



Effect of methyl jasmonate in enhanced growth, antioxidants and reduced Pb uptake in contrasting cluster bean cultivars

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Abstract

Lead is known as the second toxic heavy metal which negatively affects plant growth. Hence, there is a need to develop eco-friendly techniques to overcome the negative impacts of Pb on plants. Plant hormones play an essential role in amelioration of heavy metal toxicity. In the current study, the effects of exogenous application of methyl jasmonate (0, 10, 30 and 50 μM) on growth, antioxidant enzymes and Pb uptake of two contrasting cultivars of cluster bean (RGC 1055 and RGC 1066) were evaluated under various lead-treatments (0, 600, 800 mg/kg). In RGC 1066, the Pb uptake in roots, shoot, leaves, and pods decreased by 49.28%, 43.73%, 32.78% and 29.06%, respectively by the exogenous application of MeJA (10 μM) under Pb stress (800 mg/kg). The current findings concluded that MeJA enhanced the detrimental effect of lead by increasing growth, activity of antioxidant enzymes and reducing Pb accumulation in both cultivars. Hence, application of MeJA can be considered as an efficient method in minimizing toxic effects of lead by reducing lead uptake in cluster bean cultivars growing in contaminated soil.

Keywords Lead · Methyl jasmonate · Cluster bean · Antioxidant enzymes · Pb accumulation

Introduction

In recent years, toxicity of heavy metals is a major issue for agriculture, which highly affects the metabolic activities of plants (Hashem and El-Sherif 2019). Lead (Pb) is highly toxic environmental pollutants found in a small amount in the earth's crust (Hadi and Aziz 2015). There are various anthropogenic activities through which Pb enters into the environment, such as municipal sewage and sludge disposal, smelting of ores, pesticides, discharge from batteries, Pb-based paints, automobile exhaust and the burning of fossil fuel. Pb exerts various morphological and physiological changes in plants (Pourrut et al. 2011). Pb activates the generation of reactive oxygen species (ROS) which disrupts membrane stability by increasing lipid peroxidation (Kaur et al. 2013). Plants have antioxidant defense mechanism that

prevents the formation of ROS. However, under high metal concentration, ROS formation often exceeds plant antioxidant capacity, leading to increased oxidative damage. Hence, it is necessary to enhance the efficacy of antioxidant defense system to facilitate plant's ability to cope under stress (Kamran et al. 2021).

In recent years, modulation of heavy metal toxicity by phytohormones has become a new research approach which emphasis on the capability of plant hormones to various abiotic stress tolerance (Syta et al. 2019). Phytohormones play an important role in protecting plants from environmental stress and helping plants in their growth and development. Methyl jasmonate (MeJA) an important class of jasmonic acid (JA) plays very important role in growth, development as well as regulate the defense system under stress (Mousavi et al. 2020). JA is known as an essential plant hormone which modulates growth and immune responses to increase plant survival under stress conditions and also plays an important role in protecting against several abiotic stresses (Yang et al. 2019).

Cluster bean also known as *Cyamopsis tetragonaloba* (L.) Taub. is one of the world's major sources of high-protein livestock feed and edible vegetable oils (Jayakumar 2019). It is a multi-functional crop as the plant is used as fodder,

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Pods as vegetable and seeds as source of gum. The gum is extensively utilized in different industries including paper, cosmetics, food mining and pharmaceuticals (Sangwan et al. 2013).

The mitigating impact of MeJA on contrasting cluster bean cultivars under stress has not yet been reported. By understanding the use of MeJA in preventing plants from metal stress, the current study was planned to analyze the impact of MeJA in cluster bean cultivars under Pb stress by estimating morphological parameters at reproductive stage.

Methodology

The seeds of two cultivars of cluster bean (RGC 1055 and RGC 1066) were procured from the Krishi Vigyan Kendra (Banasthali Vidyapith, India). These two cultivars were selected on the basis of different morphological parameters examined in our previous study (Sharma et al. 2022). The mixture of soil and soilrite (1:1) was added to the plastic pots. Different concentrations of Pb solutions (0, 600, 800 mg/kg) were added to the soil in the form of lead nitrate. Sterilized seeds were soaked overnight and each pot having five seeds were grown in growth chamber at 16 h photoperiod at 25 ± 2 °C. MeJA (0, 10, 30 and 50 μ M) was sprayed on control and Pb-treated plants two weeks after sowing on both the cultivars. All the parameters were studied at reproductive stage [65 days after sowing (DAS)].

Determination growth parameters and Pb content

The morphological parameters were determined at 65 DAS. The Pb content in different plant parts were determined by atomic absorption spectrophotometer (Thermo Scientific Ice 3000) (Amin et al. 2018).

Determination of malondialdehyde (MDA)

The estimation of MDA content was calculated by the method of De Vos et al. (1991).

Determination of the activity of antioxidant enzymes

The activity of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (POD) and glutathione reductase (GR), was determined by the method of Beauchamp and Fridovich (1971), Aebi (1984), Nakano and Asada (1981), Putter (1974), Barata et al. (2000), respectively.

Statistical analysis

The obtained data were subjected to one-way ANOVA, significant differences were compared using Tukey's test. The values shown are mean \pm SD. Alphabets show significant differences among treatments at $P \leq 0.05$.

Results and discussion

Pb is a highly toxic metal that causes severe morphological and physiological changes in plants (Hadi and Aziz 2015). Phytohormones play an important role in plant physiological processes and signaling cascades of plants under biotic and abiotic stresses. The exogenous application of JA alleviates the damaging effect on plants under stress (Farooq et al. 2016).

Effects of MeJA on growth of cluster cultivars under Pb stress

All growth parameters decreased under Pb stress. However, spraying MeJA on Pb-treated plants relieved the negative effect of Pb and restored the growth of cluster bean plants. Among the different studied MeJA concentrations, the most pronounced effects were observed at 10 μ M. The root lengths were increased by 12.66% and 11.56% in cultivars RGC 1066 and RGC 1055, respectively and shoot lengths by 23.83% and 20.87% compared to Pb-stressed plants with 800 mg/kg of Pb. The dry weights were increased by 27.81% and 21.07% in RGC 1066 and RGC 1055, respectively for shoot, and by 24.25% and 23.71% for root as compared to Pb-stressed plants at 800 mg/kg. The number of pods and seeds were also increased by the application of MeJA in both cultivars. The increase in the number of pods was 22.22% and 20% by the application of MeJA (10 μ M) in RGC 1066 and RGC 1055 under 800 mg/kg of Pb stress (Tables 1, 2).

The current study showed that Pb stress affects growth parameters in cluster bean cultivars, indicating plant tolerance towards Pb toxicity is cultivar dependent. This decrease could be due to the higher deposition of Pb in plants. The study was designed to analyze the impact of foliar MeJA application on Pb-stressed cluster bean cultivars and observed the alleviation of Pb resistance in cluster bean cultivars. However, exogenous implication of MeJA increased growth and biomass in both cluster bean cultivars. The increase in growth indices might be because of the beneficial effects of MeJA on cellular redox balance and nutrient absorption. Similar ameliorative impact of MeJA was observed under arsenic stress in rice (Farooq et al. 2016) and cadmium stress in *Brassica* (Ali et al. 2018). Bali et al. (2018)

Table 1 Effect of different concentrations of MeJA on growth parameters of cluster bean cultivar (RGC 1066) under Pb stress (mean \pm SD)

Pb (mg/kg)	MeJA (μ M)	Root length (cm)	Shoot length (cm)	Root dry weight (g)	Shoot dry weight (g)	Number of pods per plant	Number of seeds per pod
0	0	58.63 \pm 0.55ef	33.76 \pm 0.40f	0.80 \pm 0.07a	1.74 \pm 0.09f	09.3 \pm 1.15f	8.3 \pm 0.57a
	10	59.46 \pm 0.58ef	35.40 \pm 1.2f	0.83 \pm 0.05b	1.77 \pm 0.05ef	10.3 \pm 0.57d	9.0 \pm 0.00b
	30	58.96 \pm 0.49f	34.36 \pm 0.80f	0.82 \pm 0.03ab	1.77 \pm 0.05c	09.6 \pm 0.57 cd	8.6 \pm 0.57bc
	50	57.33 \pm 1.10e	32.66 \pm 1.06ef	0.76 \pm 0.03c	1.75 \pm 0.04bc	09.6 \pm 1.52e	8.6 \pm 0.57 cd
600	0	41.80 \pm 0.75b	24.76 \pm 1.61bc	0.54 \pm 0.05c	1.12 \pm 0.04ab	06.6 \pm 0.57 cd	7.6 \pm 0.57ab
	10	48.06 \pm 0.70d	30.70 \pm 0.95e	0.67 \pm 0.02bc	1.41 \pm 0.03a	08.3 \pm 0.57ab	9.3 \pm 0.57a
	30	48.16 \pm 0.70d	30.00 \pm 1.37de	0.67 \pm 0.02bc	1.51 \pm 0.08b	08.0 \pm 0.00ab	9.0 \pm 1.00c
	50	45.00 \pm 0.43c	27.43 \pm 0.75 cd	0.60 \pm 0.02ab	1.37 \pm 0.07a	07.3 \pm 0.57ef	8.3 \pm 0.57d
800	0	35.80 \pm 0.52a	20.70 \pm 0.75a	0.46 \pm 0.04e	0.99 \pm 0.04ef	06.0 \pm 0.00d	7.3 \pm 0.57ef
	10	40.33 \pm 0.95b	25.63 \pm 0.90c	0.57 \pm 0.04ef	1.23 \pm 0.05bc	07.3 \pm 0.57b	8.6 \pm 0.57e
	30	39.86 \pm 0.76b	24.63 \pm 0.55bc	0.55 \pm 0.02dc	1.31 \pm 0.07abc	07.0 \pm 1.00a	8.3 \pm 0.57 cd
	50	36.96 \pm 0.47a	22.30 \pm 0.62ab	0.50 \pm 0.02bc	1.16 \pm 0.04bc	06.6 \pm 0.57bc	8.0 \pm 0.00c

Significant differences ($p < 0.05$) are represented by different alphabets

Table 2 Effect of different concentrations of MeJA on growth parameters of cluster bean cultivar (RGC 1055) under Pb stress (mean \pm SD)

Pb (mg/kg)	MeJA (μ M)	Root length (cm)	Shoot length (cm)	Root dry weight (g)	Shoot dry weight (g)	Number of pods per plant	Number of seeds per pod
0	0	60.96 \pm 0.66 fg	30.96 \pm 0.50d	0.87 \pm 0.03 cd	1.85 \pm 0.11 cd	09.6 \pm 1.15c	09.6 \pm 1.15b
	10	62.30 \pm 1.10 fg	32.50 \pm 1.21d	0.90 \pm 0.02a	2.36 \pm 0.07c	11.0 \pm 1.00 cd	10.6 \pm 0.57c
	30	61.40 \pm 0.90 g	32.20 \pm 1.60d	0.85 \pm 0.01b	2.53 \pm 0.08c	10.3 \pm 0.57b	10.3 \pm 0.57bc
	50	58.86 \pm 0.64f	29.70 \pm 0.45d	0.82 \pm 0.02bc	2.26 \pm 0.06de	10.0 \pm 0.00a	10.0 \pm 0.00ab
600	0	36.03 \pm 0.68c	19.33 \pm 0.86b	0.51 \pm 0.04c	1.16 \pm 0.08ab	06.3 \pm 0.57bc	08.0 \pm 0.00a
	10	40.40 \pm 1.10e	23.90 \pm 1.67c	0.63 \pm 0.01 cd	1.43 \pm 0.09ab	08.0 \pm 1.00c	09.6 \pm 0.57c
	30	39.73 \pm 0.41de	23.56 \pm 1.05c	0.61 \pm 0.01ab	1.51 \pm 0.06a	07.6 \pm 0.57f	09.0 \pm 0.00e
	50	37.13 \pm 0.95 cd	21.66 \pm 0.96bc	0.56 \pm 0.01a	1.39 \pm 0.03bc	07.0 \pm 1.00ef	08.6 \pm 0.57ef
800	0	28.53 \pm 1.00a	12.93 \pm 0.23a	0.32 \pm 0.05ab	0.87 \pm 0.06 cd	05.0 \pm 0.00ab	06.6 \pm 0.57b
	10	31.83 \pm 1.34b	15.63 \pm 0.76a	0.40 \pm 0.01 cd	1.05 \pm 0.06c	06.0 \pm 0.00b	07.6 \pm 0.57bc
	30	31.23 \pm 1.05b	15.30 \pm 0.30a	0.39 \pm 0.02c	1.12 \pm 0.03d	05.6 \pm 0.57bc	07.3 \pm 0.57a
	50	29.30 \pm 0.43ab	13.96 \pm 0.56a	0.35 \pm 0.03d	0.99 \pm 0.05 cd	05.3 \pm 0.57b	07.0 \pm 0.00 cd

Significant differences ($p < 0.05$) are represented by different alphabets

and Kamran et al. (2021) also reported the similar protective role of JA under Pb (tomato) and chromium (*Brassica*).

MeJA reduced accumulation of Pb in cluster bean plants

The uptake of Pb increased in root, shoot, leaves and pods with increase in concentration of Pb. Conversely, application of MeJA to Pb-stressed plants decreased Pb uptake in different parts of cluster bean plants. The results showed that accumulation of Pb was higher in cultivar RGC 1055 under all Pb concentrations. The application of MeJA (10 μ M) significantly declined the Pb uptake in root, shoot, leaves and pods by 49.28%, 43.73%, 32.78% and 29.06%, respectively in RGC 1066 and 49.83%, 43.68%, 27.57%

and 31.45% in RGC 1055 under 800 mg/kg of Pb stress (Fig. 1A, B).

JA may behave as a signaling molecule which might decrease the heavy metal transporter protein expression and ultimately reduced metal uptake. MeJA decreased Pb accumulation in all plant parts and similar results were also reported in arsenic stressed *Brassica napus* by Farooq et al. (2016). The application of JA restricted the uptake of Pb in *Wolffia arrhiza* (Piotrowska et al. 2009) and cadmium in *Vicia faba* (Ahmad et al. 2017). The reduction in content of Pb further minimizes the oxidative damage, thereby decreasing the toxic impacts of Pb on morphological parameters and lowers MDA content of cluster bean plants.

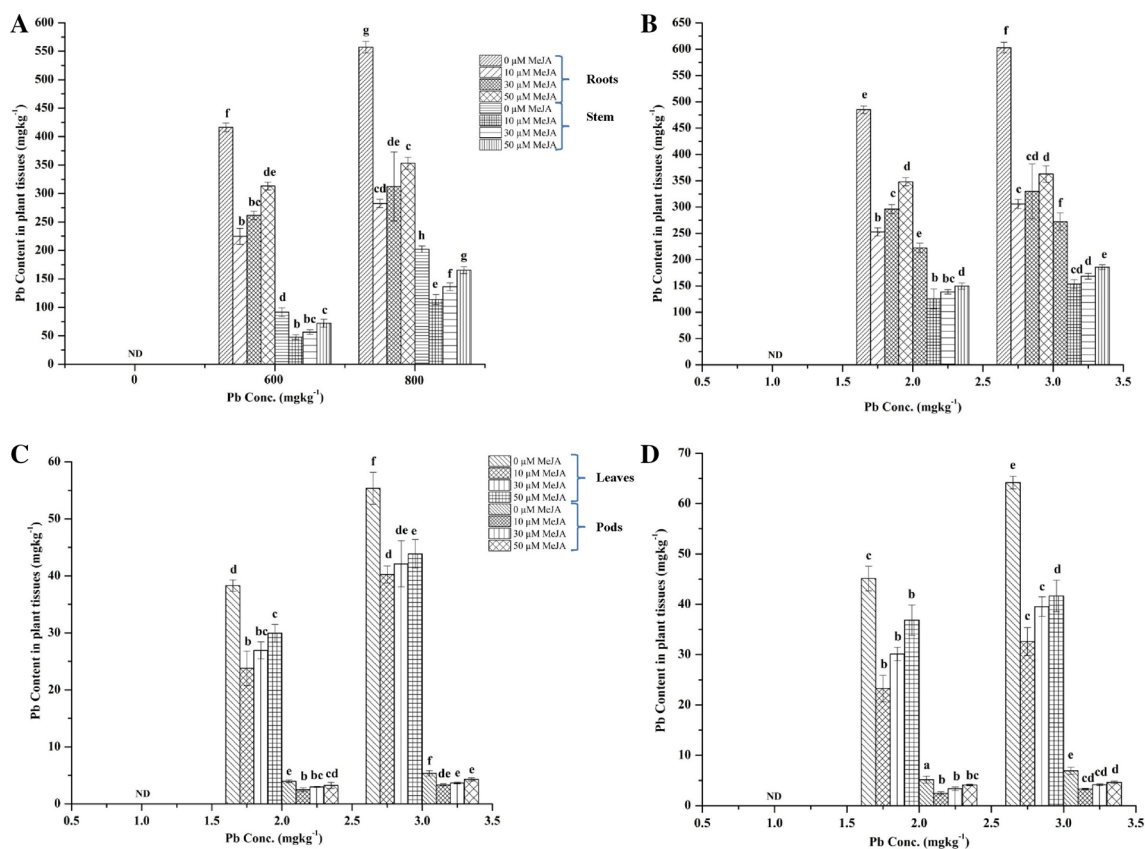


Fig. 1 Effect of MeJA on Pb content in different plant parts in RGC 1066 (A, C) and RGC 1055 (B, D) under Pb stress. Bars are represented as mean of three replicates \pm SD. Significant differences ($p < 0.05$) are represented by different alphabets

Effect of MeJA on MDA content under Pb stress

The MDA content increases under Pb stress in both cluster bean cultivars. However, the application of MeJA decreased the toxic effect of Pb by decreasing the MDA content in both the cultivars of cluster bean (Fig. 1C). The MDA content increased by 29.18% and 26.51% in RGC 1066 and RGC 1055, respectively under 800 mg/kg. The MeJA (10 μ M) supplementation decreased MDA content by 20.18% and 16.47% in RGC 1066 and RGC 1055, respectively. The exogenously applied MeJA protected the biological membrane, lower lipid peroxidation and thus enhanced tolerance to Pb stress. The results of present study are similar with the findings of Hanaka et al. (2016) and Mousavi et al. (2020) that MDA content decreased after application of MeJA under copper, cadmium and arsenic stress in bean and rice, respectively. A higher amount of MDA was found in RGC 1055 than in RGC 1066, suggested sensitivity of RGC 1055 to Pb stress.

MeJA enhanced the activity of antioxidant enzymes under Pb stress

Generally, alleviations in oxidative stress are related to increased enzymatic activity and scavenging of ROS under stress conditions (Foyer and Noctor 2011). Plants are protected against oxidative stress by their antioxidant defense system (Kamran et al. 2021). This study showed that the antioxidant enzymes activity (CAT, POD, GR, APX, and SOD) increased with an increase in Pb concentration. However, the application of MeJA also maintains the defense system by further increasing the defense system of cluster bean plants to Pb stress. The application of MeJA (50 μ M) significantly increased activities of CAT, SOD, APX and POD by 77.43%, 347.02%, 68.91%, and 77.90% in variety RGC 1066 and 57.50%, 305.58%, 62.54%, 39.66% and 62.18% in variety RGC 1055, respectively as compared to non-treated plants at 800 mg/kg of Pb (Fig. 2). The activity of enzyme GR observed to be highest at 10 μ M under 800 mg/kg of Pb stress in both cultivars.

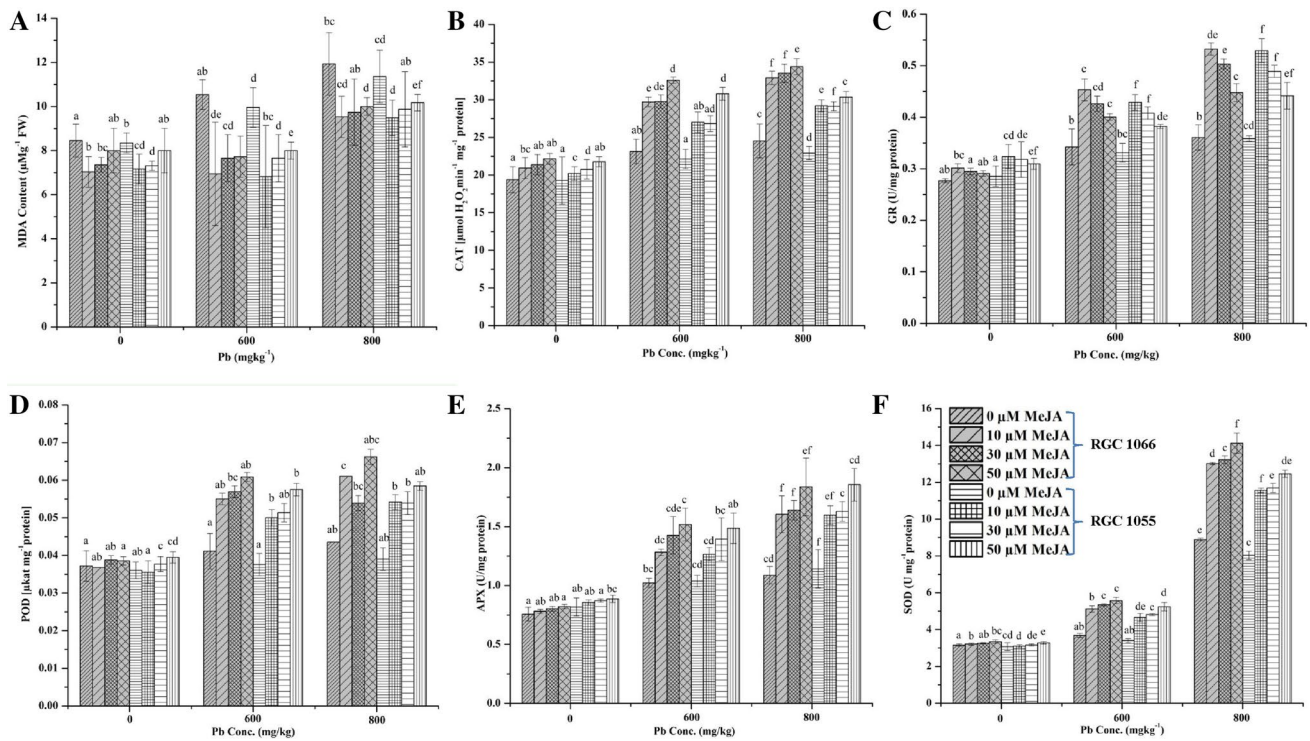


Fig. 2 Effect of MeJA on antioxidants in both cluster bean cultivars under Pb stress. Data represented as mean of three replicates \pm SD. Significant differences ($p < 0.05$) are represented by different alphabets

It is described that MeJA reduced the toxicity of metals by reducing oxidative damage in *Kandelia obovata* L. (Chen et al. 2014) and *Wolffia arrhiza* L. (Piotrowska et al. 2009). These results also explained that MeJA supplementation increased the activity of enzymes and thus reduced oxidative stress by decreasing ROS production. The increase in antioxidant enzymes activity by the application of MeJA might be because of post-transcriptional and translational changes which increase de novo synthesis and thus increase the activity of enzyme (Santino et al. 2013). The findings of present study are similar with the findings of Hanaka et al. (2016) where MeJA played a defensive role in *Phaseolus coccineus* under copper stress. MeJA increased the transcript level and ultimately inhibited arsenic-induced oxidative damage. Bali et al. (2018) and Kamran et al. (2021) also reported the similar results that JA enhanced the antioxidants enzymes in Pb-stressed tomato plants and chromium stressed *Brassica*. Fatma et al. (2021) reported that exogenous application of MeJA increased the activity of APX and GR in heat stressed wheat plants. Hence, this study proposed that increased activity of these enzymes by MeJA provided better protection against Pb-induced oxidative damage.

Conclusion

The current study described that foliar spray of MeJA modulated the toxic impact of Pb by decreasing the Pb content in different parts of plants thus showed ameliorative effect on growth parameters by increasing antioxidant enzymes. The alleviation provided by MeJA may be because of decrease in Pb accumulation in cluster bean cultivars, thus minimizing the damage caused by ROS production. Our findings demonstrated that 10 μ M of MeJA is efficient in reducing the harmful effect of Pb in cluster bean cultivars. We are working on the exogenous application of MeJA on photosynthetic efficiency of cluster bean cultivars under Pb stress.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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