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EXPLOITING BIOSTIMULANTS AND MICRONUTRIENTS FOR OPTIMAL RICE YIELD IN SODIC SOIL : A STRATEGIC APPROACH TO SALT STRESS RESILIENCE

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ABSTRACT

Sodicity, marked by elevated Na^+ over Ca^{2+} in soil can disrupt the uptake and availability of essential micronutrients, hampering potential yield of rice yield in sodicity conditions. A field experiment was undertaken to evaluate the impact of promising biostimulants and micronutrients on rice yield in sodic soil, aiming to enhance productivity in challenging conditions. The biostimulants including salt tolerant halophilic microbial consortia (HMC) isolated from sodic soils of Z.A.R.S., V. C. Farm, Mandya, Karnataka and humic acid (@ 0.2%) extracted from vermicompost, were applied solely and also in combination with foliar spray of micronutrients mixture (FS of MM- Zn:Mn: Cu:B:Mo- 14:5:4:1.2:0.2 @ 1%). Foliar spray of MM improved most of the yield parameters of rice as compared to soil application of Zn, RDF and solely application of biostimulants, however its efficacy was improved when used along with biostimulants. It was found that combined use of biostimulants and micronutrients mixture (T_9- HMC+HA+MM) most effective in increasing yield parameters *viz*., number of panicles per m^2 (284.53), panicle length (25.98 cm), panicle weight (3.33 g), total number of grains (156) and filled grains per panicle (137), along with the lowest per cent chaffyness (12.17 %). The culmination of these positive effects resulted in a significant increase in both rice's grain (48.65 q ha^{-1}) and straw yield (66.89 q ha^{-1}) in T_9 (HMC+HA+MM) followed by treatment receiving HMC+MM (T_7). Further, correlation studies revealed a negative relationship between yield and yield parameters with Na/K and Na/Ca values underscoring the importance of down regulating sodium concentration with application of biostimulants and micronutrients to optimize rice yield in sodic conditions.

Key words : Abiotic stress tolerance, Biostimulants, Halophilic microbial consortia (HMC), Humic acid (HA), Micronutrients mixture (MM).

Introduction

Sodicity poses a significant ecological challenge for cereal crop cultivation, particularly rice, leading to notable physiochemical alterations in both the qualitative and quantitative aspects of the rice-growing environment (Caliskon, 2009). In India, soils affected by alkalinity problem are reported to be 6.73 m ha of which 3.77 m ha is sodic and 2.96 m ha is saline. Studies also indicated it is likely to be increased to 11.7 m ha by 2025 (Singh, 2009). Higher concentrations of sodium than calcium is the major cause for both physical and nutritional problems in sodic soils affecting rice growth and development by creating ionic, osmotic and oxidative stresses (Munns, 2002). Salt stress reduces rice growth rate, alters metabolic activity and decreases ability to uptake water and nutrients (Munns, 2002 and Amirjani, 2011). Moreover, poor development of rice spikelets, especially inferior spikelets caused by salt stress significantly reduces rice grain yield (Shah and Jan, 2016). Several strategies have been studied to decrease the toxic effects caused by high salt stress on plant growth, including plant genetic engineering, and recently the use of biostimulants such as halophiles and Humic acids, the most promising and eco-friendly way is gaining importance to address such concerns (Yakhin *et al*., 2017).

The use of biostimulants, such as halophiles and humic acids, presents a promising approach to alleviate salt stress in rice plants (Yakhin *et al*., 2017). Halophiles possess mechanisms to expel sodium ions from cells and mitigate stress-induced ethylene production, while also producing compounds like exopolysaccharides and antioxidants (Shah and Jan, 2016). Humic acid, known for enhancing water and nutrient uptake and maintaining cell turgidity, augments plant growth under salt stress conditions (Nardi *et al*., 2021).

The micronutrients deficiency in sodic soil is another associated problem that affects plant potential to withstand salt stress. Soils affected with salts show a widespread deficiency of micronutrient due to alkaline pH that decreases their solubility and make them unavailable for plant uptake (Shivraj, 2020). Biostimulants, including humic acid, can chelate micronutrients, improving their solubility and availability to plants, thus enhancing plant resilience to sodicity stress (Shah and Jan, 2016). The main aim of this experiment was to evaluate the relative effectiveness biostimulants and micronutrients on rice plant in alleviating salt stress to achieve potential yield of rice grown in sodic soil.

Materials and Methods

The experiment was conducted during *kharif* 2020-21 at B-Block of Zonal Agricultural Research Station, CoA, V. C. Farm, Mandya, situated in Southern Dry Zone of Karnataka (Zone-6). Soil of the experimental site was sandy clay loam with an alkaline soil pH (8.80), low electrical conductivity (0.36 dS m^{-1}) and medium organic carbon content (5.89 g kg^{-1}). The available nitrogen (236.57 kg N ha^{-1}) and potassium (198.99 kg K_2O ha^{-1}) were low and phosphorus (29.88 kg P_2O_5 ha^{-1}) was found medium.

Treatment details include

T_1 : RDF
T_2 : RDF + Soil application (SA) of $ZnSO_4.7H_2O$
T_3 : RDF + Halophilic microbial consortium HMC)
T_4 : RDF + Humic acid (HA)
T_5 : RDF + Micronutrients mixture (MM)
T_6 : RDF + HMC + HA
T_7 : RDF + HMC + MM
T_8 : RDF + HA + MM
T_9 : RDF + HMC + HA + MM

Note:

- Soil application of $ZnSO_4.7H_2O$ @ 20 kg ha^{-1} at the time of transplanting
- Soil application of Halophilic microbial consortium (*Staphylococcus arlettae* and *Paraburkholderia fungorum*) @ 5 kg ha^{-1} at 10 DAT
- Foliar spray of Humic acid @ 0.2 per cent and Micronutrients mixture (Zn: Mn: Cu: B: Mo-14:5:4:1.2:0.2) @ 1 per cent at 30 and 45 DAT, respectively
- RDF: 125:62.5:62.5 kg N: P_2O_5: K_2O kg ha^{-1} and FYM @ 10 t ha^{-1}

The study was carried out using a Randomized Complete Block Design (RCBD) comprising nine treatments replicated three times, totaling 27 plots (9 treatments × 3 replications). Blocks were demarcated by 60 cm wide channels and unit plots within each block were separated by 30 cm bunds. Layout was prepared with gross plot and net plot size of 5 m × 3.2 m and 4.8 m × 2.8 m, respectively.

The experiment utilized the rice variety IR-64, suitable for the Southern Dry Zone of Karnataka and displaying semi-tolerance to alkaline soils. IR-64 is characterized by its semi-dwarf stature, medium-slender grains and early maturity in around 117 days, featuring a high yield potential with golden brown husks. Two raised nursery beds were prepared, receiving farmyard manure, urea, SSP, and MOP fertilizers uniformly. Pre-soaked rice seeds were broadcasted onto the beds, covered lightly with soil and irrigated to maintain moisture. Healthy seedlings, aged 30 days, were then transplanted from the nursery beds to the main field.

The land was ploughed initially for two times followed by puddling in standing water, reaching a depth of 5-10 cm using a country plow. After 15 days, plots were prepared according to the experiment layout, with a 60 cm wide channel for irrigation and draining excess water between replications. Fifty percent of the gypsum requirement was uniformly applied during land preparation, thoroughly incorporated into the soil through puddling to leach out excess salt. Additionally, the recommended quantity of FYM at 10 t ha^{-1} was applied to plots during experiment layout preparation, ensuring thorough mixing.

The recommended N: P_2O_5: K_2O dose for sodic soil

(125: 62.5: 62.5 kg ha^{-1}) was applied using urea, single super phosphate (SSP) and muriate of potash (MOP) respectively. Urea was split into three doses: 50% as basal and 25% each at tillering and panicle initiation stages. MOP was split into two doses, 50% each at transplanting and panicle initiation stages, while SSP was applied entirely as a basal dose. Zinc sulfate ($ZnSO_4.7H_2O$) at 20 kg ha^{-1} was only applied to T_2 treatment at transplanting. Irrigation was carried out every 6-8 days to maintain a water depth of 3-5 cm, with water drained completely 15 days before harvest. Hand weeding was performed thrice after transplanting, following integrated pest management practices for paddy cultivation. Harvesting was conducted at full maturity stage separately from the net plot of each treatment.

Preparation of halophilic microbial consortium

The salt tolerates bacterial isolates (*Staphylococcus arlettae* and *Paraburkholderia fungorum*), which were isolated and characterized from salt affected soils of V. C. Farm, Mandya were procured from Department of Agricultural Microbiology, CoA, V. C. Farm, Mandya. These isolates were grown on nutrient agar medium supplemented with 15 per cent NaCl and were picked up for enumeration using a sterile loop to transfer to fresh nutrient broth containing 15 per cent NaCl and incubated at 27°C. Broth containing bacterial isolates were further mixed with talcum powder to maintain their population @ 108 cfu per gram and left for curing. After complete curing, the talcum-based inoculum was applied to soil @ 5 kg ha^{-1} along with FYM after 10 DAT.

Morphological and plant growth promoting characteristics of halophilic microbial consortium

Staphylococcus arlettae and *Paraburkholderia fungorum* both the bacterial isolates showed growth upto 15 per cent NaCl concentration and could tolerate 20 per cent NaCl concentration. *Staphylococcus arlettae* had spherical shape, dense creamy white colony feature with gram's '+' reaction. *Paraburkholderia fungorum* had rod shape, raised clourless colony feature with gram's '-' reaction. Both the species showed positive response to HCN and ammonia production as well as for phosphate solubilization (Table 1). The IAA production by *Staphylococcus arlettae* (43.41 µg/ml) was slightly greater than *Paraburkholderia fungorum* (41.75 µg/ml) (Varuna, 2021).

Extraction of humic acid

Ten grams of air dried vermicompost was weighed and transferred to 250 ml conical flask to which 100 ml of 0.1 N NaOH was added and the content was shaken for 24 hours (Schnitzer and Skinner, 1968). The dark-colored supernatant solution was separated by centrifugation and collected. The extraction procedure was repeated three times using 50 ml of 0.1 N NaOH each time for complete extraction of the humic substances. Then 2 N HCl was added to the supernatant collected till the pH reached 2. The acidified extract was allowed to stand for 24 hours. After the specified time the humic acid fraction get precipitated and settled to the bottom, while the fulvic acid remained in solution. The supernatant was separated by centrifuging and collected in a dish. While the residue was removed from the centrifuge tube and transferred to pre-weighed porcelain dish. The dish was placed in oven and dried at 60°C to a constant weight. Then it was stored in air tight container for further use in the experiment. Using the weight, the yield of humic acid was worked out and the per cent recovery of humic acid was 5.

Table 1 : Morphological and plant growth promoting characteristics of halophilic microbial consortium (Varuna, 2021).

Characteristics	***Staphylococcus arlettae***	***Paraburkholderia fungorum***
Morphological characteristics		
Cell shape	Spherical	Rod
Colour of the colonies	Dense creamy white	Colourless
Elevation	Flat	Raised
Margin	Entire	Undulate
Gram's reaction	+	-
Plant growth promoting characteristics		
IAA production (µg/ml)	43.41	41.75
Ammonia production	+	+
HCN production	+	+
Phosphate solubilization	+	+

The extracted HA was characterized by working out E_4/E_6 ratio, is the absorbance ratio at 465 nm and 665 nm in spectroscopy. It was determined by dissolving 2-4 mg of HA sample in 10 ml of 0.05 N $NaHCO_3$ solution and maintaining the resulting pH near to 8.0. Then the absorbance was measured at 465 nm and 665 nm. The ratio of the two absorbances was worked out to get the E_4/E_6 ratio (Khaled and Hassan, 2011).

Formulation of micronutrients mixture

Micronutrients mixture containing Zn, Mn, Cu, B and Mo were prepared using various salts *viz.*, zinc sulphate, manganese sulphate, copper sulphate, boric acid and ammonium molybdate. The concentrations of micronutrients in the mixtures were formulated based on the studies of Shivraj (2020), which contain micronutrients Zn: Mn: Cu: B: Mo in the ratio of 14:5:4:1.2: 0.2 developed specifically for rice grown under sodic soil condition.

Observations recorded

Yield and yield attributes : The observations on yield attributing parameters as described in the following section were recorded from the labeled plants, where ever applicable.

Number of panicles per meter square : Panicles were counted from two randomly selected rows for one meter row length at harvest from each net plot and converted to number of panicles per square meter.

Panicle length : Randomly five panicles were selected from tagged plants and the length was measured from the neck node to the tip of the panicle and average length was recorded in centimeter (cm).

Panicle weight : The selected panicles were weighed using an electronic balance and mean weight of the panicle was calculated and expressed as grams (g).

Number grains per panicle : Grains were separated from selected five panicles and total numbers, filled and unfilled grains were counted separately. The number of grains and filled grains per panicle was expressed in terms of their mean value.

Per cent chaffyness : The ratio of number of unfilled grains per panicle to the total number of grains per panicle was calculated and expressed as percentage.

$$\text{Percent chaffyness} = \frac{\text{No. of unfilled grains per panicle}}{\text{Total no. of grains per panicle}} \times 100$$

Grain yield : Plants were harvested at their physiological maturity stage. The plants from net area were harvested separately from each plot, threshed to separate grains. Then it was sun dried to reduce the moisture content. The grain yield data from each plot was computed on hectare basis and expressed as q ha^{-1}.

Straw yield : The straw retained after threshing was dried under sun and weight was recorded. The straw yield per hectare was computed and expressed in q ha^{-1}.

Results and Discussion

Yield attributes

Number of panicles per meter square

Treatment that received HMC+HA+MM (T_9) showed significantly highest number of productive tillers per m^2 *i.e.*, 284.53 followed by HMC+MM (T_7- 276.58) and HA+MM (T_8- 274). Further, FS of MM (T_5) showed higher number of panicles per meter square *i.e.*, 263.99 followed by SA of HMC (T_3- 255.45), SA of Zn (T_2- 250.65) and FS of HA (T_4- 244.65). Treatment with RDF alone, T_1 showed least number of panicles per meter square *i.e.*, 220.18 (Table 2).

The considerable positive effect was observed through application of biostimulants and formulated grade of micronutrients mixture on number of panicles per m^2. This might be the result of higher vegetative growth *viz.*, leaf area, number of tillers, root growth and shoot growth. Stimulation of panicle initiation in rice depends on metabolic processes involving production of auxin, photosynthesis, protein synthesis and enhanced cell multiplication (division and expansion). These processes exert greater effect on flower initiation, proper maturity and timely pollination in large number of tillers to enhance number of productive tillers per m^2 (Suresh and Salakinkop, 2016 and Muhammad *et al*., 2020).

The salts stress in sodic soil deteriorates the metabolic function involved in optimum nutrient supply and plant growth promoting substances which are helpful in alleviation of this stress (Ali *et al*., 2011; Yahia *et al*., 2015 and Nadeem *et al*., 2007). Hence, foliar spray of micronutrients mixture was effective in increasing number of panicles per m^2. Also, combined application of biostimulants and micronutrients mixture had a promising effect in enhancing panicle number as compared to the treatments receiving only mineral fertilizers and biostimulants as reported by Nadeem *et al*. (2007).

Panicle length and weight

Combined application of HMC+HA+MM significantly recorded highest panicle length and weight (T_9- 25.98 cm and 3.33 g, respectively) as compared to sole application of MM (T_5- 23.54 cm and 2.60 g) or biostimulants i.e., HMC (T_3- 22.54 cm and 2.37 g) or HA (T_4- 20.87cm and 2.28 g). Further, combining MM with HMC (T_7- 24.99 cm and 3.00 g) and HA (T_8-24.65 cm and 2.99 g) enhanced panicle length and weight as compared to SA of Zn (T2- 21.87cm and 2.30 g) and RDF (T_1- 19.98 cm and 2.02 g) as indicated in Table 2.

Number of grains, filled gains and per cent chaffyness

Total number of grains and filled gains per panicle showed similar trend as that of panicle length and weight. Application of HMC+HA+MM (T_9) recorded highest total number of grains and filled grains of 156 and 137, respectively followed by HMC+MM (T_7-153 and 133) and HA+MM (T_8- 150 and 128). Further, FS of MM

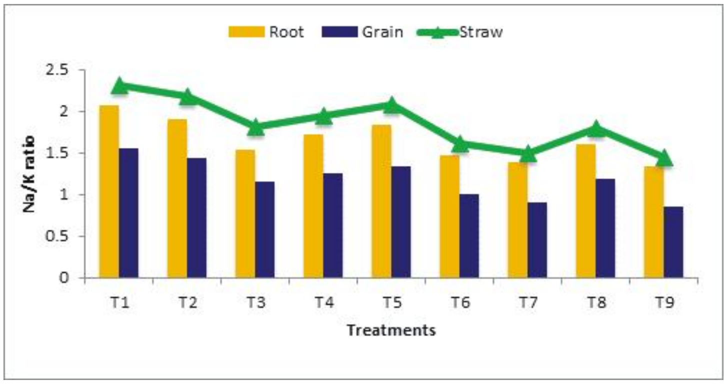

Fig. 1 : Na/K ratio in different parts of rice as influenced by biostimulants and micronutrients mixture at harvest.

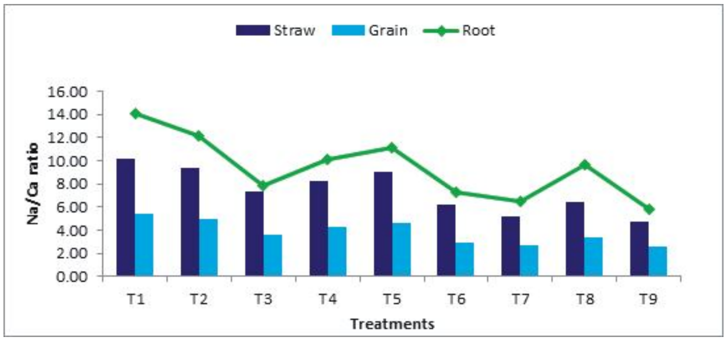

Fig. 2 : Na/Ca ratio in different parts of rice as influenced by biostimulants and micronutrients mixture at harvest. T_1: RDF, T_2: RDF + SA of Zinc, T_3: RDF + SA of HMC, T_4: RDF + FS of HA, T_5: RDF + FS of MM, T_6: RDF + SA of HMC + FS of HA, T_7: RDF + SA of HMC + FS of MM, T_8: RDF + FS of HA + FS of MM, T_9: RDF + SA of HMC + FS of HA + FS of MM.

(T_5) showed a greater number of total grains and filled grains *i.e.*, 143 and 123, respectively and followed by HMC+HA (T_6- 142 and 120), SA of HMC (T_3- 138 and 116), FS of HA (T_4- 136 and 114) and SA of Zn (T_2- 135 and 110). Treatment with RDF alone, T_1 showed a smaller number of total grains and filled gains per panicle *i.e.*, 126 and 99, respectively (Table 2).

Per cent chaffyness of grains were significantly reduced with application of MM (T_5- 15.34%) followed by application of biostimulants (HMC- 15.94 and HA- 16.17%) as compared to SA of Zn (T_2- 18.51%). The combined application of MM with HMC (T_7- 13.07%) or HA (T_8- 14.66%) further reduced the chaffyness and lowest was recorded with HMC+HA+MM (T_9-12.17%). The chaffyness of grains was highest in RDF (T_1- 21.42%).

Grain and straw yield

MM alone (T_5) and in combination with biostimulants exerted increased grain and straw yield recording maximum in HMC+HA+MM (T_9) followed by HMC+MM (T_7) and HA+MM (T_8), while RDF recording lowest grain and straw yield. Micronutrient's deficiency and salt stress are the major constrains in sodic soil that retard growth and yield of rice, this might be the reason for decreased yield in RDF (Table 3).

External application of MM along with biostimulants help in overcoming the nutrient deficiency along with salt stress alleviation in sodic soil. Mahmoud *et al.* (2011) revealed that the combined application of mineral fertilizers along with biostimulants had a promising effect in enhancing the growth and yield parameters compared to the treatments receiving only mineral fertilizers. The enhanced yield with application of biostimulants may be attributed to their stimulation effect to combat salt stress in sodic soil (Sarkar *et al.*, 2018). Biostimulant helps in tolerating salt stress induced under sodic soil condition through osmo-regulatory mechanisms as evident from the data in Figs. 1 and 2, *i.e.*, down regulating the sodium concentration in rice's root, straw and grain by enhancing the K^+ and Ca^{2+} concentration, which help in maintaining cell turgidity and enhancing metabolic activities leading to improved growth and yield during salt stress conditions. HMC form biofilms around plant roots, express ACC deaminase activity which helps to counteract the effect of stress hormone ethylene and produce growth promoting hormone IAA. Also known to secrete some osmolytes (proline, betaine) and scavenge ROS during abiotic stresses (Sarkar *et al.*, 2018). Similarly, HA serve as a growth regulating substance involve in regulating metabolic processes such as synthesis of ATPase, mitochondrial respiration, increased enzymatic activity and enhanced photosynthesis, which are helpful in maintaining cell membrance turgidity during increased salt stress and so, making it possible for plants to sustain/withstand sodicity stress along with improved performance in plants productiveness (Nardi *et al.*, 2021).

Correlation coefficients between yield and yield attributes with ratios of Na/K and Na/Ca values of index leaves as influenced by the application of biostimulants and micronutrients mixture

The outcomes of the correlation analysis as indicated in Table 4 revealed that yield parameters and yield showed negative relation with Na/K and Na/Ca values, highlighting the detrimental impact of high concentrations of Na over K and Ca on yield and quality of the harvested grains in sodic soil. Hereby, signifying the importance of application of biostimulants with/without MM is crucial

Table 2 : Yield attributes of rice as influenced by the application of biostimulants and micronutrients mixture.

Treatments	No. of panicles per m^2	Panicle length(cm)	Panicle weight(g)	No. of grains per panicle	No. of filled grains per panicle	Per cent Chaffyness
T_1	220.18	19.98	2.02	126	99	21.42
T_2	250.65	21.87	2.30	135	110	18.51
T_3	255.45	22.54	2.37	138	116	15.94
T_4	244.65	20.87	2.28	136	114	16.17
T_5	263.99	23.54	2.60	143	123	15.34
T_6	258.56	22.98	2.55	142	120	15.49
T_7	276.58	24.99	3.00	153	133	13.07
T_8	274.00	24.65	2.99	150	128	14.66
T_9	284.53	25.98	3.33	156	137	12.17
SEm ±	**11.30**	**1.01**	**0.11**	**6.13**	**5.14**	**0.81**
CD @ 5%	**33.89**	**3.03**	**0.34**	**18.39**	**15.41**	**2.42**

Table 3 : Grain and straw yield of rice as influenced by the application of biostimulants and micronutrients mixture.

Treatments	Grain yield (q ha^{-1})	Straw yield (q ha^{-1})
T_1	34.08	47.86
T_2	39.87	54.67
T_3	37.15	53.55
T_4	36.99	51.23
T_5	42.45	59.45
T_6	37.65	53.88
T_7	46.87	65.33
T_8	45.65	63.24
T_9	48.65	66.89
SEm ±	**1.80**	**2.51**
CD @ 5%	**5.40**	**7.54**

Table 4 : Correlation coefficients between yield attributes and ratios of Na/K and Na/Ca content in index leaves (WASS) as influenced by application of biostimulants and micronutrients mixture.

Traits	Na/K	Na/Ca
No. of panicle	-0.818**	-0.823**
Panicle length	-0.806**	-0.815**
Panicle weight	-0.812**	-0.820**
No. of grains	-0.855**	-0.860**
No. of filled grains per panicle	-0.865**	-0.861**
Test weight	-0.847**	-0.827**
Grain yield	-0.657	-0.657
Straw yield	-0.709*	-0.712*

Note: WASS- week after second spray of MM and HA, which was carried at 45 DAT.

in down regulating the sodium concentration in rice plants by boosting K$^+$ and Ca^{2+} concentration as evident from the data in Figs. 1 and 2. This mechanism maintains cell turgidity and enhances metabolic activities and ultimately promotes overall growth and optimum yield under salt stress conditions.

Conclusion

Soil application of halophilic microbial consortia along with foliar application of humic acid and micronutrients mixture (T_9) was most effective in enhancing rice's grain (48.65 q ha^{-1}) and straw yield (66.89 q ha^{-1}) followed by HMC+MM (T_7) and HA+MM (T_8) treatments indicating their efficiency to promote stress resilience to rice plants cultivated in sodic soil.

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Conflict of interest

The authors declare that they have no conflict of interest

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