Effects of Compost Application on Growth and Nodulation of Kidney Bean, Soybean and Alfalfa Under Salt Stress

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Abstract

The effects of two commercially prepared composts, Bark and Tenporon, on the growth and nodulation of kidney bean, soybean and alfalfa under salt stress were investigated in the greenhouse. Growth and nodulation of the legumes were improved by the composts. The inhibitory effect of NaCl and Na_2SO_4 at high concentration was also alleviated by the composts, especially in plants treated with Bark compost. The results suggest that application of some organic materials to salt-affected soils may enhance plant growth and nodulation of legumes, without desalinization with expensive chemical substances.

Introduction

Continuous irrigation with water loaded with soluble salts results in salination of arable soils and reduces crop yield in the field. According to Ben-Zioni *et al.* (1967) and Itai *et al.* (1968), crops grown under saline environment exhibit disturbed metabolism culminating in stunted growth and poor productivity. Rains (1979) outlined three basic problems encountered by plants exposed to saline environments. These are reduction in water potential of the surrounding environment making water less available, interference of toxic ions with the physiological and biochemical processes, and predominance of other ions over nutrient ions.

One way to reduce salinity is the application of compost. During composting, labile carbon compounds are lost, while more complex substances, such as humic acids, are synthesized (Riffaldi *et al.*, 1992). Besides, during decomposition of compost material in the soil, different substances are released into the soil solution. Some of these substances are leached out of the plant root absorption zone, while others remain and result in changes in the soil biological and chemical status. The substances remaining within the root absorption zone, especially cations, play a role in legume growth and nutrition under saline condition. For instance, increasing external Ca concentration has been shown to inhibit Na absorption in beans (LaHaye & Epstein, 1971), soybean (Wieneke & Lauchli, 1980), and increasing germination percentage and early seedling growth of *Phaseolus vulgaris* (Cachorro *et al.*, 1994).

In the present study, two experiments were conducted to investigate the effects of two commercially-prepared composts, Bark and Tenporon (commonly used in Japan), on the growth and nodulation of kidney bean, soybean and alfalfa at salt concentrations which significantly reduce their growth.

Materials and methods

Legume hosts and rhizobium strains

The legumes used were soybean (*Glycine max* L. cv. Enrei), kidney bean (*Phaseolus vulgaris* cv. Selina) and alfalfa (*Medicago sativa* cv. Rusan). *Bradyrhizobium japonicum* IFO 12608, *Rhizobium leguminosarum* IFO 12612 and *Rhizobium meliloti* IFO 12611 were used to inoculate the legumes, respectively. The strains were obtained from the Institute for Fermentation, Osaka (IFO), Japan.

Composts used

The composts used, Bark and Tenporon, were obtained from Fujimi Co. Ltd (Shizuoka) and Tenpoku Co. Ltd (Muroran) in Japan, respectively. Bark compost was made from barks of Japanese cedar and cypress, and the compost particle size was 1-8 mm. Tenporon is the commercial name of peat moss compost and the particle size was 0.5-2 mm. Some chemical properties of Bark compost analyzed are: organic matter: 80%, C/N: 58, total N: 12 g kg^{-1} dry wt., total Ca: 213 g kg^{-1} dry wt.; and for Tenporon compost, they are organic matter: 60%, C/N: 27, total N: 10 g kg^{-1} dry wt., total Ca, 38 g kg^{-1} dry wt.

Experiment 1

The experiments were conducted in the greenhouse of the Faculty of Agriculture, Shizuoka University, Japan. The effect of different concentrations of NaCl and Na_2SO_4 on the growth and nodulation of the three legumes was assessed under natural light conditions. The legume seeds were surface-sterilized with 90% ethanol under ultrasonic pressure for 1-3 min and washed with sterile distilled water. The soybean, kidney bean and alfalfa seeds were inoculated by the slurry method with *Bradyrhizobium japonicum* IFO 12608, *Rhizobium leguminosarum* IFO 12612 and *Rhizobium meliloti* IFO 12611, respectively. The inoculated seeds were sown into a 1.5 *l* plastic pots containing river sand with hole at the bottom. The plants were sown at two seedlings per pot at a replicate of four. The pots were placed into graduated plastic containers to collect excess salt solution that dripped from the plant culture pots.

The seedlings were irrigated with distilled water for 7 days after which salt stress treatment was imposed. Salinity treatments were imposed by adding 100 ml of 25, 50, 75, 100, 200 m*M* of NaCl and Na_2SO_4 to the culture medium once. Control treatments received 100 ml of distilled water. The excess solution collected in the graduated plastic container was used to irrigate the seedlings throughout the growth period of the plants. The 100 ml mark on the graduated containers was used as the baseline and, in cases where the volume of solution collected, was less than 100 ml; distilled water was added to make up for the difference. The seedlings were fertilized weekly with 5 ml of N-free nutrient solution [0.1 g K_2HPO_4, 0.2g $Ca(H_2PO_4)_2$, 0.1 g $MgSO_4.7H_2O$, 0.05 g $FeCl_3$, and 1 ml of trace element solution (1.43 g H_3BO_3, 1 g $MnSO_4.4H_2O$, 0.11 g $ZnSO_4.7H_2O$, 0.04 g $CuSO_4.5H_2O$ and 0.007 g $Na_2MoO_4.4H_2O$)] per 1 *l*. The plants were harvested 4 weeks after sowing. Nodulation and dry matter were determined.

Experiment 2

The effects of Bark and Tenporon composts on the growth and nodulation of the legumes were also investigated in the greenhouse under natural light conditions. The culture system described above was used. The legume seeds were surface-sterilized, inoculated with the appropriate rhizobium strains and sown into river sand mixed with compost at 4% (wt/wt dry weight basis). Control treatments were not treated with compost. The seedlings were irrigated with distilled water for 7 days, after which salt stress treatment was imposed. Based on the growth results of the legumes at different salt concentrations in Experiment 1, salinity treatment was imposed by irrigating the plants at the following salt concentrations: kidney bean (50 m*M* NaCl, 50 m*M* Na_2SO_4), soybean (100 m*M* NaCl, 100 m*M* Na_2SO_4), and alfalfa (150 m*M* NaCl, 100 m*M* Na_2SO_4). The plants were also treated with composts and irrigated with salt solution at concentrations that inhibited the plant growth in Experiment 1. The inhibited salt concentrations were 100 m*M* NaCl and 150 m*M* Na_2SO_4 for kidney bean, 200 m*M* NaCl and 150 m*M* Na_2SO_4 for soybean, and 200 m*M* Na_2SO_4 for alfalfa. The seedlings were fertilized weekly with the N-free nutrient solution as described in Experiment 1, and harvested 4 weeks after sowing for dry matter determination and nodule number count.

Results and discussion

The growth of kidney bean, soybean and alfalfa at different salt concentrations is shown in Fig. 1. Significant ($P < 0.05$) decrease in total dry matter (TDM) production of kidney bean occurred with increase in NaCl concentration, and growth was inhibited at 100 m*M* NaCl. There was also significant ($P < 0.05$) decrease in TDM production between 25 m*M* and 100 m*M* Na_2SO_4. Significant ($P < 0.05$) decrease in TDM production of soybean was observed at 100 m*M* for both salts, and growth was inhibited at 200 m*M* NaCl and 150 m*M* Na_2SO_4. The growth trend of alfalfa plant was different from the other legumes. Significant ($P < 0.05$) decrease in plant growth was observed at 150 m*M* NaCl and 75 m*M* Na_2SO_4, and growth was inhibited at 200 m*M* Na_2SO_4. Based on these results, the sensitivity of the legumes to both salts is in the order: kidney bean > soybean > alfalfa. Legumes have been recognized to be sensitive or only moderately resistant to salinity (Mess & Hoffman, 1977).

Nodulation in kidney bean and soybean was more sensitive to salt stress than plant growth (Table 1). There was significant ($P < 0.05$) decrease in nodule number in kidney bean at the lowest salt concentration (25 m*M*). Nodule formation was inhibited at 50 m*M* NaCl and 75 m*M* Na_2SO_4. In soybean, there was no significant ($P > 0.05$) change in nodule number between 25 and 75 m*M* NaCl. However, there was significant ($P < 0.05$) increase in nodule number at 50 m*M* Na_2SO_4. Nodule formation was inhibited at 100 m*M* NaCl and 75 m*M* Na_2SO_4. For alfalfa, there was no difference in nodule number between 25 and 100 m*M* but significant ($P < 0.05$) decrease in nodule number was observed at 150 m*M* for both salts. In several legumes, such as soybean (Singleton & Bohlool, 1984), pea (Siddiqui *et al.*, 1985), cowpeas and mungbeans (Bulasubramanian and Sinha, 1976) and faba beans (Yousef & Sprent, 1983), nodule formation was reported to decrease with

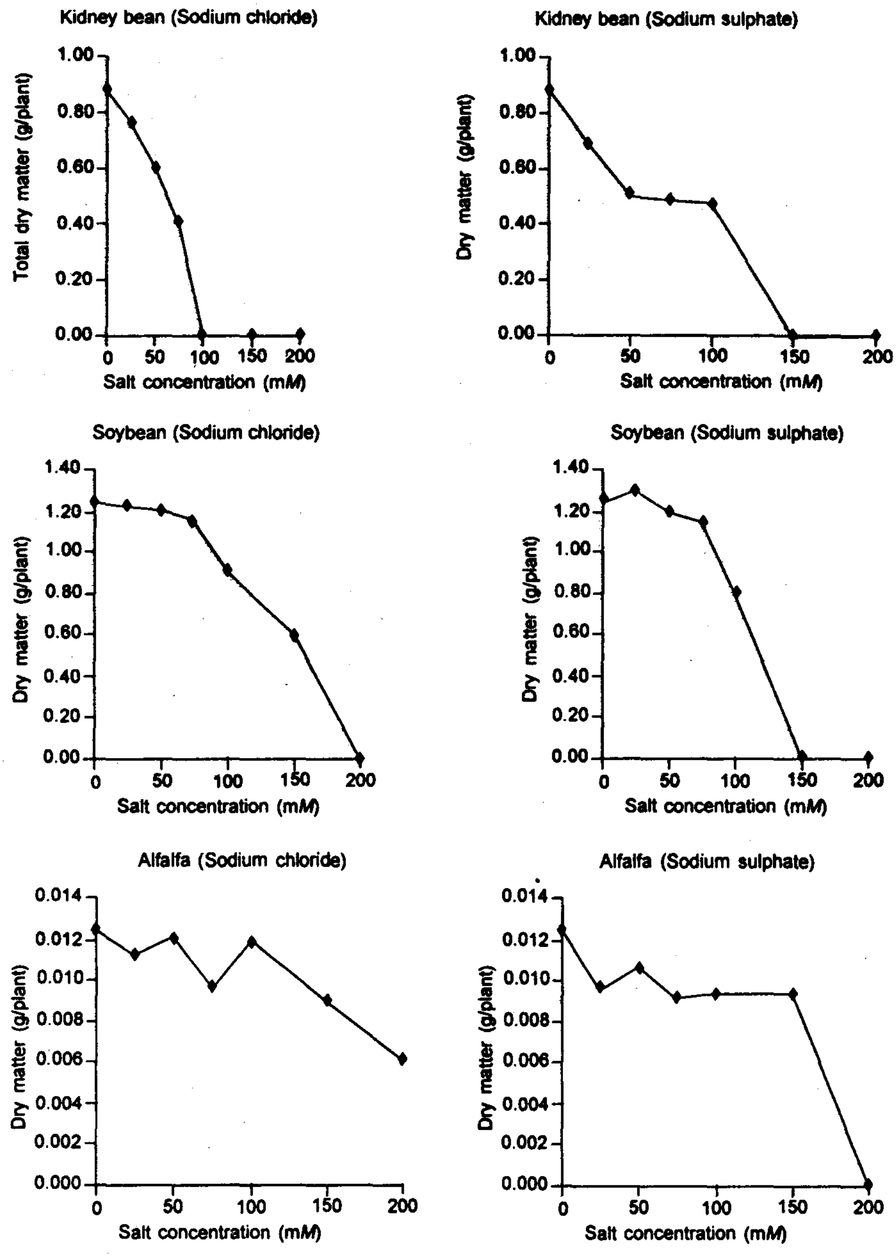

Fig. 1. Total dry matter of kidney bean, soybean and alfalfa at different salt concentrations

increasing salinity. In the present study, no significant ($P > 0.05$) decrease in nodule number was observed in alfalfa between 25 and 100 m*M*. However, there was significant ($P < 0.05$) increase in nodule number in soybean at 50 m*M* Na_2SO_4.

The effect of compost application on plant growth is shown in Fig. 2. Application of Bark and Tenporon composts improved the shoot growth of kidney bean at 50 m*M*

TABLE 1

Nodule number of kidney bean, soybean and alfalfa plants grown under different salt concentrations

		Salt concentration (mM)							
Legume	*Salt*	*0*	*25*	*50*	*75*	*100*	*150*	*200*	*LSD(5%)*
Kidney bean	NaCl	12	2	2	2	-	-	-	2.07
	Na_2SO_4	12	6	1	0	0	-	-	1.88
Soybean	NaCl	1	1	2	2	0	0	-	1.18
	Na_2SO_4	1	1	5	0	0	-	-	3.67
Alfalfa	NaCl	15	18	13	16	13	9	5	4.89
	Na_2SO_4	15	18	13	14	13	5	-	4.21

Dash line (-) indicates no plant growth

NaCl, however, the increase in shoot growth was only significant ($P < 0.05$) in plants treated with Tenporon compost. At 50 m*M* Na_2SO_4, Bark and Tenporon composts significantly ($P < 0.05$) enhanced shoot growth by 25% and 46%, respectively. Besides, application of compost had no effect on root development of kidney plants irrigated with NaCl solution. However, compost application caused significant ($P < 0.05$) reduction in root development of kidney bean plants irrigated with Na_2SO_4. Application of compost increased shoot growth of soybean at 100 m*M* NaCl and 100 m*M* Na_2SO_4 but significant ($P < 0.05$) increase was observed at 100 m*M* NaCl. However, root growth was not affected by application of compost for both salts. Shoot growth of alfalfa was significantly ($P < 0.05$) improved by compost application at 150 m*M* NaCl. Increase in shoot growth of alfalfa at 100 m*M* Na_2SO_4 was observed for both composts but significant ($P < 0.05$) increase was observed for Tenporon compost. Significant increase in root growth was only observed in alfalfa plant treated with Tenporon compost at 100 m*M* Na_2SO_4. Application of Bark compost significantly ($P < 0.05$) increased nodule numbers in soybean and alfalfa (Table 2).

Results showed that Bark compost enabled kidney bean to grow at inhibitory concentration of 100 m*M* NaCl (Fig. 3), however, nodule formation was not observed (Table 3). Both composts enabled alfalfa to grow at inhibitory concentration of 200 m*M* Na_2SO_4 and there was nodule formation as well (Table 3). The number of nodules formed in Bark-treated plants was almost twice that of Tenporon-treated plants. Both composts also enabled soybean to grow at inhibitory concentration of 200 m*M* NaCl (Fig. 3), however, only Bark-treated plants showed nodule formation (Table 3). Bark compost enabled soybean to grow at inhibitory concentration of 150 m*M* Na_2SO_4 (Fig. 3) and nodules were also formed. There appears to be no published work indicating growth and nodule formation in legumes at salt concentrations inhibitory to legume growth as a result of organic matter application. In a previous work (Lawson *et*

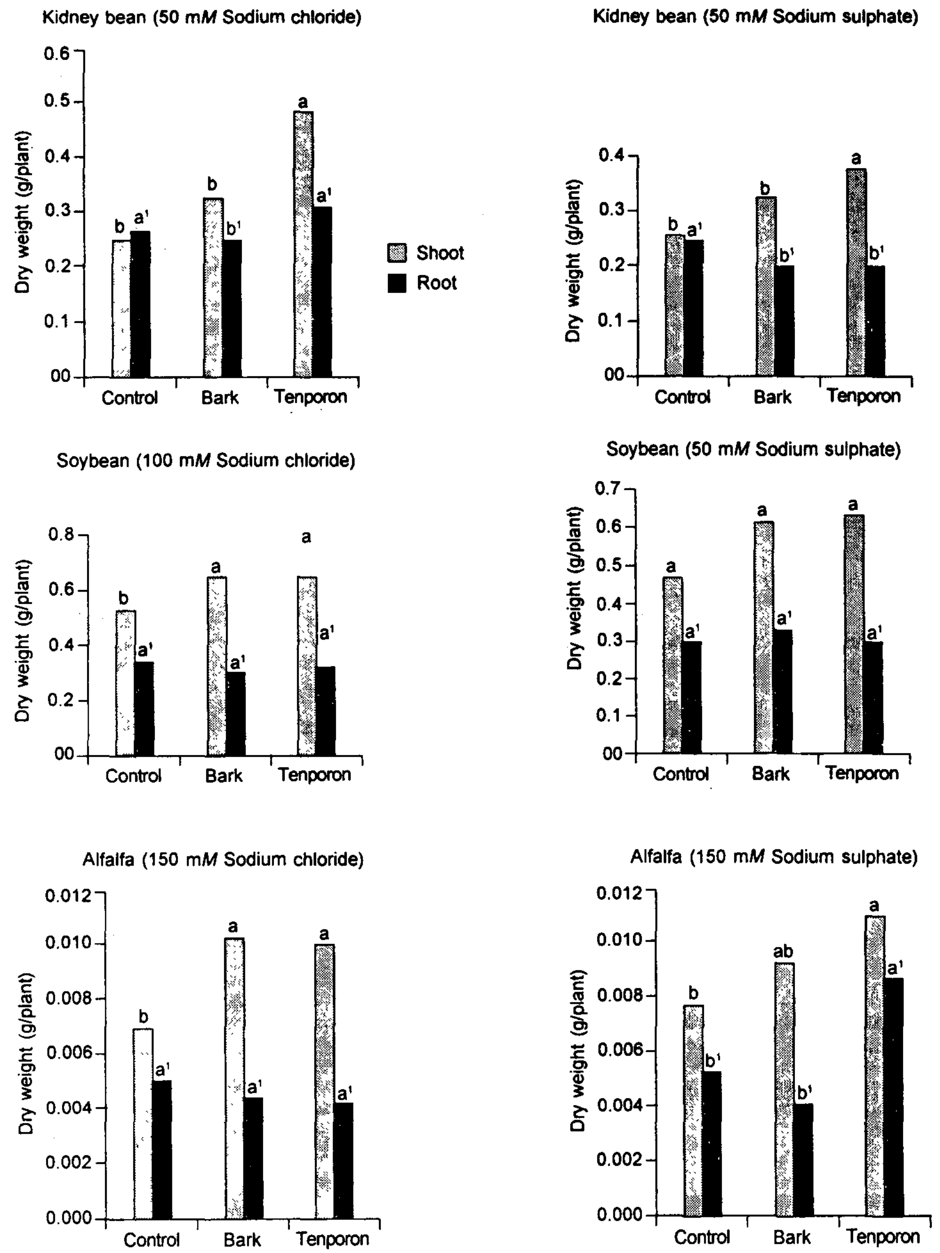

Fig. 2. Effect of compost application on plant growth of kidney bean, soybean and alfalfa at salt concentrations that caused significant reduction in growth. Treatments sharing common alphabet(s) are not significantly different at $P = 0.05$.

al., 1995), the application of Bark and Tenporon composts improved soil moisture status and exchangeable Ca, however, the increase in exchangeable Ca was more in Bark-treated soil than Tenporon-treated soil. Besides, analysis showed that Bark

TABLE 2

Effect of compost application on nodule number of kidney bean, soybean and alfalfa grown under saline condition

Legume	Salt(conc.)	Control	Bark	Temporon	LSD (5%)
Kidney bean	NaCl (50 m*M*)	0	0	0	
	Na_2SO_4 (50 m*M*)	0	0	0	
Soybean	NaCl (100 m*M*)	6	16	7	4.6
	Na_2SO_4 (100 m*M*)	9	15	8	4.9
Alfalfa	NaCl (150 m*M*)	9	23	10	3.7
	Na_2SO_4 (100 m*M*)	11	21	10	4.7

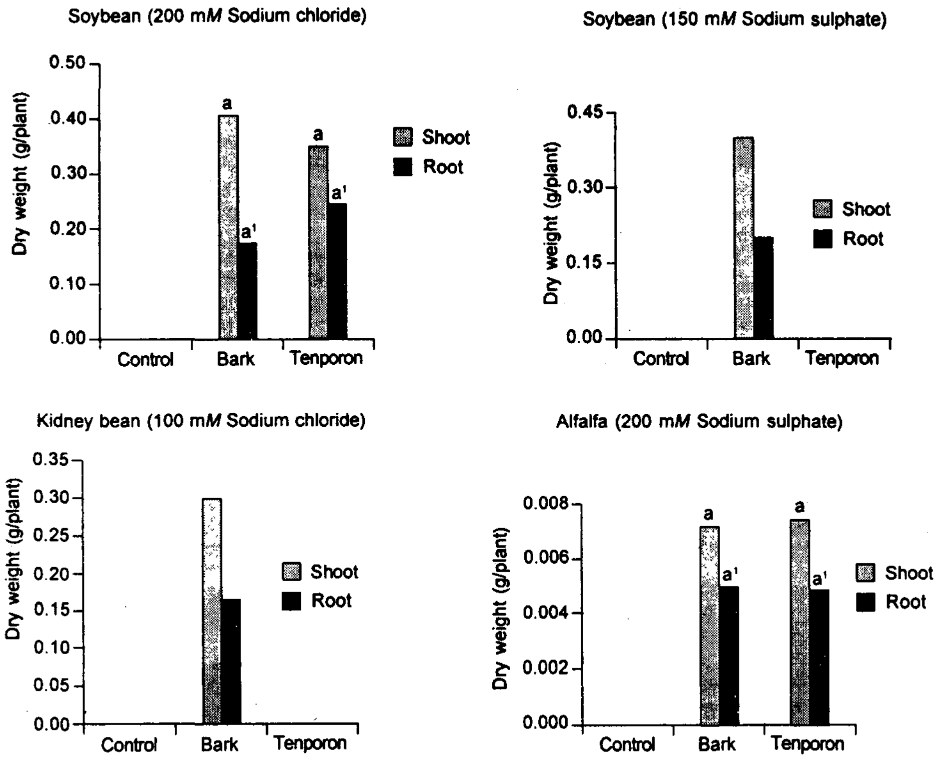

Fig. 3. Effect of compost application on plant growth of kidney bean, soybean and alfalfa at salt concentrations inhibitory to plant growth. Treatments sharing common alphabet(s) are not significantly different at *P* = 0.05.

compost contained total Ca of 213 kg^{-1} dry wt. while Tenporon compost contained total Ca of 38 kg^{-1} dry wt. Cachorro *et al.* (1994) indicated that high Ca concentration could decrease Na toxicity under salinity. Ashraf & Naqvi (1991) found that addition of Ca

TABLE 3

Effect of compost application on nodule number of kidney bean, soybean and alfalfa grown at inhibitory concentrations

Legume	*Salt(conc.)*	*Control*	*Bark*	*Temporon*	*LSD (5%)*
Kidney bean	NaCl(100 m*M*)	-	0	-	
	Na_2SO_4(150 m*M*)	-	-	-	
Soybean	NaCl(200 m*M*)	-	3	0	
	Na_2SO_4(150 m*M*)	-	6	-	
Alfalfa	Na_2SO_4(200 m*M*)	-	17	9	6.5

Dash line (-) indicates no plant growth.

increased the Ca/Na and K/Na ratios in shoots and improved germination and growth. Increasing external Ca concentrations were shown to inhibit Na absorption in bean (LaHaye & Epstein, 1971) and soybean (Wieneke & Lauchli, 1980) and, thus, may be an important factor in controlling salinity response of legumes. The possible improvement of plant growth by Bark and Tenporon composts in the present study may be due to improved moisture and availability of plant nutrients, especially Ca.

The enhanced nodulation in soybean and alfalfa by Bark compost may be due to high increase in exchangeable Ca, as shown in previous work (Lawson *et al.*, 1995) because Ca concentration has been shown to affect the attachment of rhizobia to root (Smit *et al.*, 1987). Smit *et al.* (1991) also observed that the growth of *Rhizobium leguminosarum* var. *viciae* cells under Ca^{2+}-limitation caused a loss of attachment to pea root hair tips. The inability of Bark compost to induce nodulation in kidney bean at the inhibitory concentration may be due to the sensitivity of this legume to salt stress. Further studies on the mechanisms responsible for the improved plant growth and nodulation from genetical and physiological point of view are necessary. The present study indicated that the application of some organic materials to saline soils may enhance nodulation and plant growth of some legumes for increased yield without desalinization with expensive chemical substances.

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