

ISSN : 1812-5379 (Print)
ISSN : 1812-5417 (Online)
<http://ansijournals.com/ja>

JOURNAL OF AGRONOMY



ANSI*net*

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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Phosphorus and Zinc Uptake and Their Interaction Effect on Dry Matter and Chlorophyll Content of Sweet Corn (*Zea mays var. Saccharata*)

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Abstract: Zinc and Phosphorus have antagonistic effect on the absorption and translocation of each other in plants. P-induced Zn deficiency is more common than Zn-induced P deficiency because growers commonly apply large amounts of P fertilizer as compared to Zn fertilizer. This research was conducted to examine the effect of different levels of Zn and P on the yield, Zn and P uptake and chlorophyll contents of corn plants. Sweet corn was grown in nutrient culture containing all combinations of Zn as $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ at levels of 0.0, 5.0, 10.0 and 20.0 mg L^{-1} and of P as KH_2PO_4 at levels of 0.0, 20.0, 40.0 and 80.0 mg L^{-1} . Zn_0P_{20} treatment produced the highest yield and the yields were decreased with P application in combination with Zn. The lowest dry weight of young corn plants was recorded under Zn_0P_{80} treatment at both harvesting times due to both Zn deficiency and P toxicity. Chlorophyll content decreased with high Zn and P applications and this can be attributed to the interactions of Zn and P with iron in the growth medium. Zn_0P_{80} treatment had the lowest Zn and the highest P uptake by shoot at 14 days after transplanting. The study has shown that Zn deficiency can enhance P uptake and translocation to such an extent that P may accumulate to toxic level in leaves. $\text{Zn}_{20}\text{P}_{80}$ treatment produced the highest Zn and P uptake by roots. Zn and P uptake by roots increased with increased Zn and P supply.

Key words: Zinc, phosphorus, corn, uptake, chlorophyll

INTRODUCTION

Zinc and Phosphorus are two essential nutrients for normal plant growth. Nutrient deficiencies in plant tissue usually occur when a nutrient is available in inadequate amounts. In this case, the nutrient is available in marginal to normal amounts but the excessive rates of antagonizing ions can cause the deficiency of the other nutrient ions in plant tissue. High available P can emphasize visual Zn deficiency symptoms in plants. This is called P-induced Zn deficiency. Zn-induced P deficiency is very rare because growers commonly apply the large amounts of P fertilizer as compared to Zn fertilizer (Edwards and Kamprath, 1974; Loneragan *et al.*, 1982; Schwartz *et al.*, 1987; Cakmak and Marschner, 1987).

Zn translocation from roots to shoots can be reduced by high levels of P application. High P concentration in roots causes Zn to be tied up within the root cells. Zn becomes part of the fabric of the root and thus, becomes unavailable for transport to the leaves. Under conditions of high Zn application, P may circumvent Zn in roots by the formation of Zn-phytate (Singh *et al.*, 1988; Loneragan and Webb, 1993; Hopkins *et al.*, 1998;

Rupa *et al.*, 2003). High amounts of Zn may be kept in the roots in this condition. Formation of sparingly soluble Zn phosphates in the apoplast of the root cortex might be a reason for uneven Zn distribution between roots and upper plant parts (Cakmak and Marschner, 1987). Youngdahl *et al.* (1977) stated that Zn-P interaction takes place within the plant. High levels of P supply, causes an increment of Zn concentration in the roots and a reduction of Zn concentration in the shoot. This suggest that Zn×P interaction occurs within the root, due to the rupture of sidelong Zn transport to the vascular tissue or linear transport from root to upper plant parts.

Snehi Dwivedi *et al.* (1975) in a pot experiment with maize suggested that at high P level, Zn is immobilized not only within the roots but also within the nodes of the stem. According to the authors, the P concentration reduced in the node and increased in the internode. It was suggested that the P formed organic substances which accumulated at the internodes. This accumulation weakens the translocation of Zn and when the Zn status increases, it competes with synthesized organic complex and moves upwards. For this reason, Zn accumulated at the nodes when P is available in low concentration.

Cakmak and Marschner (1987) found that in cotton grown in nutrient solution, excessive amounts of P had no significant effect on total Zn concentration in plant tissue but reduced the water-soluble Zn (which is physiologically active Zn) as a result of sparingly soluble Zn phosphates and increased the uptake and particularly P translocation to the shoots induced by Zn deficiency. Consequently, P accumulates in the leaves of Zn-deficient plants up to toxic levels and simultaneously reduces the physiological availability of Zn and emphasizes Zn deficiency.

Low Zn supply along with high P supply in the soil increases P concentration in plant tissues specifically old leaves which may induce P toxicity and contribute to symptoms resembling Zn deficiency (Loneragan *et al.*, 1982; Webb and Loneragan, 1988; Zhao *et al.*, 1998; Gill *et al.*, 2004). Results with subterranean clover (Loneragan *et al.*, 1979), corn and potato (Christensen and Jackson, 1981), okra (Loneragan *et al.*, 1982) and cotton (Cakmak and Marschner, 1993) support this hypothesis.

When both Zn and P are marginal in soils, P fertilization can promote plant growth and cause dilution in tissue Zn and consequently cause Zn deficiency in plant tissue (Loneragan *et al.*, 1979; Farah and Soliman, 1986; Singh *et al.*, 1988). Zn and P application together can remedy their deficiencies along with increasing plant growth (Gianquinto *et al.*, 2000; Stukenholtz *et al.*, 1966) demonstrated that increasing crop yield due to P application can reduce Zn concentration and uptake at upper plant parts. Loneragan *et al.* (1979) in their study on subterranean clover on siliceous sand observed that, when P concentrations in the plant were low, P application promoted growth and reduced Zn concentrations in upper plant parts by dilution effect. Increment of P concentrations more than 1% in the leaves, depressed plant growth and might cause P toxicity and contribute to symptoms resembling Zn deficiency. In these situations, application of Zn could alleviate P toxicity by promoting growth (Christensen and Jackson, 1981; Cakmak and Marschner, 1987).

There is a physiological relation between high membrane permeability and excessive accumulation of P. Zn deficiency can cause a physiological P deficiency which is shown as accumulation of P in the form of organic P fraction, including phospholipids needed for normal membrane structure and function (Marschner and Rimmington, 1986; Alou, 1989; Lu *et al.*, 1998). Rosell and Ulrich (1964) demonstrated that the soluble P fraction constitutes approximately 80% of the total P concentration of the normal leaves while, this proportion reduces to approximately 100% in Zn-deficient

plants. Zn containing enzymes necessary for phosphate metabolism may not be formed in deficient plants or the more stable organic P compounds may not be formed during Zn deficiency and were decomposed upon drying of the tissues for chemical analysis. Zn might also serve in membrane formation or stabilization, thus preventing collapse and loss of turgor of interveinal blade tissues.

This study was designed to investigate the effects of different levels of P and Zn on the concentration of these nutrients in different plant parts and dry matter yield and chlorophyll content at different vegetative growth stages.

MATERIALS AND METHODS

Experimental design: The nutrient culture method was used in this experiment. The experiment was performed at the Department of Land Management, Faculty of Agriculture, UPM. Sweet corn seeds hybrid 926 from Green World Genetics in Malaysia was used as the indicator plant. The seeds were soaked in water for 24 h and then germinated in rolled paper towels saturated with deionized water in the Laboratory at 24°C. The paper towels were kept saturated for 5 days and after this time, 5 days old sweet corn seedlings were transplanted into 2 L capacity plastic pots containing nutrient solution at the rate of four seedlings per pot.

The basic nutrient solution was according to Trostle *et al.* (2001) which contained 0.75 mM K₂SO₄, 0.65 mM MgSO₄, 0.1 mM KCl, 2 mM Ca(NO₃)₂, 1×10⁻⁴ mM CuSO₄, 0.1 mM EDTAFe, 1×10⁻³ mM MnSO₄, 1×10⁻² mM H₃BO₃ and 5×10⁻⁶ mM (NH₄)₆Mo₇O₂₄. pH was kept at 6.8 by using 0.1 M KOH or HCl solution. All combinations of Zn treatments (in the form of ZnSO₄·7H₂O) at levels of 0.0, 5.0, 10.0 and 20.0 mg L⁻¹ and of P treatments (in the form of KH₂PO₄) at levels of 0.0, 20.0, 40.0 and 80.0 mg L⁻¹ were included. The nutrient solution was changed every three days. The experimental design was a randomized complete block consisting of 5 blocks (replications). The plants were grown at ambient sunlight. The temperature and humidity were 24-33°C and 70-88%, respectively.

Measurements: Two plants per treatment were harvested 7 (V2- plants had 2 leaves) and 14 days (V5- plants had 5 leaves) after transplanting. The roots, stems and leaves were then separated. These harvesting times are mentioned as 7 and 14 DAT, respectively. The plant samples were rinsed with distilled water, dried at 70±2°C for 48 h, weighed and ashed at 300°C for 3 h followed by 500°C for 2 h in a muffle furnace. The ash was dissolved in concentrated HCl and 20% HNO₃. Phosphorus was measured using an autoanalyzer (8000 series, Lachat QuickChem FIA+, USA) and Zn was determined by

inductively coupled plasma optical emission spectrometry (ICP-OES) (Optima 8300, PerkinElmer, USA).

For chlorophyll content analysis, the fresh leaves (0.2 g) were put into the 20 mL glass vials. A 5 mL concentrated dimethyl sulfoxide (DMSO) was added and the glass vials placed in an oven at 70°C for 1 h. Under the wavelength of 645 and 663 nm, the content of chlorophyll b and chlorophyll a were measured, respectively using spectrophotometer (1000 series, Cecil CE 1011, Auckland, New Zealand). Arnon's (1949) equation was used to convert absorbance measurements to mg chlorophyll g⁻¹ in leaf tissue.

Calculations and statistical analysis: The dry weight of shoots and roots were multiplied by the concentrations of Zn and P to measure Zn and P uptake by shoots and roots, respectively.

Data were analyzed statistically by using SAS 9.2 software (SAS institute, Cary, NC, USA).

RESULTS AND DISCUSSION

Corn plants showed the highest dry matter yield at both 7 and 14 DAT in Zn₀P₂₀ treatment (Table 1). The yields were slightly lower in sweet corn with P application in combination with Zn than those without applied Zn and this is related to Zn×P interaction. The lowest dry weight of 16.4 and 35.2 mg plant⁻¹ recorded at 7 and 14 DAT, respectively under Zn₀P₈₀ treatment was due to both Zn deficiency and P toxicity (Table 1). The reduction in yield owing to low Zn concentration in the leaves may be

Table 1: Dry weight (mg plant⁻¹) and total chlorophyll content (mg g⁻¹ fresh weight) in sweet corn plants in nutrient solution with different P and Zn levels at 7 and 14 DAT

Treatment (mg L ⁻¹)		Dry weight (mg plant ⁻¹)		Total chlorophyll content (mg g ⁻¹ fresh weight)	
Zn	P	7 DAT	14 DAT	7 DAT	14 DAT
0	0	23.0 ^{cd}	48.8 ^{abc}	0.735 ^{ab}	0.695 ^{abc}
	20	34.4 ^a	61.0 ^a	0.739 ^{ab}	0.724 ^{ab}
	40	24.8 ^{bcd}	49.8 ^{abc}	0.845 ^a	0.725 ^a
	80	16.4 ^d	35.2 ^c	0.817 ^a	0.728 ^a
5	0	22.2 ^{cd}	47 ^{abc}	0.608 ^{abc}	0.659 ^{abc}
	20	20.4 ^{cd}	46.2 ^{abc}	0.444 ^{bc}	0.566 ^{abc}
	40	28.2 ^{abc}	53.4 ^{abc}	0.551 ^{abc}	0.589 ^{abc}
	80	21.8 ^{cd}	39.8 ^{bc}	0.578 ^{abc}	0.565 ^{abc}
10	0	23.0 ^{cd}	49.4 ^{abc}	0.645 ^{abc}	0.675 ^{abc}
	20	33.0 ^{ab}	57.2 ^{ab}	0.613 ^{abc}	0.645 ^{abc}
	40	24.0 ^{bcd}	55.4 ^{ab}	0.491 ^{bc}	0.599 ^{abc}
	80	25.6 ^{abc}	54.2 ^{ab}	0.505 ^{bc}	0.481 ^{abc}
20	0	27.0 ^{abc}	49.2 ^{abc}	0.601 ^{abc}	0.684 ^{abc}
	20	25.4 ^{abcd}	39.8 ^{bc}	0.514 ^{bc}	0.587 ^{abc}
	40	21.8 ^{cd}	55.6 ^{ab}	0.476 ^{bc}	0.436 ^{bc}
	80	27.0 ^{abc}	48.2 ^{abc}	0.401 ^c	0.412 ^c

within each column, same letter indicates no significance difference between treatments (p>0.05)

attributed to the decrease in the activity of the CO₂-fixing enzymes, ribulose 1-5 diphosphate carboxylase and carbonic anhydrase during the process of photosynthesis. These plants had also the highest leaf P/Zn ratio (Table 2). This ratio can be a better indicator of Zn nutritional status than Zn concentration alone but is dependent on plant species. P/Zn ratio of 25-154 was detected as the critical range for corn plants depending on experimental conditions (Prasad *et al.*, 1971). The highest P/Zn ratio was found in Zn₀P₈₀ treatment which was 117.2 and 227.0 at 7 and 14 DAT, respectively. This ratio was higher than the critical range at 14 DAT. The consequence of such an unfavorable ratio was a decrease of dry weight in comparison to other treatments. In Zn₂₀P₀ treatment, the P/Zn ratio dropped to 18.0 and 19.8 at 7 and 14 DAT, respectively. These ratios were lower than the critical range at both harvesting times. The P/Zn ratio was increased by the enhancement of plant growth. On the other hand, P-induced Zn deficiency intensified with plant age. Loneragan *et al.*, (1982) working with okra and Welch *et al.* (1991) on wheat, barley, tomato and subterranean clover demonstrated that the enhancement of P application in solution culture low in Zn accentuated symptoms resembling Zn deficiency in plants. They also observed that low Zn decreased dry weight of plant tops more markedly under high level of P supply than under low level of P supply.

The results showed that chlorophyll contents were increased in Zn₀P₄₀ and Zn₀P₈₀ treatments, while, it was decreased in Zn₅P₂₀, Zn₁₀P₄₀, Zn₁₀P₈₀, Zn₂₀P₂₀, Zn₂₀P₄₀ and Zn₂₀P₈₀ treatments at 7 DAT (Table 1) but at 14 DAT enhanced in Zn₀P₂₀, Zn₀P₄₀ and Zn₀P₈₀ treatments, while, its reduction was observed in Zn₂₀P₄₀ and Zn₂₀P₈₀ treatments. The decline of the chlorophyll content in

Table 2: Leaf P/Zn concentration ratio and root/shoot Zn uptake ratio in sweet corn plants in nutrient solution with different P and Zn levels at 7 and 14 DAT

Treatment (mg L ⁻¹)		P/Zn ratio		Root/shoot Zn Uptake Ratio	
Zn	P	7 DAT	14 DAT	7 DAT	14 DAT
0	0	107.2 ^{ab}	80.6 ^{bc}	0.84 ^{cd}	0.62 ^c
	20	106.2 ^{ab}	201.4 ^a	0.28 ^e	0.72 ^a
	40	78.2 ^{abcd}	119.0 ^b	0.44 ^e	0.78 ^{ab}
	80	117.2 ^a	227.0 ^a	0.36 ^e	1.84 ^{abcd}
5	0	32.6 ^{de}	33.0 ^d	0.68 ^{de}	0.78 ^{de}
	20	52.0 ^{bcde}	54.6 ^{cd}	0.98 ^{cd}	0.98 ^{bc}
	40	55.6 ^{bcde}	62.8 ^{cd}	1.16 ^{cde}	2.52 ^{abc}
	80	89.2 ^{abc}	49.4 ^{cd}	2.08 ^{abc}	1.36 ^{de}
10	0	28.0 ^{de}	29.0 ^d	0.90 ^{cde}	1.90 ^{abcd}
	20	31.2 ^{de}	55.6 ^{cd}	1.44 ^{bcde}	1.90 ^{abcd}
	40	46.2 ^{de}	44.4 ^{cd}	2.54 ^{ab}	1.62 ^{bcde}
	80	46.6 ^{de}	45.8 ^{cd}	1.74 ^{abcd}	1.10 ^{de}
20	0	18.0 ^f	19.8 ^d	1.34 ^{bcde}	1.10 ^{de}
	20	26.0 ^{de}	35.0 ^{cd}	1.82 ^{abcd}	3.04 ^{ab}
	40	29.6 ^{de}	32.6 ^{cd}	2.86 ^a	2.40 ^{abcd}
	80	30.2 ^{de}	25.4 ^d	2.56 ^{ab}	3.42 ^a

within each column, same letter indicates no significance difference between treatments (p>0.05)

Table 3: Zn uptake ($\mu\text{g plant}^{-1}$) and P uptake ($\mu\text{g plant}^{-1}$) by shoots in sweet corn plants in nutrient solution with different P and Zn levels at 7 and 14 DAT

Treatment (mg L^{-1})		Zn uptake ($\mu\text{g plant}^{-1}$)		P uptake ($\mu\text{g plant}^{-1}$)	
Zn	P	7 DAT	14 DAT	7 DAT	14 DAT
0	0	2.42 ^d	4.83 ^b	120 ^f	280 ^{cdef}
	20	2.56 ^d	2.26 ^b	280 ^{ab}	420 ^{abc}
	40	2.55 ^d	3.75 ^b	200 ^{abc}	350 ^{abcde}
	80	2.69 ^d	2.18 ^b	220 ^{abc}	460 ^f
5	0	4.15 ^{cd}	6.36 ^b	130 ^f	190 ^g
	20	3.01 ^d	6.42 ^b	170 ^{bc}	330 ^{abcdef}
	40	7.05 ^{abc}	5.96 ^b	340 ^a	330 ^{abcdef}
	80	2.75 ^d	6.09 ^b	190 ^{bc}	290 ^{bcdef}
10	0	5.04 ^{bcd}	26.25 ^{ab}	130 ^f	210 ^f
	20	6.95 ^{abc}	10.97 ^b	190 ^{bc}	450 ^f
	40	4.27 ^{cd}	9.01 ^b	200 ^{abc}	390 ^{abcd}
	80	4.07 ^{cd}	9.89 ^b	180 ^{bc}	450 ^f
20	0	9.41 ^a	45.50 ^a	160 ^{bc}	220 ^{ef}
	20	7.36 ^{abc}	8.73 ^b	200 ^{abc}	280 ^{cdef}
	40	6.53 ^{abc}	9.66 ^b	190 ^{bc}	270 ^{def}
	80	8.44 ^{ab}	17.97 ^b	240 ^{abc}	420 ^{ab}

within each column, same letter indicates no significance difference between treatments ($p>0.05$)

these treatments was possibly caused by the interactions of Zn and P with iron in the growth medium. Fe is required for the activity of ALA synthase, which catalyzes the first identified step of the tetrapyrrole biosynthetic pathway leading to chlorophyll formation. The results were in agreement with those of Alam and Shereen (2002) in wheat.

The highest ratio of the uptake of Zn in roots and shoots was observed in $\text{Zn}_{20}\text{P}_{40}$ and $\text{Zn}_{20}\text{P}_{80}$ treatment at 7 and 14 DAT, respectively (Table 2). It demonstrated that P application adversely affects translocation of Zn from root to shoot. Precipitation of Zn phosphate contributed to Zn deficiency, consequently, P and Zn accumulated in roots and stems near the point of entry and aforementioned ratio was increased. An enhancement of this root/shoot Zn uptake ratio can demonstrate Zn precipitation in roots or reduced Zn translocation from roots to shoots. This ratio decreased with increased Zn supply at both 7 and 14 DAT (Table 2) which proves that Zn×P interaction in roots may be overcome to some extent with higher levels of Zn application.

Zn_0P_{80} treatment had the lowest Zn and the highest P uptake by shoot at 14 DAT (Table 3). It shows that Zn deficiency can enhance P uptake and translocation to such an extent that P may accumulate to toxic level in leaves. Cakmak and Marschner (1987) stated that P concentration was increased in cotton leaves under low Zn and high P supply in solution culture. This high P concentration in leaves reduced the physiological availability of Zn in plant tissue and enhanced the Zn deficiency symptoms. Gianquinto *et al.* (2000) showed the same results in dwarf bean in a glasshouse experiment. In contrast, Parker *et al.* (1992) demonstrated that enhancement of solution P depressed growth slightly on

Table 4: Zn uptake ($\mu\text{g plant}^{-1}$) and P uptake ($\mu\text{g plant}^{-1}$) by roots in sweet corn plants in nutrient solution with different P and Zn levels at 7 and 14 DAT

Treatment (mg L^{-1})		Zn uptake ($\mu\text{g plant}^{-1}$)		P uptake ($\mu\text{g plant}^{-1}$)	
Zn	P	7 DAT	14 DAT	7 DAT	14 DAT
0	0	0.75 ^f	4.41 ^{de}	90 ^f	130 ^{defg}
	20	0.63 ^f	1.44 ^e	190 ^{abc}	250 ^{abc}
	40	1.22 ^f	3.32 ^{de}	150 ^{abc}	290 ^{ab}
	80	0.70 ^f	5.04 ^{cde}	120 ^{bc}	240 ^{abcd}
5	0	2.96 ^f	5.07 ^{de}	190 ^{abc}	90 ^{fg}
	20	3.29 ^f	6.61 ^{cde}	130 ^{abc}	180 ^{cdef}
	40	4.40 ^{def}	10.11 ^{bcd}	170 ^{abc}	240 ^{abc}
	80	3.82 ^{ef}	12.52 ^{bcde}	160 ^{abc}	280 ^{abc}
10	0	4.35 ^{def}	10.69 ^{bcde}	140 ^{abc}	70 ^g
	20	10.31 ^{cd}	19.14 ^{abcd}	150 ^{abc}	230 ^{abcd}
	40	10.21 ^{cde}	15.10 ^{bcde}	210 ^{ab}	240 ^{abcd}
	80	7.08 ^{cdef}	18.22 ^{bcd}	170 ^{abc}	250 ^{abc}
20	0	13.00 ^{bc}	17.20 ^{bcde}	170 ^{abc}	70 ^g
	20	13.36 ^{bc}	24.35 ^b	210 ^{ab}	240 ^{abcd}
	40	18.47 ^{ab}	21.32 ^{bc}	170 ^{abc}	180 ^{bcde}
	80	20.48 ^a	63.30 ^a	240 ^a	310 ^a

within each column, same letter indicates no significance difference between treatments ($p>0.05$)

tomato grown in chelator-buffered nutrient solution but the increment of P or reduction of Zn concentration in tissue was not observed.

Zn uptake by shoot was increased with the increase in Zn application at both harvesting times (Table 3). The highest Zn uptake by shoot was observed in Zn_{20}P_0 treatment which was 1.9 and 4.1 times higher than the average Zn uptake by shoot at 7 and 14 DAT, respectively and Zn uptake by shoot was decreased with P application.

$\text{Zn}_{20}\text{P}_{80}$ treatment had the highest Zn uptake by roots which was 20.48 and 63.30 $\mu\text{g plant}^{-1}$ at 7 and 14 DAT, respectively. This treatment also had the highest P uptake by roots which was 240 and 310 $\mu\text{g plant}^{-1}$ at 7 and 14 DAT, respectively (Table 4). It showed that Zn and P uptake by roots increased with increased Zn and P supply. In potato (Christensen and Jackson, 1981), okra (Loneragan *et al.*, 1982) and cotton (Cakmak and Marschner, 1987), Zn deficiency increased total P content and either had no effect on root dry matter or depressed it under certain conditions of treatment or harvest; thus, there can be no doubt that Zn deficiency enhanced P uptake by roots and played some part in enhancing P concentration in these species. By contrast, corn in the present experiment did not show an increased P uptake when grown without Zn. Sankhyan *et al.* (1997) demonstrated that P does not have an inhibitory effect on the root Zn uptake but obstructs its plant translocation.

CONCLUSION

The present study shows that Zn×P interaction can cause a reduction in yield of sweet corn plants. P/Zn ratio can be a better indicator of Zn nutritional status than Zn

concentration alone. P-induced Zn deficiency was intensified with enhancement of plant growth.

ACKNOWLEDGMENTS

The author wishes to express his thanks to Ms. Zabedah Tumirin, Department of Land Management, Faculty of Agriculture, University Putra Malaysia, for providing him the Laboratory facilities.

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