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ORIGINAL PAPER

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ALLEVIATION OF ADVERSE EFFECTS OF SALT STRESS ON LETTUCE (*Lactuca sativa* var. *crispa*) BY APPLICATION OF VERMICOMPOST

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ABSTRACT

In arid and semiarid regions, soil salinity causes the salt stress in plants and negatively affects the crop production. In this respect, organic farming practices are becoming the foreground as applications that are sensitive to the environment and support sustainability in agriculture. This study aimed to explain the role of vermicompost (VC) for reducing the effect of salt stress on lettuce (*Lactuca sativa* var. *crispa*) plant. The experiments were performed with different concentrations of VC (0, 2.5 and 5% VC on a weight per weight basis of soil-w/w) and salt stress treatments (4 and 8 dS m^{-1} NaCl levels). Under salt stress, shoot height, relative water content (RWC), stomatal conductance (g_s), chlorophyll *a* (Chl*a*) content decreased, while electrolyte leakage (EL), malondialdehyde (MDA) contents and superoxide dismutase (SOD) and catalase (CAT) activities significantly increased in parallel with the severity of the stress. In this study, VC under the salinity stress significantly increased the RWC, g_s and Chl*a*, Chl*t* and carotenoid contents in the leaf tissues; however, MDA and EL contents decreased and SOD and CAT activities increased compared to the controls. In all levels of NaCl, the 5% ratio of VC significantly reduced the negative effects of salinity.

Key words: antioxidant enzymes, lipid peroxidation, organic material, plant growth, salinity

INTRODUCTION

Salt stress affects plant development and productivity negatively. Higher amounts of salt and soil water and excessive irrigation cause physiological and biochemical changes in the plant [Munns and Tester 2008]. The reduction in the osmotic potential of the soil solution, ion toxicity and the imbalance of the nutrients are the consequences of salinity affecting plant growth negatively [Parvaiz and Satyawati 2008]. During the initiation and continuation of salt stress, the plant is affected by all its physiological and biochemical. Biomass, relative water content (RWC), chlorophyll content and stomatal conductance (g_s) were affected in the plants grown under saline conditions [Tavakkoli et al. 2011, Jacoby et al. 2016, Yarsi et al. 2017]. Reactive oxygen species (ROS) production, which occurs in salt stress conditions, damages cellular components, including membrane lipids [Mittova et al. 2004]. The plants have antioxidant defense systems consisting of enzymatic and nonenzymatic components that hold the ROS balance under the stress in the cell. For example, they activate superoxide dismutase (SOD) and catalase (CAT) enzymes for sweeping different types of ROS [Zhu et al. 2004]. However, the effect of salt stress on plants varies depending on the duration and severity of stress [Tavakkoli et al. 2010]. In addition,

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the genotypic characteristics of plants play a decisive role in strese tolerance.

Organic substances increase the nutrient content of the soil and improve its structure, thus contributing to the increase of the yield and quality of the soil [Ayyobi et al. 2014]. Because of these properties, the use of organic fertilizers has become a widely used method in recent years to heal saline soils [Lakhdar et al. 2009]. Vermicompost (VC) is obtained by the conversion of organic materials into humus-like materials using worms [Arancon et al. 2003]. VC is applied to support sustainability in agriculture as well as in the processing of solid organic wastes and debris at the same time. VC has a very positive effect on plant growth and soil rehabilitation [Chinsamy et al. 2013]. Previous studies have shown that VC decreases the negative effects of salt stress on chickpea and bean [Ahmadpour et al. 2016, Beykkhormizi et al. 2016]. Bidabadi et al. [2017] have show that VC might provide anti-stress effects in *Punica granatum* under stress conditions by reducing the toxic elements.

Lettuce is a sensitive vegetable to salt stress [Qin et al. 2013]. Since salted lettuce grown fields are considered to be problematic it is important to determine VC effects on plants. The aim of this study was to explain the role of VC in terms of morphological, physiological and biochemical properties in order to reduce the effect of salt stress on lettuce.

MATERIALS AND METHODS

Plant materials and treatments

The study was carried out under the automatic controlled greenhouse conditions [relative humidity, 50-55% and temperatures, of 24/20°C (day/night)] until the end of 24 March 10 July 2017 (total 109 days). The greenhouse is located in Soil Fertilizer and Water Resources Central Research Institute in Ankara, Turkey (latitude 40°04'35.27"N, longitude 33°36'28.69"E, altitude 894 m). Lettuce seeds (*Lactuca sativa* var. *crispa*) were planted in the media containing vermiculite and perlite (1 : 1 v/v). The seedlings with 3−4 true leaves were transplanted (one plant per pot) to the a 7 L pots (25 cm diameter, by 22 cm deep) containing VC at different rates. The VC ratios used in the study are: 0% (control), 2.5% VC, 5% VC (on a weight per weight basis of soil-w/w). Chemical properties of soil and VC used in the study are given in Table 1. The salt stress treatments were imposed 2 weeks after transplanting of seedlings to the pots (24 Mai). The plants were irrigated at the level of field capacity with tap water until stress started. In order to produce salt stress in the plants, plants were irrigated with water containg sodium chloride (NaCl) (4 and 8 dS m^{-1}) during the growing season. The control plants were irrigated with tap water (EC: 0.42 dS m^{-1}). Both control and salt plants were irrigated under free drainage conditions (field capacity + 20% wash water). Plants were kept under these conditions for 46 days until harvest. After separating out the shoot and root systems of each plant at harvest, they were used for the morphological measurements. The leaf samples were frozen in liquid nitrogen and stored at −80°C until the physiological and biochemical analysis.

Measurements and analyzes

Shoot height, fresh and dry weight of plants were measures 60 days after planting. The dry weights of shoot samples were determined after drying at 65°C in the oven for 48 hours. Photosynthetic pigments, electrolyte leakage percentage, RWC and stomatal conductance, were measured 40 days after seedling planting. Fresh leaf samples were first weighed to determine RWC. After 8 hours in water, turgor weight was determined. The dry weight of the same leaves was recorded after drying at 65°C for 48 hours. RWC was calculated using the following formula: RWC = 100 × [(fresh weight – dry weight) / (turgor weight – dry weight)]

Stomatal conductance (g_s) was determined by porometry (Model SC-1, Decagon). Measurements were made between 12.00 am and 13.00 pm.

The method proposed by Lichtenthaler [1987] was used to measure chlorophyll (*a*, *b*, *total* (*a* + *b*)) and carotenoid contents. Fresh leaves (0.5 g) were extracted for 12 h with acetone (80%). The extract was centrifuged at 4,000 rpm for 15 min and the absorbance of the supernatant read at 647, 664, and 470 nm by using a spectrophotometer (Perkin Elmer Lambda − EZ200). Chlorophyll and carotenoid pigment contents (mg g^{-1}) were calculated by the following formulas. Chl*a* = 12.21 (A664) – 2.79(A647), Chl*b* = 21.21 (A647) – 5.1(A664), Chl*t* (total) = Chl*a* + Chl*b*, Carotenoid = (1000 A470 – 1.8 Chl*a* – 85.02 Chl*b*)/198.

Table 1. Properties of soil and VC used in study

Properties	VC	Soil
Soil texture		sand clay loam
pH	8.1	7.75
Electrical conductivity (dS m^{-1})	6.5	1.28
Organic matter (%)	65.5	0.54
Nitrogen (%)	2.2	0.18
Phosphate (%)	1.7	3.60
Potash (%)	1.5	0.86

Electrolyte leakage was determined in 0.1 g leaf sample. Electrolyte leakage (EL) is calculated by the following formula [Dionisio-Sese and Tobita 1998]. EL = EC1/EC2 × 100.

Lipid peroxidation was determined by the malonyldialdehyde (MDA) method in the 200 mg fresh leaves [Lutts et al. 1996]. The amount of MDA was determined by measuring the absorbance of the supernatant at 532 and 600 nm.

To perform the enzyme extraction, 1 g of wet leaf sample was homogenized in 5 ml of 0.1 mol L^{-1} potassium phosphate buffer containing 0.1 mmol L^{-1} EDTA. The supernatant was recovered after centrifugation at 12,000 rpm for 20 minutes at 4°C and used for enzyme analysis. SOD and CAT activitya was performed according to the methods described by Beyer and Fridowich [1987] and Cakmak and Horst [1991].

The data obtained from this study were analyzed the two-factor completely randomized analysis of variance design using MSTAT-C. Each treament was repated three times. The differences between the means were compared with Duncan's multiple range test ($p < 0.05$).

RESULTS

Effects of VC on the shoot height, fresh and dry weight of shoots of lettuce plants under different salt levels are presented in Table 2. The effect of "salinity and VC" treatments interaction on shoot height was significant ($p < 0.05$), while this interaction effect on shoot fresh and dry weights was not significant ($p > 0.05$). Shoot height, shoot fresh and dry weights decreased significantly with all salinity levels (Tabs 3 and 4). Shoot height of 5% VC treated plants in 4 dS m^{-1} salt level was significantly higher than the respective control (non-VC) (22.5 cm). VC applications increased plant height at the 8 dS/m salt level, but these increases were lower than the 4 dS m^{-1} salt level.

Table 2, presents the effect of "salinity and VC" treatments interaction on RWC. RWC showed a significant decrease in parallel with the increase in salt level ($p < 0.05$). The 2.5 and 5% VC doses significantly increased the RWC content of plants at the level of 8 dS m^{-1} salt. However, in practice, VC applications at the 4 dS m^{-1} salt level did not provide significant increases in the RWC, compared to non-applied plants. g_s was significantly reduced by increased salinity ($p < 0.05$) (Tab. 3). On the other hand, VC increased g_s under salt stress conditions (Tab. 2). The 2.5 and 5% VC treatments, had similar effects on g_s at the 4 dS m^{-1} salt level (131.18 and 140.73 mmol m^{-2} s^{-1}). However, the 5% VC treatment resulted in a marked increases in g_s at the 4 dS m^{-1} salt level, compared to the control (non-VC) (respectively 97.42 and 140.73 mmol m^{-2} s^{-1}) (Tab. 3).

The effects of saliniyt and VC interactions on Chl*a*, Chl*t* and carotenoid contents were found to be significant ($p < 0.05$), but the effect treatments on Chl*b* was not significant ($p > 0.05$) (Tab. 5). Chl*a*, Chl*b*, Chl*t* and carotenoid concentrations decreased in response to salt stress. The lowest values of Chl*a*, Chl*t* and carotenoid were determined to be 3.35, 5.52 and 2.13 mg g^{-1} respectively at the 8 dS m^{-1} salt level (non-VC) (Tab. 3). VC treatments led to increase in Chl*a*, Chl*t* and cartenoid values of lettuce plants under salt stress

Table 2. ANOVA for the effects of salt and vermicompost treatments on the measured traits

SOV	*df*	SH	SFW	SDW	RWC	g_s	EL
Salinity (S)	2	**	**	**	**	**	**
Vermicompost (VC)	2	**	**	**	ns	**	**
S ×x VC	4	**	ns	ns	**	**	**
Error	18	0.63	30.59	5.81	90.62	84.19	4.91
CV(%)		3.92	4.45	17.60	13.30	8.25	9.47

ns: Not not significant. * P 0.05, **P 0.01. SOV: Source source of the variation; CV: Coefficient coefficient of variation; SH: Shoot shoot height; SFW: Shoot shoot fresh weight; SDW: Shoot shoot dry weight; RWC: Relative relative water content; g_s: Stomatal stomatal conductance, EL: Electrolyte electrolyte leakage

Table 3. Vermicompost effect on shoot height (SH), relative water content (RWC), stomatal conductance (g_s), Chlorophyll *a* (Chl*a*), Chlorophyll *total* (Chl*t*) and carotenoid of lettuce under salt stress

Salinity	VC	SH cm	RWC %	g_s mmol m^{-2} s^{-1}	Chl*a* mg g^{-1}	Chl*t* mg g^{-1}	Carotenoid mg g^{-1}
0	0	20.50 ^{c}	96.35 ^{a}	111.85 ^{c}	9.15 ^{d}	12.83 cd	3.70 $^{c–e}$
	2.5%	23.00 ^{b}	82.66 ^{ab}	134.90 ^{b}	12.78 ^{c}	16.93 b	4.58 ^{b}
	5%	25.50 ^{a}	85.87 ^{ab}	172.96 ^{a}	17.79 ^{a}	22.62 a	5.56 ^{a}
4 dS m^{-1}	0	17.00 ^{ef}	70.22 ^{bc}	81.85 ^{de}	5.94 ^{e}	8.48 e	3.31 ^{e}
	2.5%	20.00 ^{c}	71.15 ^{bc}	131.18 ^{b}	9.93 ^{d}	13.52 c	4.31 ^{bc}
	5%	22.50 ^{b}	62.78 ^{c}	140.73 ^{b}	15.58 ^{b}	20.88 a	4.09 $^{b–d}$
8 dS m^{-1}	0	15.67 ^{f}	37.56 ^{d}	60.20 ^{f}	3.35 ^{f}	5.52 f	2.13 ^{f}
	2.5%	19.50 ^{cd}	64.99 ^{c}	69.31 ^{ef}	8.32 ^{d}	10.90 d	3.54 ^{de}
	5%	18.33 ^{de}	72.37 ^{bc}	97.42 ^{cd}	9.64 ^{d}	14.12 c	4.06 $^{b–d}$

Within each column, means followed by the same letter do not differ significantly at $p < 0.05$

Table 4. Mean comparisons of salt and vermicompost treatments main effects on lettuce traits

Treatment	SFW g $plant^{-1}$	SDW g $plant^{-1}$	Chl*b* mg g^{-1}
Salinity			
0	160.72 ^{a}	16.60 ^{a}	4.22 ^{a}
4 dS m^{-1}	114.47 ^{b}	13.62 ^{b}	3.81 ^{ab}
8 dS m^{-1}	97.81 ^{c}	10.86 ^{c}	3.08 ^{b}
Vermicompost			
0	99.72 ^{c}	9.11 ^{b}	2.80 ^{b}
2.5%	131.67 ^{b}	16.07 ^{a}	3.44 ^{b}
5%	141.61 ^{a}	15.91 ^{a}	4.87 ^{a}

Within each column, means followed by the same letter do not differ significantly at $p < 0.05$. SFW: Shoot shoot fresh weight; SDW: Shoot shoot

Table 5. The results of ANOVA for the effects of different treatments on the measured traits

SOV	*df*	Chl*a*	Chl*b*	Chl*t*	Carotenoid	MDA	SOD	CAT
Salinity (S)	2	**	*	**	**	**	**	**
Vermicompost (VC)	2	**	**	**	**	**	**	**
S x VC	4	**	ns	*	*	**	**	**
Error	18	17.11	0.79	1.350	0.14	2.84	57.50	77.18
CV(%)		9.49	23.94	8.31	9.39	13.02	7.22	7.12

ns: Not not significant. * p 0.05, **p 0.01. SOV: Source of the variation; CV: Coefficient coefficient of variation; Chl*a*: Chlorophyll chlorophyll *a*, Chl*b*: Chlorophyll chlorophyll *b*,Chl*t*: Chlorophyll chlorophyll *total*, MDA: Malondialdehydemalondialdehyde, SOD: Superoxide superoxide dismutase, CAT: Catalasecatalase

Table 6. Vermicompost (VC) effect on electrolyte leakage (EL), malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) of lettuce under salt sress

Salinity	VC	EL %	MDA µmol g^{-1} FW	SOD $Umin^{-1}$ mg^{-1}FW	CAT µmol min^{-1} mg^{-1}FW
0	0	28.36 ^{c}	6.84 ^{ef}	60.59 ^{d}	55.24 ^{e}
	2.5%	22.62 ^{cd}	6.34 ^{ef}	70.58 ^{d}	60.53 ^{e}
	5%	18.37 ^{d}	5.56 ^{f}	87.48 ^{c}	89.41 ^{d}
4 dS/m	0	36.63 ^{b}	25.56 ^{b}	90.85 ^{c}	90.99 ^{d}
	2.5%	33.87 ^{b}	12.48 ^{c}	88.67 ^{c}	120.53 ^{c}
	5%	23.22 ^{cd}	8.90 ^{de}	140.19 ^{b}	188.67 ^{b}
8 dS/m	0	52.69 ^{a}	29.41 ^{a}	92.75 ^{c}	119.85 ^{c}
	2.5%	55.18 ^{a}	10.21 ^{cd}	149.86 ^{b}	174.84 ^{b}
	5%	34.61 ^{b}	11.24 ^{cd}	164.54 ^{a}	210.28 ^{a}

Within each column, means followed by the same letter do not differ significantly at $p < 0.05$

relative to control (non-VC) plants (Tab. 3). The highest value of carotenoid under salt stress was found to be in the 2.5% VC and 4 dS m^{-1} salt treatment (4.31 mg g^{-1}) (Tab. 3). The 2.5% and 5% VC treatments have a similar effect at the 8 dS m^{-1} salt level without alleviating the adverse effects of salt stress on Chl*a*. On the other hand, VC provided increases in the content of Chl*b*.

According to the results, EL was affected significantly by interaction effects of "salinity and VC" treatments ($p < 0.05$) (Tab. 2). EL has increased in parallel with the increase in salt doses (Tab. 6). However, VC treatments were effective in reducing the electrolytic leakage of stressed cells. The 5% VC rate was very successful in reducing EL in high salt stress, 2.5% VC was partially satisfactory. The lowest EL under stress was recorded at 5% VC treatment at 4 dS m^{-1} salt level (23.22%).

Table 5 shows that VC treatments under saline stress are significant on MDA ($p < 0.05$). MDA values increased in parallel with the increase in salt concentration (Tab. 6). On the other hand, VC treatments have shown a beneficial effect on the MDA values of the cells and contribute to the reduction of the MDA amounts. Accordingly, the lowest MDA under salt stress was determined in combination of "4 dS m^{-1} salt and 5% VC" treaments (8.90 µmol g^{-1} FW). On the other hand, in the highest salinity condition (8 dS m^{-1}), there was no difference in MDA between 2.5% and 5% doses of VC (10.21 and 11.24 µmol g^{-1} FW) (Tab. 6).

SOD and CAT enzyme activities in lettuce leaves were significantly influenced by salinity and VC applications ($p < 0.05$) (Tab. 5). Increased salinity has led to an increase in the activation of SOD and CAT enzymes (Tab. 6). In VC applied plant leaves, SOD and CAT activities increased under salt stress. In particular, the 5% VC resulted in significant increases relative to control plants (non-VC) in SOD and CAT activities. These increases were similar for both enzymes. According to this; the highest SOD and CAT values were recorded as 164.54 U $min^{-1}mg^{-1}$ FW and 210.28 µmol min^{-1} mg^{-1} FW, respectively, in the leaves induced with the highest salinity level (8 dS m^{-1}).

DISCUSSION

In this study, shoot height, shoot fresh and dry weights decreased significantly with the increasing intensity of salt stress, In plants subjected to VC under salt stress, shoot size increased compared to control plants (non-VC). This means an increase in the surface area of the plant. On absence of VC application the osmotic stress affected plant growth negatively, such a consequence of high salinity in plants. Mahajan and Tuteja [2005] interpret the acceleration of processes such as leaf aging and the abscission under stress as an adaptation mechanism. In addition, factors such as ion toxicity and nutritional imbalance can have negative effects on plant development [Jacoby et al. 2016]. Improved root capacity in VC treated plants may have led to improvement of plant growth parameters. The increase in root surface area stimulates plant growth and development due to the intake of nutrients. VC may have led to root and consequently plant development by increasing the content and porosity of the nutrient material in the soil [Chaoui et al. 2003]. Chinsamy et al. [2013] reported an improvement in the morphological properties of tomato seedlings treated with VC under high salt stress.

RWC, was reduced in the leaves of lettuce plants under salt stress in our study. Salt stress inhibits plant water intake, causing osmotic effects leading to reduced plant growth and ion toxicity and damage to cells that transport water to the foliage [Sathee et al. 2015]. However, the RWC values increased significantly with VC applications under salt stress. VC contributes to the uptake of water from the soil and to the increase in leaf RWC values by increasing the water permeability of the soil and increasing the water holding capacity [Ansari 2008]. In response to increased salinity, the gs is significantly reduced. Our results are in agreement with the findings of Campos et al. [2012]. The water stress induced by salt accumulation promotes the lowering of the osmotic potential of the xylem. Plants are affected by osmotic stress, therefore, exhibit a rapid decrease in gs [Zhu 2002]. On the other hand, VC application under salt stress also prevented reduction in gs. VC increases water uptake of roots due to its capacity of holding water and the microorganisms including mycorrhizal fungi. Improvement of leaf water content of VC allowed stomata to remain open longer and increased CO_2 uptake.

Chl*a*, Chl*t* and carotenoid were significantly reduced under salt stress compared to control plants (non-VC). Toxic ion accumulation in the leaves may have affected the chlorophyll content negatively [Yeo and Flovers, 1983]. The application of VC to lettuce plants resulted in the increases in Chl*a*, Chl*b* and carotenoid pigments when compared to control treatment. Consistent with our results, increases in Chl*a*, Chl*t* and carotenoid contents due to organic matter treatment have been reported on different plants [Bidabadi et al. 2017]. This positive effect of VC on Chl*a*, Chl*t* and carotenoid pigments can be attributed to an increase in photosynthetic rate and CO_2 assimilation which improve mineral uptake by the plant [Ayyobi et al. 2014]. In addition, the delay in the degradation of chlorophyll pigment by VC application may also be related to the enhancement of water productivity. Amiri et al. [2017] reported that VC treatments protects the proportion of chlorophyll content to carotenoid content. Thus, the increase in carotenoid concentration following VC treatments may be due to the increase in chlorophyll.

EL represents cellular damage and is considered a reliable physiological indicator of plant cell damage under stress conditions [Gao et al. 2011]. The salt stress levels greatly increased the EL. This is due to toxic ions found in salt stressed leaves [Lutts et al. 1996]. VC treatment caused a decrease in electrolyte leakage in plants exposed to stress. Decrease in EL, modification of membrane fluidity and permeability may be due to VC [Aydın et al. 2012]. VC may also have contributed to increased leaf water content and reduced toxic ion concentration by increasing water use efficiency.

The end product of lipid peroxidation causes negative consequences such as MDA, ion permeability and alteration of enzyme activity [Distelbarth et al. 2012]. The content of MDA in lettuce leaves were strongly affected by both salinity and VC. As expected, salinity increased MDA content of the leaves of plants. Butt et al. [2016] in mustard indicate that lipid peroxidation increases with salt stress. The MDA content of VC treated plants were less than those of the untreated plants under all levels of salt stress. An adaptive mechanism in tomato plant due to VC treatment repoted by Chinsamy et al. [2013] indicates the positive effects of VC on physiological capacity of plants under salt stress. However, the positive effect of VC on MDA is limited if the ambient salinity increases. This may indicate that the low-level VC is inadequate to overcome the toxic effect of high salinity. In our study, both applications of VC had similar effect on MDA at the highest salinity level.

As a result of salt stress, antioxidant enzyme activities convert ROS into harmless compounds, which constitute the most important resistance mechanisms of plants against oxidative stress. Salt stress has a significant effect on SOD and CAT enzyme activities. The correlation between salinity tolerance and the increase of SOD activity has been demonstrated in many previous studies [Tavakkoli et al. 2010, Hand et al. 2017]. In our study, a marked increase in SOD and CAT activities have occurred in parallel with the increase in salt levels, in order to provide tolerance of the plant to salt stress. The VC treated plants were found to have significant SOD and CAT activities that play an active role in the sweep of ROS when compared to control plants (non-VC). In addition, the increase in activity of antioxidant enzymes following VC administration may be indicative of the formation of a protective mechanism to reduce the oxidative damage caused by salt stress [Khan et al. 2010]. Bidabadi et al. [2017] have shown that antioxidant enzyme mechanisms are stimulated when VC is applied to pomegranate plants exposed to salt stress. These results confirm the efficacy of VC in inducing antioxidant enzyme mechanisms in plants.

CONCLUSIONS

As a consequence of salt stress, osmotic and oxidative stress lead to some disadvantages on plant morphology and physiology. VC was a suitable soil amendment alternative as it improved plant growth and development under high saline conditions. The results of this study show that VC application on lettuce plants growing under salt stress conditions improves some growth, physiological and biochemical properties and increases the tolerance of plants to salt stress. For this reason, it is possible to recommend VC application under salt stress conditions.

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