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Effect of phosphate solubilizing bacteria and blue green algae on yield and NPK content in hybrid rice

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Abstract
A pot experiment was conducted during *kharif* season of 2020 on sandy loam soil in texture and classified as mixed hyperthermic typic haplustepts to investigate the impact of phosphate solubilizing bacteria (PSB) and blue green algae (BGA) on crop yield and NPK nutrient uptake in hybrid rice cultivation. Thirteen treatments laid in completely randomized design with two different doses of chemical fertilizers (100% and 75%), two isolates of PSB (*Enterobacter cloacae* strain BAU3 and *Bacillus aryabhattai* strain BAUMS8) and blue green algae had been used in the study. The grain and straw yield of hybrid rice and nutrient uptake in both grain and straw increased significantly when supplied with 100% recommended dose of inorganic fertilizers (RDF) + PSB @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1} as compared to 100% RDF. It had been observed that combined application of PSB and BGA along with 100% RDF, enhanced grain and straw yield upto 11.17 and 6.53 percent, respectively in comparison to 100% RDF. Increase in the concentration of grain and straw N in treatment T_8 was 8.65% and 11.42%, respectively, when compared with treatment T_2 (100% RDF). The concentration of grain P in treatment T_8 were 5.80% when compared with treatment T_2 (100% RDF) while the magnitude of increase in rice straw was 7.28 percent. A similar trend of result was obtained in the case of K content in grain and straw of hybrid rice but the K concentration was higher in straw. Finally, application of 100% RDF along with PSB @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1} significantly augmented the yield and nutrient content in hybrid rice.

Keywords: *Bacillus*, biofertilizers, *Enterobacter*, nutrient, soil fertility

Introduction

The current world population is expected to reach 10 billion people by the end of 2050 (UN DESA 2017), therefore, the annual grain production has to be increased by about 50% in order to be able to supply everyone with food at this time (FAO 2009). Increased pressure on the agricultural sector leads to the ruthless use of inorganic fertilizers, which ultimately affects the fertility and productivity of the soil. Thus, the choice to increase soil fertility and agricultural productivity through the use of better ecological management tools ensures successful food security (Aarti Yadav 2019) [1]. In Southeast Asia, rice is one of the staple foods and centre of the agricultural production system. Hybrid rice has the capacity of producing 15-20% more grain yield than that of the best inbred high yielding variety grown under similar conditions (Virmani 1996) [36]. Therefore, hybrid rice cultivation in India should be expanded to alleviate hunger and increased production of rice is essential for food security. Important strategies to increase rice productivity are the use of local hybrids and better nutrient management through the integration of organic fertilizers, inorganic fertilizers and bio-fertilizers to supply plants with nutrients according to the their demands (Maiti *et al*. 2006; Kamble *et al*. 2008; Talathi *et al*. 2009; Mondal *et al*. 2016) [18, 13, 33, 19]. We contend that healthy and productive soils are critical to ensuring global food security in the future. The use of hybrid rice offers a great opportunity to increase productivity, but the excessive extraction of available nutrients by these varieties degrades the health of the soil and its fertility.

Phosphorus (P) is the second most important macronutrient, after nitrogen (N), required for plant growth and development and is involved in various essential metabolic pathways, including photosynthesis, biological oxidation, nutrient uptake and cell division (Kalayu 2019) [12]. Even though large amount of organic and inorganic form of P present in soil, only 0.1% of total soil P exists in a soluble form for plant uptake. This occurs due to the fixation and low solubility of P containing compounds in soil (Castro and Pereira 2014) [5].

The lack of available P in the soil leads to an imbalance in the use of synthetic fertilizers, which have an undesirable effect on soil health, thus maintaining the health of the soil and supplying the plants with nutrients at an optimal level, without affecting crop productivity, leads to the development of ecological alternative biotechnological tools.

Among the various phosphatic biofertilizer, phosphate solubilizing bacteria (PSB) are the most important with a population of about 1 to 50% out of total soil microbial populations (Alam *et al*. 2002) [2]. Many phosphate solubilizing bacteria (PSB) belonging to genera *Pseudomonas, Enterobacter, Bacillus, Rhizobium, Burkholderia*, *Serratia*, *Rhodococcus* and *Arthrobacter* have been isolated from the rhizosphere (Castro and Pereira 2014) [5]. These microbes can convert insoluble forms of P into available forms for plant uptake by secretion of organic and inorganic acids, phosphatase enzymes and exopolysaccharides (Vazquez *et al*. 2000; Richardson 2001; Emami *et al*. 2020) [35, 26, 8]. *Bacillus* sp. and *Enterobacter* sp. are well-known plant growth promoting rhizobacteria (PGPR) that accelerate plant growth by solubilizing P minerals and by producing metabolites such as siderophores and phytohormones (Prakash and Arora 2019) [22]. Like PSB, another alternative is use of blue green algae (BGA), prokaryotes that perform oxygenic photosynthesis and liberates various plant growth promoting substance like vitamins, IAA and amino acids, which help in solubilization of insoluble phosphosphates and make it available to the plant (Satapathy 1999) [28]. Although they are known to primarily increase the availability of nitrogen (N) in paddy fields through the mechanisms of biological nitrogen fixation but there are few reports which show that they have ability to solubilize insoluble P, like, rock phosphate, $Ca_3(PO_4)_2$, $FePO_4$, $AlPO_4$, and hydroxyl-apatite ($Ca_5(PO_4)3OH$) (Yandigeri *et al*. 2011) [38] in soils by releasing low molecular weight organic acids. The uses of biofertilizers are an economical, environmentally friendly and renewable supply of soil nutrients that play a vital role in maintaining long-term sustainability and soil fertility (Aarti Yadav 2019) [1] but information on the joint use of inorganic and bio-fertilizers for hybrid rice in Bihar, India is limited. Considering all these points, an investigation was carried out to evaluate the impact of phosphate solubilizing bacteria and blue green algae on yield and NPK uptake in hybrid rice.

Materials and Methods

Experimental site

A pot experiment was conducted in the net house of the Department of Soil Science and Agricultural Chemistry, Bihar Agricultural University, Sabour, Bhagalpur ($25^0$23' N latitude; $78^0$07' E longitude and an elevation of 25 m from sea level). The experimental site falls under a sub-humid zone of Bihar, India. The experiment was conducted during *kharif* season of 2020 with the aim of studying the effect of phosphate solubilizing bacteria (PSB) and blue green algae (BGA) application with the recommended dose of fertilizers in soil on yield and NPK uptake in hybrid rice of variety Arize-6129. The experimental soil was sandy loam in texture (52.40% sand, 28.00% silt and 19.60% clay) and could be classified as mixed hyperthermic typic haplustepts. The average rainfall in 2019 and 2020 during the *kharif* (July - October) was recorded to be 366.90–22.10 mm. The maximum temperature of 2019 and 2020 ranged from 33.65 °C to 32.30 °C and the minimum temperature varied from 26.45 °C to 22.15 °C. The average relative humidity of 2019 and 2020 varied from 74.65% to 81.92% during the cropping season (Figure 1). Overall, the average temperature and relative humidity during the crop growing period didn't differ significantly from the two-year averages.

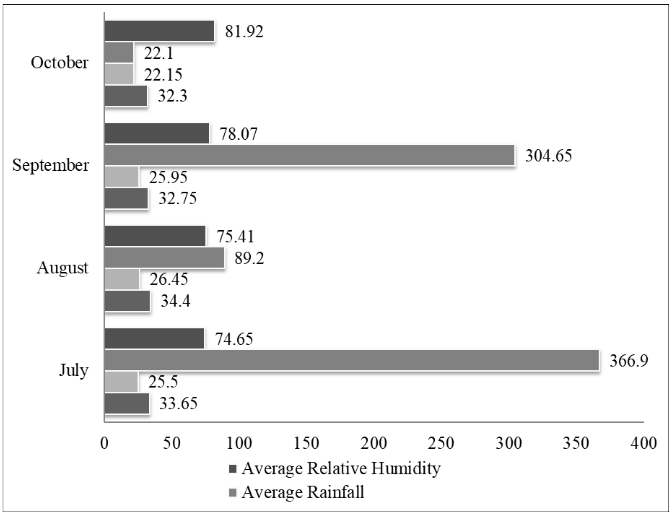

Fig 1: Average metrological report of two years (2019 and 2020) during the cropping period

Experimental details

The experiment was laid out in a completely randomized design (CRD) comprising with thirteen nutrient management practices (T_1: Control, T_2: 100% RDF, T_3: 75% RDF, T_4: 100% RDF + PSB_1 @ 750 ml ha^{-1},T_5: 75% RDF + PSB_1 @ 750 ml ha^{-1}, T_6: 100% RDF + BGA @ 10 kg ha^{-1}, T_7: 75% RDF + BGA @ 10 kg ha^{-1}, T_8: 100% RDF + PSB_1 @ 750 ml/ha + BGA @ 10 kg ha^{-1}, T_9: 75% RDF + PSB_1 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}, T_{10}: 100% RDF + PSB_2 @ 750 ml ha^{-1}, T_{11}: 75% RDF + PSB_2 @ 750 ml ha^{-1}, T_{12}: 100% RDF + PSB_2 @ 750 ml ha^{-1}+ BGA @ 10 kg $ha^{-1}$and T_{13}: 75% RDF + PSB_2 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}**)** replicated thrice in pots filled with 15 kg soil pot^{-1}. The recommended dose of inorganic fertilizers (RDF) for hybrid rice was 120 kg N, 60 kg P_2O_5, 40 kg K_2O and 5 kg $ZnSO_4.7H_2O$ ha^{-1} while among biofertilizers; BGA and two isolates of phosphate solubilizing bacteria *i.e.* PSB_1 (*Enterobacter cloacae* strain BAU3) and PSB_2 (*Bacillus aryabhattai* strain BAUMS8) were procured from Biolab, Bihar Agricultural University, Sabour, Bhagalpur.

Crop management practices

The rice seedlings were raised in a dry nursery bed prepared by crosswise ploughing followed by subsequent flooding. The manures and fertilizers were applied in the form of FYM, urea, DAP, MOP. A full dose of FYM P, K and one third dose of N were applied as basal before planting. The remaining N fertilizer was applied in two equal splits at mid-tillering and heading stage. Transplanting was done in the pot one seedling per hill of 21 day old seedlings. Chemical and biofertilizers *viz*., BGA @10 kg ha^{-1} and PSB_1/PSB_2 @ 750 ml ha^{-1} (1×10^9 cfu ml^{-1}) were applied as per the treatments by dipping the seedling roots before transplanting. The crop received four irrigation in the stages of early tillering (20 DAT), panicle initiation (40 DAT), grain formation (60 DAT) and grain filling (80 DAT) with 70 mm of water per irrigation for maintaining the soil under saturated conditions from planting to 25 days after flowering. Dusting of Fenvalerate 0.4% DP @ 25 kg ha^{-1} was done at heading stage to protect the crop against the attack of *Leptocorisa varicornis* during the cropping period.

Observations recorded

The grain and straw yield were recorded from each pot after harvesting. Initial fertility status of experimental soil collected from Research farm of Bihar Agricultural University, Sabour was determined from a composite soil sample (collected at 0–20 cm depth) before the initiation of the pot experiment. The experimental soil was low in oxidizable carbon content (4.3 g kg^{-1} soil) with having soil reaction neutral (pH 7.39) and EC (0.11 dSm^{-1}). The experimental soil was categorized as medium in terms of soil fertility with available N, P and K values of 213.25 kg ha^{-1}, 13.45 kg ha^{-1} and 180.40 kg ha^{-1} respectively. The pH and EC of the experimental soil was determined with the help of pH meter and EC meter in a 1:2.5 soil: water suspension (Jackson 1973) [11]. The organic carbon content (%) was determined by the wet digestion method (Walkey and Black 1934) [37], available N by alkaline potassium permanganate method (Subbiah and Asija 1956) [32], available P by the Olsen method (Olsen *et al*. 1954) [20] and available K by the neutral ammonium acetate method (Black 1965) [3]. The N content in grain and straw samples was estimated by Kjeldahl digestion method (Bremner and Mulvaney 1982) [4]; while P and K content were estimated by the di-acid digestion method (Koening and Johnson 1942) [16].

Statistical analysis

The experimental data were subjected to analysis of variance. The least significant difference (LSD) test was used to separate significantly differing treatment means at $p<0.05$. Correlation analysis was executed by computation of Pearson's correlation coefficient.

Results and Discussion

Crop productivity

The yield of grain and rice straw, as shown in Table (1), increased positively with the application of 100% NPKZn (T_2) showing the value of 60.18 and 76.60 qt ha^{-1}, respectively, in comparison to 51.50 and 73.80 qt ha^{-1}, respectively, in the control (T_1). The highest grain yields (66.90 qt ha^{-1}) and straw (81.60 qt ha^{-1}) were recorded in T_8 (100% RDF + PSB_1 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}) which were almost at par in treatment T_4 (100% RDF + PSB_1 @ 750 ml ha^{-1}), T_9 (75% RDF + PSB_1 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}), T_{12} (100% RDF + PSB_2 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}) and T_{13} (75% RDF + PSB_2 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}) (Table 1). This could be apparently visualized from the Figure (2) that the magnitude of crop productivity were positively increased under the combined and balanced integration of PSB and/or BGA and inorganic fertilizers as compare to RDF nutrient management treatments (T_2) (without PSB and/or BGA) while it was negative in control (T_1) and 75% RDF treatments (T_3, T_5, T_7 and T_{11}) in case of grain yield. The values were also negative in control (T_1) and 75% RDF treatments (T_3 and T_{11}) in case of straw yield. A significant increase in the treatment T_8 upto 11.17 and 6.53 per cent in grain and straw, respectively in comparison to 100% RDF had been observed, while treatment T_4, T_9, T_{12} and T_{13} in the case of grain yield and treatment T_4, T_6, T_7, T_9, T_{10}, T_{12} and T_{13} in the case of straw yield were statistically comparable to T_8 treatment. These parameters attained the highest value with increasing RDF levels (100% RDF) in the presence of the combined use of PSB and BGA which confirmed the findings of Rana *et al*. (2015) [24] and Singh and Shrivastava (2015) [15]. Distinct positive effects of PSB and BGA in the presence of different doses of RDF levels were noticed and this might be due to fixation of atmospheric nitrogen by BGA and the increased solubility of phosphorus by the application of PSB increased nutrient uptake by crop leading to increase in biological yield of rice (Chittora *et al*. 2020) [7]. Increase in yield components might also be due to higher photosynthetic activity, release of growth promoting substances like IAA, vitamins, etc. control of plant pathogens and proliferation of beneficial organisms in the rhizosphere which ultimately promoted dry matter production resulting higher grain and straw yield has also been reported by (Prasanna *et al*. 2012; Garai *et al*. 2014; Singh *et al*. 2016; Kang *et al*. 2021) [13, 10, 31].

Table 1: Effect of Phosphate Solubilizing Bacteria and Blue Green Algae on grain and straw yield of rice

Treatments	Grain yield		Straw yield	
	qt ha^{-1}	g $hill^{-1}$	qt ha^{-1}	g $hill^{-1}$
T_1: Control	51.50	15.45	73.80	22.14
T_2: 100% RDF	60.18	18.05	76.60	22.98
T_3: 75% RDF	54.90	16.47	75.80	22.74
T_4: 100% RDF + PSB_1 @ 750 ml ha^{-1}	62.80	18.84	78.40	23.52
T_5: 75% RDF + PSB_1 @ 750 ml ha^{-1}	59.10	17.73	77.20	23.16
T_6: 100% RDF + BGA @ 10 kg ha^{-1}	61.39	18.42	79.10	23.73
T_7: 75% RDF + BGA @ 10 kg ha^{-1}	57.78	17.33	77.70	23.31
T_8: 100% RDF + PSB_1 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}	66.90	20.07	81.60	24.48
T_9: 75% RDF + PSB_1 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}	64.83	19.45	80.80	24.24
T_{10}: 100% RDF + PSB_2 @ 750 ml ha^{-1}	61.43	18.43	78.80	23.64
T_{11}: 75% RDF + PSB_2 @ 750 ml ha^{-1}	57.81	17.34	76.20	22.86
T_{12}: 100% RDF + PSB_2 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}	66.74	20.02	81.30	24.39
T_{13}: 75% RDF + PSB_2 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}	64.20	19.26	80.30	24.09
S.Em (±)	1.44	0.43	1.37	0.30
LSD (0.05)	4.20	1.26	3.97	0.86

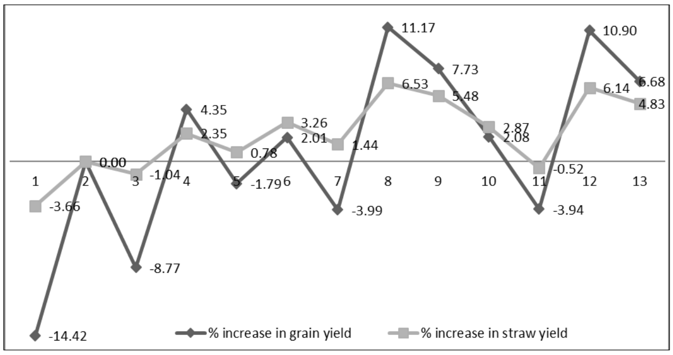

Fig 2: Percent changes in grain and straw yield of different treatments as compare to 100% RDF treatment

NPK content in grain and straw

Crop nutrition plays an important role in escalating the nutrient removal by grain and straw of hybrid rice. The results indicate that there were non-significant differences between inorganic treatments and the combined use of organic and inorganic treatments for N, P and K contents in grain and straw, however, these parameters were lower when the rice seedlings inoculated without bacterial and/or algal bio-fertilizers (Table 2).

The N-concentration of rice in grain and straw enhanced from 1.101% and 0.408% in the control treatment (T_1) to a highest of 1.294% and 0.459% respectively, in the treatment T_8 (100% RDF + PSB_1 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}). It had also been observed that the treatment T_4 (100% RDF + PSB_1 @ 750 ml ha^{-1}), T_6 (100% RDF + BGA @ 10 kg ha^{-1}), T_9 (75% RDF + PSB_1 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}), T_{10} (100% RDF + PSB_2 @ 750 ml ha^{-1}), T_{12} (100% RDF + PSB_2 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}) and T_{13} (75% RDF + PSB_2 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}) were statistically similar with treatment T_8 in both grain and straw of rice. Increase in the concentration of grain and straw N respectively in treatment T_8 was 17.49% and 12.43% when compared with treatment T_1 (control) and 8.65% and 11.42% with treatment T_2 (100% RDF).

Table 2: Effect of Phosphate Solubilizing Bacteria and Blue Green Algae on nitrogen, phosphorus and potassium content in grain and straw

Treatments	N		P		K	
	Grain (%)	Straw (%)	Grain (%)	Straw (%)	Grain (%)	Straw (%)
T_1: Control	1.10	0.41	0.26	0.13	0.41	1.64
T_2: 100% RDF	1.19	0.41	0.27	0.14	0.42	1.65
T_3: 75% RDF	1.14	0.41	0.26	0.14	0.41	1.64
T_4: 100% RDF + PSB_1 @ 750 ml ha^{-1}	1.22	0.43	0.27	0.14	0.44	1.68
T_5: 75% RDF + PSB_1 @ 750 ml ha^{-1}	1.19	0.42	0.27	0.14	0.43	1.68
T_6: 100% RDF + BGA @ 10 kg ha^{-1}	1.23	0.44	0.27	0.14	0.43	1.67
T_7: 75% RDF + BGA @ 10 kg ha^{-1}	1.20	0.42	0.27	0.14	0.42	1.66
T_8: 100% RDF + PSB_1 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}	1.29	0.46	0.27	0.15	0.45	1.69
T_9: 75% RDF + PSB_1 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}	1.26	0.45	0.27	0.14	0.44	1.69
T_{10}: 100% RDF + PSB_2 @ 750 ml ha^{-1}	1.23	0.43	0.27	0.14	0.44	1.68
T_{11}: 75% RDF + PSB_2 @ 750 ml ha^{-1}	1.20	0.42	0.27	0.14	0.42	1.67
T_{12}: 100% RDF + PSB_2 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}	1.29	0.46	0.27	0.15	0.45	1.69
T_{13}: 75% RDF + PSB_2 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}	1.26	0.45	0.27	0.14	0.44	1.68
S.Em (±)	0.03	0.01	0.00	0.00	0.01	0.01
LSD (0.05)	0.08	0.04	0.01	0.01	0.02	0.02

The P-concentration in rice showed similar trend to that of N and the lowest content in grain and straw respectively, had been recorded in control treatment (T_1) 0.259% and 0.132% where no fertilizer was applied and highest in the treatment (T_8), 0.274% and 0.147% when the combined application of 100% RDF + PSB_1 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1} was done. The increase in concentration of grain P in treatment T_8 were 5.80% and 2.63% when compared with treatment T_1 (control) and T_2 (100% RDF), respectively while the magnitude of increase in rice straw was 11.34 and 7.28 per cent. However, a similar trend of result was obtained in the case of K content in grain and straw of hybrid rice but the K concentration was higher in straw. The raised K content were 9.50 and 8.01% had been observed in grain while the enormity of increase in straw was 3.34 and 2.38% in treatment T_8 when compared with treatment T_1 (control) and T_2 (100% RDF), respectively.

Crop management input nutrient sources supply through combination of PSB, BGA and inorganic fertilizers tended to increase the nutrient content in both grain and straw as compare to sole supply of nutrients through chemical fertilizers. This could be due to a balanced supply of plant nutrients from bio-fertilizers and chemical sources (Panigrahi *et al*. 2014) [21]. The combined application of RDF along with PSB and BGA was significantly superior to other treatments in terms of NPK content (%) in grain and straw and its total intake. It was observed that treatment T_8 (100% RDF + PSB_1 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}) was significantly superior for NPK content and its uptake. Also, treatment T_{12} (100% RDF + PSB_2 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}) showed statistical similarity in almost all of these parameters. The N and P content in grain were much higher than that of straw due to increased efficiency and cumulative synergistic effect of combined application of PSB and BGA (Chinnusamy *et al*. 2006) [6], whereas K content in straw was higher than that of grain might due to luxury consumption of K (Mondal *et al*. 2016) [19]. The results are also in conformity with the findings of Raut and Mahapatra (2006) [25], Khadayate *et al*. (2005) [15], Kumar and Yadav (2009) [17], Sharma *et al*. (2009) [29] and Sahu *et al*. (2009) [27].

Conclusions

The combined application of phosphate solubilizing bacteria (PSB) and blue green algae (BGA) significantly influenced NPK content in hybrid rice and increased the yield upto 11% as compared to 100 percent recommended dose of fertilizers (RDF). Biofertilizers are cost effective, eco-friendly and renewable supply of soil nutrient which play a vital role in maintaining a long term sustainability and fertility of soil. Thus, applications of PSB and BGA have appreciable impact on the NPK nutrition to hybrid rice in modern intensive agriculture.

Disclosure statement

No potential conflict of interest was reported by the authors.

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