

HERBICIDAL EFFECTS ON BIOCHEMICAL COMPONENTS AND YIELD IN RICE PLANTS (*Oryza sativa* L.)

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ABSTRACT

This investigation was carried out in a demonstrated field at Al-Ibrahimia district, El-Sharkia Governorate, Egypt, during 2019 season to study the effect of four postemergence herbicides namely, halosulfuron-methyl, fenoxaprop-p-ethyl, bispyribac-sodium and bensulfuron-methyl compared with control treatment under four rice cultivars (Egyptian hybrid rice 1" EHR1", Sakha 104, Giza 179 and Giza 178) on photosynthetic pigments and total carbohydrate, protein content, essential and non-essential amino acids.

The obtained results revealed that, no health problems were noticed for all rice varieties on the different experimental area. The lowest values of photosynthetic pigments and total carbohydrate were recorded for halosulfuron-methyl herbicide compared with other herbicides or control treatment. Addition of herbicides led to significant decrease in non-essential amino acids and protein content but, Aspartic amino acid was increased with application of bensulfuron-methyl herbicide. But, rice yield components were significantly increased due to foliar application of herbicide. On other hand, EHR1 gave the highest values for physiological and biochemical parameters as compared with the other varieties. On other side, Sakha 104 cv. appeared to produce the highest number of panicle per m², seed index and grain yield/fad. The interaction between factors under study had significant effects on physiological and biochemical parameters and yield components of rice were the results suggested that there a complementary effect between weed control treatments and rice cultivars by sowing Sakha 104 cv. and EHR1 with application of the tested herbicides.

The physiological and biochemical models of dual-herbicide-tolerant rice cultivars adds further informations to the knowledge of the crop herbicide tolerance degree for sustainable weed management in recent agricultural system.

Conclusively, The results of the study showed a decrease in the activity of physiological and biochemical content as response to the tested herbicide treatment at the recommended rates, and this was reflected in the values of essential and non-essential amino acids in the tested rice varieties. The rice crop components were also highly significant due to the application of herbicides.

Keywords: Biotoxic, herbicides, cultivars, rice, yield components, protein, carbohydrate, photosynthetic.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important strategic cereals not only in Egypt but in almost worldwide. In Egypt, rice area is about half million hectares with average yield of 10 tons/ ha. This significantly high productivity is mainly due to the improvement of short duration varieties with a higher yield potential, as well as, resistance to a biotic and biotic stresses (Khattab, 2019). Weeds are one type of biotic stress which led to cause severe reduction in rice productivity. Traditional methods of weed control with manual weeding increases the cost of cultivation (Singh *et al.*, 2016).

In rice agricultural systems, the aim of herbicide is to kill or decrease weed infestation allowing the rice to grow. Herbicides are the major methods of weed control in rice fields in Egypt but there are certain negative impacts on some environmental systems. The other important role of herbicides, can provide effective and economic control in rice field, it is saves labour cost up to 40% (Saha *et al.*, 2018). Chlorophyll is the main instrument responsible for photosynthesis. Carotenoids are natural pigments with substantial applications in nutraceutical and pharmaceutical, food industries (Jinendiran *et al.*, 2019), it is provide several biological functions in micro-organisms and plants such as in photoprotection (Britton *et al.*, 2004). Herbicides exhibited some phytotoxic effects on crop plants (Sundaru, 1983, Nandihalli and Bhowmik, 1992, Singh *et al.*, 1996, Kushwaha and Bhowmik, 1999, Thapa, 2012, Singh *et al.*, 2016, Tagour and Mosaad, 2017, Saha *et al.*, 2018). A number herbicides such as halosulfuron-methyl, fenoxaprop-p-ethyl, bispyribac-sodium and bensulfuron-methyl etc. have been recommended as post-application against rice weeds (Saha *et al.*, 2018). These herbicides are useful in growth rice field to control the early weeds. All these herbicides have differential biotoxic effects on the target crops and their associated weeds flora.

The effects of herbicides were physiological changes and disturbances in cell division (Singh, 2007, Kumar and Singh, 2010), carbohydrate content and caused changes in gene mutation (Kumar, 2012), inhibits the synthesis of

protein (Egli *et al.*, 1985 and Benmoussa *et al.*, 2000). Also, Ahmed *et al.*, (2019) determined the effects of glyphosate herbicide on the total chlorophyll, percent of death and injury level in Alfalfa plants. Plants can synthesize everything they need to survive, including amino acids (Shaner, 1989). Sands and Pilgeram (2009) found that glyphosate and imidazolinone herbicides can be inhibited by the exogenous addition of certain amino acids. Also, Fernández-Aparicio *et al.* (2017) who stated that, certain amino acids induce inhibitory effects as herbicides in plant growth due to feedback inhibition of metabolic pathways and amino acids. For efficacious action on weeds flora, the herbicides are applied on the foliage of herbicides-tolerant crop cultivars. Herbicides application or hand weeding had the highest rice yield and yield attributes compared with control treatment (Pasha *et al.*, 2012, Shukla *et al.*, 2015).

With respect to varietal differences, several investigators reported significant differences between rice cultivars in photosynthetic pigments and total carbohydrate, protein content and amino acids (Kamara *et al.*, 2010, Thapa, 2012, Zayed *et al.*, 2014, Zhou *et al.*, 2018, and Khattab, 2019). Also, several authors reported significant increase of grain yield and yield attributes due to rice varietal differences (Abou-Khalifa, 2012, Abou Khalifa *et al.*, 2014, Gomaa, 2018 and Khattab, 2019).

Therefore, this study towards to understand the biotoxic effects of the foliar selective post-emergence herbicides on tolerance of potential rice cultivars, which may be the important step for designing the integrated weed management program.

MATERIALS AND METHODS

An open field experiment was implemented in a demonstrated field at Al-Ibrahimia district, El-Sharkia Governorate, Egypt, during 2019 season. The analyses of the soil, showed that the soil was clay in texture, organic matter (2.0%), total N (0.15%), available P (17 ppm), available K (140 ppm), and pH (7.9 “moderately alkaline”).

The present study aimed to find out the effect of four selective post-emergence herbicides (halosulfuron-methyl, fenoxaprop-p-ethyl, bispyribac-sodium and bensulfuron-methyl) compared with control treatment under four rice cultivars (EHR1, Sakha 104, Giza 179 and Giza 178) on photosynthetic pigments, total carbohydrate, protein content and essential and non-essential amino acids. The tested herbicides were halosulfuron-methyl (Inpul 75% WG)^R, at rate 20g /fad., supplied by Shoura Chemicals, Egypt. fenoxaprop-p-ethyl (Whip- super 7.5% EW)^R, at rate 350 cm³/ fad., supplied by Bayer Crop Science, Egypt, bispyribac-sodium (Vogal 30 % WP)^R, at rate 100 g/

fad., supplied by Shoura Chemicals, Egypt and bensulfuron-methyl (Reto 60 % WG)^R, at rate 50 g/ fad., supplied by Shoura Chemicals, Egypt.

Five treatments were sprayed against the weeds associated with cultivars rice plants using a knapsack sprayer (5 liters/plot) where, the selective herbicides were applied once at based on the recommendations of the Egyptian Ministry of Agriculture for each herbicide after sowing and in control treatment. Phosphorus at a level of 15.5 kg P₂O₅/fad, as ordinary superphosphate (15.5% P₂O₅) was incorporated at the time of seeding. Rice grains, sowing at the rate of 60 kg/fad and were soaked in water for 24h, then drained and incubated for 12h to motivate and enhance germination percentage. In wet leveled plots, Per-germination seeds were manually planting in hills at 20 cm between rows × 20 cm between hills on 20th May. Nitrogen fertilizer was applied as urea (46.5% N) in three splits after 20, 40 and 55 day after sowing (DAS).

The preceding crop was wheat (*Triticum aestivum*). A split plot design of four replications was used, where the herbicides treatments were allocated in the main plots whereas cultivars were allocated in the sub plots (9 m²). Soil samples were collected from the experimental sites at the depth of 0 -30 cm before planting to determine soil physical and chemical properties at the Central Laboratory of Faculty of Agriculture, Zagazig University, Zagazig, Egypt.

Measuring phytotoxicity of the tested herbicides on the rice cultivars.

After 72 h of treatment fresh leaves were collected to determine chlorophyll a (Chl. a), chlorophyll b (Chl. b) and carotenoids according to Fideel's method (Fideel, 1962). The optical density was measured using spectrophotometer Jenway-6405-UV/VIS at 662, 644 and 440 nm to determine the photosynthetic pigments (Chl a, Chl. b and carotenoids) as mg/g fresh weight(FW), respectively. The pigments concentration was calculated using the formula adapted by Wettstein (1957).

$$\text{Chl. a (mg/L)} = (9.784 \times E_{662}) - (0.99 \times E_{644}).$$

$$\text{Chl. b (mg/L)} = (21.426 \times E_{644}) - (4.64 \times E_{662}).$$

$$\text{Carotenoides (mg/L)} = (4.695 \times E_{440.5}) - [0.268 \times (\text{Chl. a} + \text{Chl. b})].$$

Where, E is the reading of the optical density at given wave length.

Estimation of total carbohydrate, protein content and amino acids

After 72 h from spraying, samples of the tested rice cultivars leaves were randomly taken from each plot to determine each total carbohydrate and crude protein content (A.O.A.C., 2000). Amino acids were extracted from oven dry leaves using ethyl alcohol (80%, v/v). The quantitative and qualitative amino acids determination was carried out according to Christias *et al.* (1975).

The reduction percentage of phytotoxicity of herbicides was calculated according to Thapa(2012) by the formula.

$$\text{Reduction percentage} = (\text{Control} - \text{Treatment}) / \text{Control} \times 100$$

At harvest (end of August), the number of panicle per m² and the following yield attributes were recorded on ten panicles, *i.e.*, filled grains no./ panicle and 1000 grain weight. Also, the following final yield traits were recorded from a central area of 1 m² per plot: grain yield at grain moisture content of 14 % and harvest index (%).

Statistical analysis

The obtained data were statistically analyzed as mentioned by Gomez and Gomez (1991) using the computer MSTAT statistical analysis package (MSTAT-C, 1991).

Least significant differences (LSD) method was used to test the differences between treatment means at 5% level of probability as described by Steel *et al.* (1997).

RESULTS AND DISCUSSION

Photosynthetic pigments and total carbohydrate content

The effects of herbicides and cultivars and their interaction on physiological components, photosynthetic pigments; chlorophyll a, chlorophyll b and total chlorophyll (a + b), carotenoids, as well as, total carbohydrate in rice plants are shown in Tables (1 and 1 continued).

Insignificant ($P < 0.05$) highly differences were observed in photosynthetic pigments and total carbohydrate were observed due to application of herbicides treatments under the four cultivars of rice. It is clear from the results that, spraying herbicides (halosulfuron-methyl, fenoxaprop-p-ethyl, bispyribac-sodium and bensulfuron-methyl decreased significantly chl. a (18.10, 15.36, 1.86 and 4.99%), chl. b (41.52, 37.59, 27.64 and 30.52%) and total chl. (a + b) (32.12, 28.66, 17.24 and 20.22%), carotniods were recorded (14.77, 11.90, 2.20 and 13.52%) as well as total carbohydrate (10.51, 9.77, 8.17 and 6.57%) as reduction percentage in rice leaves compared with control treatment, respectively. Moreover, the lowest values for photosynthetic pigments and total carbohydrate were recorded by foliar application of halosulfuron-methyl herbicide than those obtained herbicides or control treatment. On other hand, the results revealed that, varietal differences

were significantly ($P < 0.05$) affected on photosynthetic pigments and total carbohydrate where, EHR1 gave the highest values for either photosynthetic pigments or total carbohydrate content as compared with those recorded by Sakha 104, Giza 179 and Giza 178 cvs., respectively.

According to the interaction effect between herbicides treatments and cultivars, it is interesting to mention that, under the check treatment for herbicides with sowing of EHR1 significantly increased photosynthetic pigments and total carbohydrate content as compare with foliar application herbicides under the other cultivars. Contrary to this, Giza 178 cv. was the most varieties negatively affected by herbicides where, the lowest values for photosynthetic pigments and total carbohydrate achieved by foliar application of halosulfuron-methyl herbicide. These results are in harmony with Ahmad *et al.*, (2007), Saleem *et al.*, (2011) and Hanci and Cebeci (2014) they suggested that the chlorophyll and carotenoids contents usually decreases and reduction in the plants exposed to environmental stress. Also, Kumar (2012) who showed that, application of herbicides (2, 4-D and IPU) were able to reduce total soluble carbohydrate in plant. Butachlor slightly affected rice leaves chlorophyll and amino acids and although exhibited phytotoxic effects on plants, but, the symptoms disappeared 14 DAS (Thapa, 2012).

Rate of photosynthesis affected adversely due to inactivation of chlorophyll (Amirjani and Mahdiyeh, 2013). On other hand, Giza178 cv. significantly had higher plant pigments and photosynthesis comparing to other varieties; Giza177 and Sakha 104 (Zayed *et al.*, 2014). Also, Giza 178 cv. achieved the highest photosynthetic pigments content (Chl. a & b and Carotenoids) followed by Giza 182 cv. which recorded the lowest value (Khattab, 2019).

Essential amino acid and total essential amino acid

Application of herbicides caused significant decrease in both the essential amino acids namely, Lysine, Methionine, Cysteine, Threonine, Isoleucine, Leucine, Tyrosine, Valine and total essential amino acids in rice plant (2 and 2 continued). The lowest values for essential amino acids and total essential amino acids achieved by foliar application of halosulfuron-methyl herbicide compared with other herbicides or control treatment, may be due to the effect of halosulfuron-methyl inhibition of acetolactate synthase (ALS) responsible for biosynthesis of the branched chain amino acid such as valin, leucin and isoleucine (Saari *et al.*, 1990). At contrary, Phenylalanine amino acid was increase by application of halosulfuron-methyl

herbicide as a compared with the other treatments. Otherwise, the essential amino acids and total of its where appeared to be increased by control

treatment. These results are quite interesting as they indicate that, addition of herbicides were effective to reduce the photosynthetic pigments (chl. a & b and carotenoids) (Tables 1 and 1 continued) and were reflected on essential amino acids and total essential amino acids in rice plants.

Respecting varietal differences results, highly significant differences ($P < 0.05$) were observe, mean through, EHR1 gave the highest values for essential amino acids and total essential amino acids in rice plant as compared with those recorded by the other cultivars *viz.* Sakha 104, Giza 179 and Giza 178, respectively. Concerning the influence of interaction between herbicides treatments and cultivars on essential amino acids, as well as, total essential amino acids, the results revealed highly significant differences. It is important to note that, the Giza 178 cv. was the most varieties severe affected by herbicides where, the lowest values for almost essential amino acids and total essential amino acids achieved by application of halosulfuron-methyl herbicide, which followed by Giza 179, Sakha 104, and EHR1, respectively.

However, the highest values for almost essential amino acids and total essential amino acids were achieved through by the control treatment and planting of EHR1. Similar significant effects were reported by Scheel and Casida (1985) who showed that, chlorsulfuron and sulfometuronmethyl decreased the amino acids synthesis (isoleucine, leucine and valine) by blocking the acetolactate synthase pathway. Also, Zarzecka and Gugala (2003) showed that, the herbicides can have certain effects on changes in plant physiological process. Souahi *et al.* (2016) stated that, herbicides effect on all aspects of primary and secondary metabolisms in the tested crops when given to control the weeds. (Fernández-Aparicio *et al.*, 2017) found that when assayed the highest herbicides concentration showed sensitivity to alanine, cysteine, histidine, isoleucine, leucine, methionine and tyrosine and the inhibition percent depend on plant species and the plant growth stage. Zayed *et al.* (2014) who found that, Giza178 cv. significantly had higher amino acids comparing to other varieties; Giza177 and Sakha 104.

Non- essential amino acids and protein content

With respect to the influence of herbicides on non- essential amino acids and protein content in rice plants, the results showed that insignificant highly differences were observed in non- essential amino acids *viz.* Aspartic, Serine, Proline, Glycine, Alanine, Histidine and Arginine, as well as, protein content were observed due to application of herbicides treatments under the four cultivars of rice (Tables 3 and 3 continued). Addition of herbicides led to

significant decrease in non-essential amino acids and protein content, by contrast, Aspartic amino acid was increased with application of bensulfuron-

methyl herbicide. Otherwise, most non-essential amino acids, as well as, protein content appears to increase with the control treatment. On other hand, the results revealed that, varietal differences were significantly affected on non- essential amino acids and protein content in rice. A same trend, EHR1 gave the highest values for either non-essential amino acids or protein content as compared with those recorded by Sakha 104, Giza 179 and Giza 178 cvs., respectively (Tables 3 and 3 continued). Similarly, Giza 178 cv. was the most varieties affected by herbicides as compare with other rice cultivars where, the lowest values for non-essential amino acids and protein content caused by foliar application of halosulfuron-methyl herbicide which followed by Giza 179, Sakha 104 cvs., and EHR1, respectively. However, the highest values for the same traits were achieved through by control plot and planting of EHR1.

Generally, our results indicated that, exposure to the herbicides causes significant variation in essential, non-essential amino acids, protein content. Also, post-emergance herbicides tolerant-varieties are not always available for many crop species. Early, Omokawa *et al.* (1988) found that, The N-(2,6-Diethylphenyl)-N- nutoxymethyl 2-chloro-acetamide inhibits the protein synthesis in the rice plant. The noticeable decrease in carbohydrates and protein content of different plants in relation to applied herbicides might be due to the disturbing influence of such toxicants on enzymes involved in metabolic pathway of above mentioned components and supported the various reports (Yin *et al.*, 2008, Hasaneen *et al.*, 2009, Rosenbom *et al.*, 2010, Hala *et al.*, 2011 and Morais *et al.*, 2011). Singh *et al.* (2016) found that, crop injuries for rice cultivars when applied with azimsulfuron. According to Souahi *et al.* (2016) stated that, the decrease in protein content is an indication of reduction in the growth of the plants. On other hand, rice varietal differences were significantly affected on protein content and proline amino acid (Zayed *et al.*, 2014 and Zhou *et al.*, 2018). The ratio of aspartate-derived concentration to glutamate-derived free amino acid determined for the japonica group was significantly lower than that for the indica group (Kamara *et al.*, 2010).

Yield attributes

The effects of herbicides and cultivars and their interaction on rice grain yield and yield attributes are shown in Tables (4 and 4 continued). Insignificant ($P < 0.05$) highly differences were observed in number of panicle per m^2 , number of filled grains per panicle, 1000-grains weight, grain yield per fad and harvest index were observed due to application of herbicides

treatments under the four cultivars of rice. Contrarily to the negative impact on the physiological and biochemical parameters affected negatively by herbicides

application (Tables 1-3). photosynthetic pigments were significantly reduced in the herbicide treated rice plots in the early stage of growth but the crop recovered later on. It is clear from the results that, spraying herbicides (halosulfuron-methyl, fenoxaprop-p-ethyl, bispyribac-sodium and bensulfuron-methyl increased significantly rice yield and yield attributes compared with control treatment. The obtained results are in accordance with those reported by Pasha *et al.* (2012), Thapa (2012) and Shukla *et al.* (2015). On other hand, the results revealed that, varietal differences were significantly ($p < 0.05$) affected on rice yield and yield attributes where, Sakha 104 cv. gave the highest values for number of panicle per m^2 , 1000-grains weight, grain yield per fad and harvest index as compared with those recorded by EHR1, Giza 179 and Giza 178 cvs. Mean through, EHR1. gave the highest number of filled grains per panicle. Similar significant effects were reported by (Abou-Khalifa, 2012, Abou Khalifa *et al.*, 2014, Gomaa, 2018, Khattab, 2019 and Tefera *et al.*, 2019). Also, studied by Khwaja and Deva (2014) showed that fenoxaprop-p-ethyl, pyrasulfuron-ethyl, metasulfuron-methyl and bispyribac-sodium caused increasing tillers per hill, panicle growth, panicle weight, test weight, number of filled grain, grain yield and straw yield of rice field. Perhaps the reason for this is that there was more space to the crop competition in terms of dry matter production of weed as well as good source sink relationship which allow crop to absorb required amount of nutrient, water and sunlight for it is growth, better transfer of photosynthesis which contributes to increase the weight of of panicles and other parameters. According to the interaction effect between herbicides treatments and cultivars indicated that Sakha 104 cv. appeared to produce the highest seed index (30.22 g) and grain yield/fad., (5.08 ton) when were foliar application with Nominee while the lowest grain yield/ fad., (2.62 ton) was recorded by unweeded treatment with sowing Giza 179 cv.

Conclusively, from these results, it is concluded that the activity of photosynthetic pigments, total carbohydrate, protein content decreased as response to the herbicides at recommended rates and reflected on essential amino acids and non-essential amino acids under rice varieties but the crop recovered later on. Contrarily, rice yield components were significantly increased due to foliar application of herbicide. On other side, Giza 178 cv. of rice was most sensitive among the selected varieties as maximum reduction in the tested biochemical content while, EHR1 gave the highest values for almost recorded physiological attributes under the current study.

Moreover, Sakha 104 cv. appeared to produce the highest number of panicle per m², seed index and grain yield/fad.

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تأثير بعض مبيدات الحشائش على المكونات البيوكيميائية والمحصول في نباتات الأرز (*Oryza sativa* L.)

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أجريت تجربة حقلية في حقل إرشادي بمركز الابراهيمية بمحافظة الشرقية خلال الموسم الزراعي 2019 بهدف دراسة تأثير أربعة مبيدات حشائش بعد الانبثاق مقارنة بمعاملة الكنترول وذلك تحت أربعة أصناف أرز (جيزة 178 ، جيزة 179 ، سخا 104 و هجين مصر 1) على بعض المؤشرات الفسيولوجية والبيوكيميائية. وكانت مبيدات الحشائش المختبرة: هالوسلفيرون ميثيل (انبول 75 % WG) بمعدل 20 جم / فدان ، فينو كسابروب - بي - إيثيل (ويب سوبر 7.5 % EW) بمعدل 350سم³ / فدان ، بيسبيراك - صوديوم (فوجال 30 % WP) بمعدل 100 جم / فدان. و بنسلفيرون - ميثيل (ريتو 60 % WG) بمعدل 50 جم / فدان.

أظهرت النتائج وجود فروق معنوية بين معاملات مبيدات الحشائش. حيث لوحظ انخفاض محتوى أوراق نباتات الأرز من صبغات التمثيل الضوئي (كلورفيل ا,

كلورفيل ب والكاروتينات) الكربوهيدرات الكلية، البروتين وكذلك الأحماض الأمينية الأساسية وغير الأساسية، حيث سجل مييد هالوسولفورون - ميثيل أدنى القيم للمؤشرات الفسيولوجية والبيوكيميائية المختبرة مقارنة بمعاملة الكنترول. علي النقيض من ذلك، كان لمقاومة الحشائش تأثيراً معنوياً وإيجابياً في مؤشرات محصول الأرز مقارنة بمعاملة الكنترول. من ناحية أخرى تفوق هجين أرز مصر 1 في جميع المؤشرات الفسيولوجية والبيوكيميائية المدروسة مقارنة بالأصناف الأخرى. علاوة علي ذلك حقق الصنف سخا 104 أعلى عدد من السنابل/ م²، وزن 1000 حبة ومحصول حبوب للقدان. لوحظ تداخل فعل معنوي بين مبيدات الحشائش وأصناف الأرز المنزرعة في الصفات تحت الدراسة والتي أظهرت تأثير تكاملياً بين المعاملة بمبيدات الحشائش مع زراعة الصنف سخا 104 و هجين أرز مصر 1 مع تطبيق مبيدات الحشائش. تضيف النماذج الفسيولوجية والبيوكيميائية لأصناف الأرز التي تتحمل مبيدات الحشائش معلومات إضافية إلى معرفة درجة تحمل المحاصيل لمبيدات الحشائش من أجل الإدارة المستدامة للحشائش في النظام الزراعي الحديث.

التوصية: أوضحت نتائج الدراسة انخفاض نشاط صبغات التمثيل الضوئي، الكربوهيدرات الكلية، ومحتوى البروتين استجابة للمعاملة بمبيدات الحشائش المختبرة بالمعدلات الموصى بها، وانعكس ذلك على قيم الأحماض الأمينية الأساسية وغير الأساسية في أصناف الأرز المختبرة. كما أعطت مكونات محصول الأرز معنوية بدرجة كبيرة بسبب تطبيق مبيدات الحشائش.

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