	2.80	3.46	2.43	3.08
Mean	2.24	2.32	2.46	2.70	
	T		P		TXP
SE	0.04		0.04		0.08
C.D at 5%	0.12		0.12		0.25

Table 4. Seed yield of chickpea as influenced by phosphate solubilizing microorganisms and phosphorus levels

Phosphorus levels (P_2O_5 kg ha ⁻¹)					
PSM	P0	P45	P60	P75	Mean
Seed Yield kg ha⁻¹					
T0	1198.85	1232.67	1303.35	1342.52	1269.35
T1	1344.64	1308.21	1354.48	1370.79	1344.53
T2	1345.15	1401.31	1416.74	1463.33	1406.63
T3	1402.44	1442.52	1538.42	1627.34	1502.68
Mean	1322.77	1346.18	1403.25	1451.00	
	T		P		TXP
SE	12.59		12.59		25.18
C.D at 5%	36.35		36.35		72.71

the interactions. This was mainly due to the fact that *Aspergillus awamori* and application of phosphorus @ 75 kg P_2O_5 ha⁻¹ increase in the availability of N and P caused better root

development, better growth and development of plants and better diversion of photosynthates towards sink Tagore et al. [17]. Kumar et al. [18] reported that the straw yield of chickpea

increased due to increase in phosphorus levels might be because of increase in the microbial activity in the root environment which accelerates cell division and formation of meristem.

3.3 Effect of Phosphate Solubilizing Microorganisms and Phosphorus Levels on Quality Parameters of Chickpea

3.3.1 Protein yield

The data pertaining to protein content and protein yield influenced significantly and presented in Table 6. The maximum protein yield (426.96 kg ha⁻¹) and protein content (23.62 %) was recorded with seed inoculation of *Aspergillus awamori* followed by *Aspergillus niger* (392.47 kg ha⁻¹ and 23.24 % respectively) and *Bacillus megaterium* (362.27 and 22.42% respectively). Whereas minimum protein yield (312 kg ha⁻¹) and protein content (23.62 %) was recorded in control. The data on effect of different levels of phosphorus application shows that maximum protein yield and protein content was noticed with the application of phosphorus @ 75P₂O₅ kg ha⁻¹ (412.15 kg ha⁻¹ and 23.62% respectively). The grain yield and protein content reduced significantly with decrement in phosphorus levels up to application of phosphorus @ 60 kg P₂O₅ ha⁻¹ (378.87 kg ha⁻¹ and 22.44 %) and 45 kg P₂O₅ ha⁻¹ (344.48 kg ha⁻¹ and 21.25 %). The minimum protein yield (357.26 kg ha⁻¹) and protein content (22.38 %) was recorded with no application of phosphorus. The interaction effect

of phosphate solubilizing microorganisms and phosphorus levels shows significant effect on protein yield and protein content. This may be due to both nutrients plays main role in protein metabolism. Similarly increase in protein content in seed due to application of phosphorus resembles to increase in higher uptake of N by plant which is main constituent of amino acid and building block of protein. Similar results are also noted by Mir et al. [19] and Singh et al. [13].

3.3.2 Test weight

The data furnished in Table 7 revealed that, the high value of test weight (158.00 g) was recorded with seed inoculation of *Aspergillus awamori* followed by *Aspergillus niger* (157.62 g) and *Bacillus megaterium* (157.14 g). The minimum test weight (156.99 g) was noticed in control. The application of @ 75 kg P₂O₅ ha⁻¹ recorded maximum test weight (158.49 g), followed by application of 60 kg P₂O₅ ha⁻¹ (157.71 g) and 45 kg P₂O₅ ha⁻¹ (156.49 g). The combined effect of phosphate solubilizing microorganisms and varied levels of phosphorus influenced and the increase in test weight due to combined application of phosphate solubilizing microorganisms and phosphorus levels attribute to increase in the symbiotic nitrogen fixation by adding more phosphorus which help to seeds for their development and ultimately increase the size of seed [17]. Dutta and Bandyopadhyay [14] reported that seed inoculation of *Rhizobium* and *Phosphobacterium* increase the test weight of chickpea. These findings are in line with the findings reported by Prajapati et al. [1].

Table 5. Straw yield as influenced by phosphate solubilizing microorganisms and phosphorus levels in chickpea

PSM	Phosphorus levels (kg P ₂ O ₅ ha ⁻¹)				Mean
	P0	P45	P60	P75	
	Straw Yield kg ha⁻¹				
T0	1438.6	1479.2	1564.0	1611.0	1523.2
T1	1613.6	1569.8	1625.4	1644.9	1613.4
T2	1614.2	1681.6	1700.1	1756.0	1688.0
T3	1682.9	1731.0	1846.1	1952.8	1803.2
Mean	1587.32	1615.41	1683.90	1741.19	
	T		P		TXP
SE	15.11		15.11		30.22
C.D at 5%	43.63		43.63		87.26

Table 6. Protein content and protein yield as influenced by phosphate solubilizing microorganism and phosphorus levels in chickpea

PSM	Phosphorus levels (kg P ₂ O ₅ ha ⁻¹)				
	P0	P45	P60	P75	Mean
	Protein Content (%)				
T0	18.92	19.97	20.71	22.04	20.41
T1	22.21	20.84	23.30	23.33	22.42
T2	24.16	22.75	22.43	23.63	23.24
T3	24.21	21.46	23.33	25.47	23.62
Mean	22.38	21.25	22.44	23.62	
	T		P		TXP
SE	0.12		0.12		0.25
C.D at 5%	0.36		0.36		0.73
	Protein Yield (kg ha⁻¹)				
T0	272.68	295.78	324.31	355.47	312.06
T1	358.61	327.56	378.88	384.05	362.27
T2	390.20	382.92	381.46	415.32	392.47
T3	407.57	371.66	430.85	497.77	426.96
Mean	357.26	344.48	378.87	413.15	
	T		P		TXP
SE	3.04		3.04		6.09
C.D at 5%	8.80		8.80		17.6
					0

Table 7. Test weight of chickpea seed as influenced by phosphate solubilizing microorganisms and phosphorus levels

PSM	Phosphorus levels (P ₂ O ₅ ha ⁻¹)				
	P0	P45	P60	P75	Mean
	Test Weight (g)				
T0	156.35	157.59	156.77	157.27	156.99
T1	155.88	156.86	157.70	158.13	157.14
T2	155.94	157.37	158.14	159.04	157.62
T3	157.77	156.45	158.25	159.51	158.00
Mean	156.49	157.07	157.71	158.49	
	T		P		TXP
SE	0.07		0.07		0.15
C.D at 5%	0.21		0.21		0.43

4. CONCLUSION

It can be inferred and concluded that incorporation of phosphate solubilizing microorganisms *viz. Aspergillus awamori* in combination with application of phosphorus @ 75 kg P₂O₅ ha⁻¹ improved growth attributes, yield attributes and quality of chickpea.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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