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RESEARCH ARTICLE

Comparison of the trapping effect and antioxidant enzymatic activities using three different light sources in cockchafers

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Abstract Light traps have been widely used for controlling underground pests. However, very little is known regarding the relationship between trapping effect and antioxidant enzymatic activities using light irradiation in underground pests. Thus, we determined the trapping effect of three light sources of the frequoscillation pest-killing lamp on two species of cockchafers, *Serica orientalis* Motschulsky (Coleoptera: Melolonthidae) and *Anomala corpulenta* Motschulsky (Coleoptera: Rutelidae), and evaluated the effect of the same three light sources on the activities of their antioxidant enzymes. The catches of *S. orientalis* were significantly higher compared to *A. corpulenta* using light source A in peanut fields in China. After irradiation by light source A, the malondialdehyde (MDA) contents and activities of superoxide dismutase (SOD) and glutathione S-transferases (GST) in *S. orientalis* were significantly and marginally significantly lower compared to *A. corpulenta*. Taken together, these results indicated a weaker antioxidant enzyme activity response to light stress and a larger quantity of trapping catches using light irradiation in cockchafers. Thus, we proposed a potential negative relationship between trapping effect and antioxidant enzymatic activities in response to light irradiation in cockchafers.

Keywords *Serica orientalis* Motschulsky · *Anomala corpulenta* Motschulsky · Antioxidant enzyme systems · Trapping catches · Light irradiation · Underground pests

Introduction

Light traps can effectively control insect pests without releasing pollutants. They have been widely used to monitor pest population dynamics and to control for pests in agriculture, forestry, and the warehousing industry (Neethirajan et al. 2007; Allsopp 2010; Zhang et al. 2011). Currently, the frequoscillation pest-killing lamp, which emits different light sources, is a very popular tool for pest management in China. The effect of light irradiation on insect behavior, physiology, and biochemistry has been previously reported (Gunn 1998; A-H-Mackerness et al. 1999; Mazza et al. 2002; Jing and Lei 2004; Jing et al. 2005; Meng et al. 2009). However, the phototactic mechanism of the insect remains poorly understood. Many hypotheses have been proposed to explain the phototactic behavior of the insect, among which light interference (Robinson and Robinson 1950; Robinson 1952), light orientation (Baker and Sadovy 1978; Atkins 1980), and biological antenna hypotheses (Callahan 1965a, 1965b) have been reported. A deeper understanding of the phototactic mechanism of the insect can promote the application of the light trap.

Similar to other eukaryotes, insects have developed a suite of antioxidant enzyme systems, which demonstrate various antioxidant enzymes to counteract the toxicity of reactive oxygen species (ROS) and to reduce oxidative damage

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(Ahmad 1992; Felton and Summers 1995). The major ingredients of the antioxidant enzyme system of insects include superoxide dismutase (SOD) and glutathione S-transferases (GST) (Felton and Summers 1995; Wang et al. 2001; Gao et al. 2013). In enzymatic ROS-cleaning pathways, SOD can convert superoxide into oxygen and hydrogen peroxide (Fridovich 1978). GST can scavenge the lipid peroxidation or hydroperoxide products from cells (Dubovskiy et al. 2008; Meng et al. 2009). Several studies have shown that the antioxidant systems of insects are coordinated with each other, and some changes in antioxidant enzymatic activity can result in changes in other systems (Shigeoka et al. 2002). Thus, a comprehensive analysis of the activities of various antioxidant enzymes is important to correctly assess the antioxidant capacity of insects (Meng et al. 2009).

Cockchafers, adult white grubs, are major pests of peanuts in China. Two important species, *Serica orientalis* Motschulsky (Coleoptera: Melolonthidae) and *Anomala corpulenta* Motschulsky (Coleoptera: Rutelidae), have been shown to cause serious losses in Chinese peanut production. These two cockchafers are nocturnal insects and exhibit obvious phototactic behavior to light irradiation. Increasing evidence has indicated that trap lamps are useful for the control of cockchafers in agriculture (Wood and Yew 1969; Jones 1990; Švestka 2007). Moreover, several studies have been performed on oxidative stress induced by light irradiation in insects (Heck et al. 2003; Lopez-Martinez et al. 2008; Meng et al. 2009, 2010; Sang et al. 2012), but the potential relationship between trapping effect and antioxidant enzymatic activities using light irradiation in cockchafers has not been reported.

In this study, the trapping effects of the three light sources of the frequoscillation pest-killing lamp on *S. orientalis* and *A. corpulenta* were investigated. Moreover, the effects of the same three light sources on the activities of antioxidant enzymes of *S. orientalis* and *A. corpulenta* were also evaluated. The purpose of this study was to analyze the potential relationship between trapping effect and antioxidant enzyme activities using light irradiation in cockchafers.

Materials and methods

Light source and insects

Light sources A, B, and C (Hebi Jiaduo Science, Industry and Trade Co. Ltd., Henan, China) were used as light sources in this study. Light source A emits light with wavelengths in the range of 350.91–461.44 nm and 544.43–548.6 nm. Light source B emits light with wavelengths in the range of 399.56–449.12 nm and 544.43–548.4 nm. Light source C emits light with wavelengths in the range of 356.42–386.57 nm, 434.28–437.79 nm, and 544.43–548.2 nm.

S. orientalis and *A. corpulenta* were collected from peanut fields in Dawu County, Hubei Province, China. Next, the two species of cockchafers were reared in buckets. The buckets were covered with a piece of black cloth and the soil moisture was 18–25%. *S. orientalis* and *A. corpulenta* fed on fresh poplar leaves and were used for further experiments 8 days later.

Field experiment

The trapping effect on *S. orientalis* and *A. corpulenta* among the three light sources of the frequoscillation pest-killing lamp was investigated in Dawu County, Hubei Province, China, from May to August, 2011. The experiment was repeated three times for each light source, and thus, there were nine lamps used in total. The lamps were placed in the field according to the manufacturer's protocols. All lamps for each replicate experiment were randomly installed in a straight line. The vertical height of the lamps was 1.5 m, and the distance between two lamps was approximately 25 m. All lamps were lit every 2 days. Insect bags were hung at 18:00–19:00 and collected at 5:00–6:00 the next day. Then, the catches for *S. orientalis* and *A. corpulenta* were quantified.

Light irradiation treatment and oxidative stress measurement

The collected *S. orientalis* and *A. corpulenta* were divided into three different groups. Sixteen samples of *S. orientalis* and four samples of *A. corpulenta* per treatment were randomly selected and exposed to the three light sources or dark condition (CK) for 30 min (Meng et al. 2009; Zhou et al. 2013). The temperature in the irradiating area was maintained at 25 °C. After irradiation, all of the samples were immediately frozen in liquid nitrogen and stored at − 80 °C for further determination.

Prior to homogenization, the wings of *S. orientalis* and *A. corpulenta* were removed and the remaining parts were weighed. The tissues were homogenized in ice-cold buffer (1/150 mol/mL Na_2HPO_4-KH_2PO_4, 0.1% Triton, pH 7.4), and the ratio of body weight and buffer volume was 1:10. The crude homogenates were centrifuged at 10,000×*g* for 10 min at 4 °C and the supernatant was used for further analysis. According to the Bradford method (1976), protein concentrations were measured using bovine serum albumin as the standard (Bradford 1976). In this study, the activities of malondialdehyde (MDA), SOD, and GST were measured spectrophotometrically using assay kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu Province, China) according to the manufacturer's protocols, with some modifications (Meng et al. 2009). Lipid peroxidation was measured by determining the MDA produced by reacting with thiobarbituric acid (TBA) to provide a red species with a maximum wavelength at 532 nm. The MDA concentration was expressed as

nanomole of MDA produced per milligram protein. The SOD activity was measured spectrophotometrically at 550 nm using the xanthine and xanthine oxidase systems. One unit of SOD activity was expressed as the number of enzyme required for 50% inhibition of the xanthine and xanthine oxidase system reaction in 1 mL enzyme extraction of 1 mg protein. The SOD activity was expressed as U mg^{-1} protein. The GST activity was measured using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate. The formation of GSH-CDNB conjugate was monitored using the variance in absorbance at 412 nm. One unit of GST activity was expressed as the number that catalyzes the conjugation of 1 mmol/L GSH with CDNB per minute per milligram protein. The GST activity was expressed as U mg^{-1} protein. Each experiment was repeated four times.

Statistical analysis

All statistical analyses were carried out in IBM SPSS Statistical 18.0 (SPSS Inc., Chicago, IL, USA). The results in the graphs represent the mean values ± S.E. We applied the paired *t* test which was used to compare trapping effect and antioxidant system effect between the two cockchafers for each light source. Meanwhile, the one-way ANOVA was used to compare the effect of the three light sources (A, B, and C) and dark condition (CK) on the antioxidant system in *S. orientalis* and *A. corpulenta,* respectively. Significant differences were analyzed using Tukey's multiple range test.

Results

Trapping effect of the three light sources on *S. orientalis* and *A. corpulenta*

The catches of *S. orientalis* and *A. corpulenta* by light source A were 4476 and 191, respectively (Fig. 1). The catches of *S. orientalis* and *A. corpulenta* by light source B were 3396 and 187, respectively (Fig. 1). The catches of *S. orientalis* and *A. corpulenta* by light source C were 4153 and 260, respectively (Fig. 1). The catches of *S. orientalis* were significantly higher compared to *A. corpulenta* by light sources A (t = 6.275, df = 2, p = 0.024) and C (t = 5.565, df = 2, p = 0.031). However, there were no significant differences in the catches between *S. orientalis* and *A. corpulenta* by light source B (Fig. 1; t = 2.220, df = 2, p = 0.157).

Effect of the three light sources on the antioxidant system of *S. orientalis* and *A. corpulenta*

The protein concentrations in *S. orientalis* were significantly higher compared to *A. corpulenta* after irradiation by light source A (t = 5.891, df = 3, p = 0.010) (Fig. 2). However, there were no significant differences in the protein concentrations between *S. orientalis* and *A. corpulenta* after irradiation by light sources B (t = 1.509, df = 3, p = 0.228) and C (t = 3.057, df = 3, p = 0.055) and in dark condition (t = − 0.379, df = 3, p = 0.730) (Fig. 2). Meanwhile, the protein concentrations irradiation by light source A were significantly higher compared to dark condition (CK) in *S. orientalis* (p = 0.001) (Fig. 2).

The MDA contents in *S. orientalis* were significantly lower compared to *A. corpulenta* after irradiation by light sources A (t = − 4.762, df = 3, p = 0.018) and C (t = − 4.051, df = 3, p = 0.027) (Fig. 3). However, no significant differences were detected in the MDA contents between *S. orientalis* and *A. corpulenta* after irradiation by light source B (t = − 1.346, df = 3, p = 0.271) and in dark condition (t = 0.637, df = 3, p = 0.570) (Fig. 3). The MDA contents irradiation by light source A were significantly higher than dark condition (CK) in *A. corpulenta* (p = 0.015) (Fig. 3). We also observed statistically non-significant but suggestive decreasing trends in the MDA contents between the three light sources and dark condition (CK) in *S. orientalis* (Fig. 3).

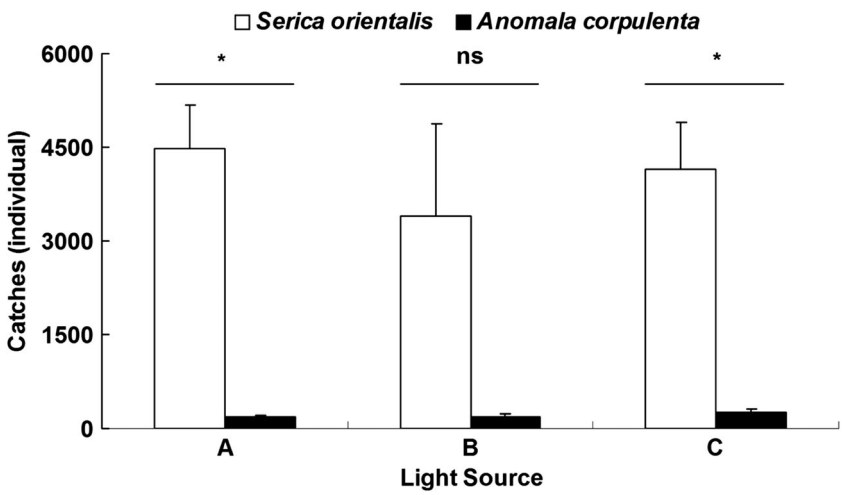

Fig. 1 Comparison of the catches between *S. orientalis* and *A. corpulenta* after irradiation by the three light sources. Values were expressed as the Mean ± SE (n = 3). ns, not significant; *, $p < 0.05$

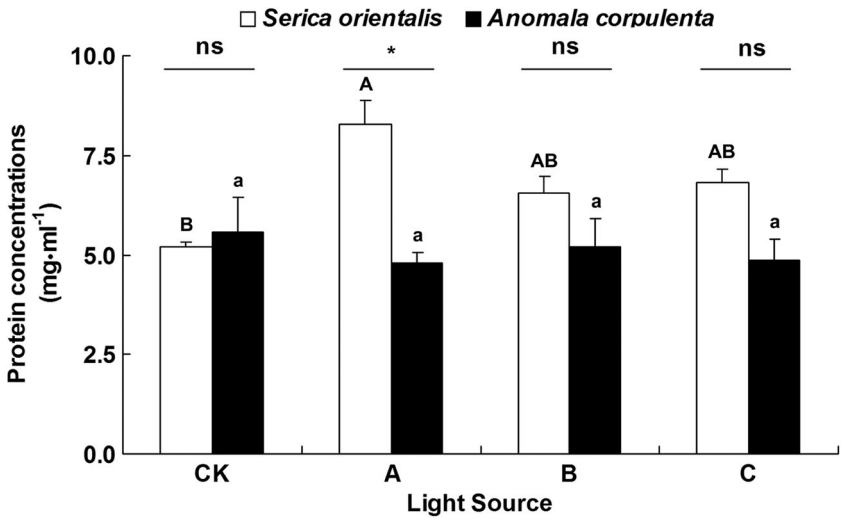

Fig. 2 Comparison of the protein concentrations between *S. orientalis* and *A. corpulenta* after irradiation by the three light sources. Values are expressed as the Mean ± SE ($n = 4$). Different letters over the bars denote significant differences ($p < 0.05$; capital letters: *S. orientalis*; lowercase letters: *A. corpulenta*). ns, not significant; *, $p < 0.05$

The SOD activity in *S. orientalis* was significantly lower compared to *A. corpulenta* after irradiation by light source A ($t = -9.675$, $df = 3$, $p = 0.002$) (Fig. 4). However, there were no significant differences in the SOD activity between *S. orientalis* and *A. corpulenta* after irradiation by light sources ($t = -2.111$, $df = 3$, $p = 0.125$) and C ($t = -3.015$, $df = 3$, $p = 0.057$) and in dark condition ($t = -0.078$, $df = 3$, $p = 0.943$) (Fig. 4). Also, the SOD activity irradiations by the three light sources were significantly lower compared to dark condition (CK) in *S. orientalis* (light source A $p < 0.001$; light source B $p = 0.042$; light source C $p = 0.006$) (Fig. 4).

The GST activity in *S. orientalis* was marginally significantly lower compared to *A. corpulenta* after irradiation by the light sources A ($t = -2.981$, $df = 3$, $p = 0.059$), B ($t = -2.649$, $df = 3$, $p = 0.077$), and C ($t = -2.809$, $df = 3$, $p = 0.067$) (Fig. 5). However, there were no significant differences in the GST activity in dark condition (CK) between *S. orientalis* and *A. corpulenta* (Fig. 5; $t = -1.111$, $df = 3$, $p = 0.348$). We also observed statistically non-significant but suggestive decreasing trends in the GST activity between the three light sources and dark condition (CK) in *S. orientalis* (Fig. 5).

Discussion

UV irradiation is known to cause the production of ROS and has been considered to be one of the environmental stresses in animals (Rebollar et al. 2006; Schauen et al. 2007) and insects (Meng et al. 2010). In response, insects have an antioxidant system, which is the key for their ability to remove ROS to protect cells from oxidative damage (Meng et al. 2009; Yang et al. 2010). The induction of antioxidant enzymes as a result of UV irradiation may also indicate the over-production of ROS (Meng et al. 2009; Wang et al. 2012). In the present study, our results indicated that there was a potential negative relationship between trapping effect and antioxidant enzyme activities in response to light source A. We proposed a weaker response to light stress and a larger quantity of catches using

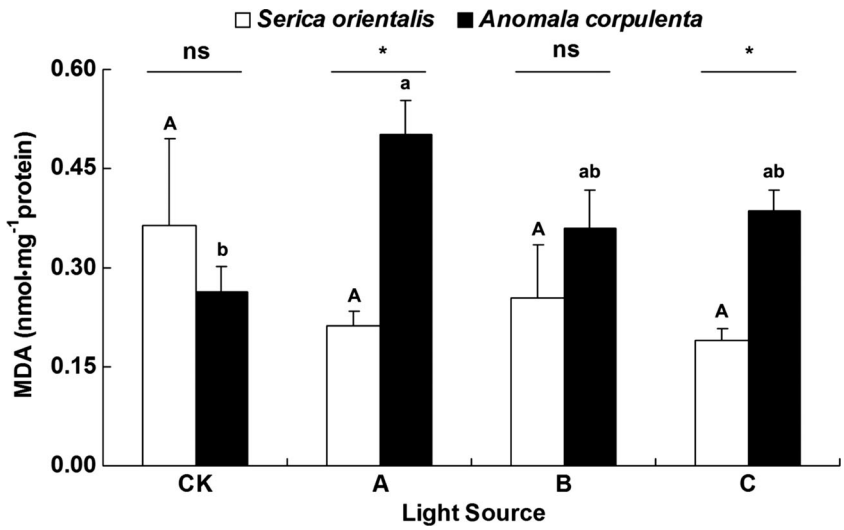

Fig. 3 Comparison of the MDA contents between *S. orientalis* and *A. corpulenta* after irradiation by the three light sources. Values were expressed as the Mean ± SE ($n = 4$). Different letters over the bars denote significant differences ($p < 0.05$; capital letters: *S. orientalis*; lowercase letters: *A. corpulenta*). ns, not significant; *, $p < 0.05$

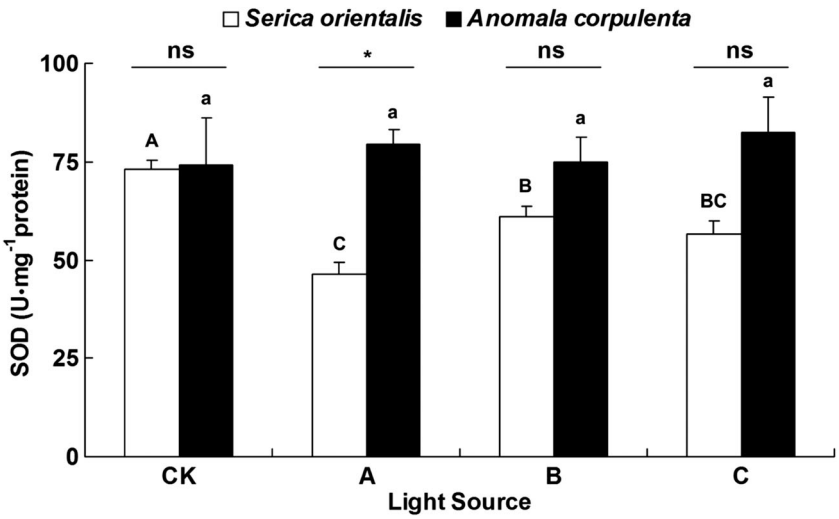

Fig. 4 Comparison of the SOD activity between *S. orientalis* and *A. corpulenta* after irradiation by the three light sources. Values were expressed as the Mean ± SE ($n = 4$). Different letters over the bars denote significant differences ($p < 0.05$; capital letters: *S. orientalis*; lowercase letters: *A. corpulenta*). ns, not significant; *, $p < 0.05$

light irradiation in the cockchafers. We found that the response of *S. orientalis* to light stress was weaker compared to *A. corpulenta* under light source A, which resulted in a significantly higher number of catches of *S. orientalis* compared to *A. corpulenta*. Most likely, the degree of resistance of *S. orientalis* against the frequoscillation pest-killing lamp was lower compared to *A. corpulenta*. In addition, there were no significant differences in the catches between *S. orientalis* and *A. corpulenta* by light source B. A possible explanation for this result is that some unpredictable environmental factors might affect our results in the field experiments. Thus, the catches of *S. orientalis* and *A. corpulenta* had big fluctuation by light source B among replications.

As a biological marker of oxidative stress, MDA induces oxidative stress in insects exposed to light irradiation and has been used to determine the degree of lipid peroxidation (Del Rio et al. 2005; Meng et al. 2009). In our study, irradiation of light sources A and C generated significantly more ROS damage to lipids in *A. corpulenta* compared to *S. orientalis*. The higher MDA levels in *A. corpulenta* might be due to the stronger response to light irradiation.

SOD is the first line of defense against ROS in insects and plays an important role in scavenging intracellular superoxide radicals induced by external stimulus such as light irradiation. In this study, we observed that SOD activity in *A. corpulenta* was higher compared to *S. orientalis* after irradiation by light source A. In previous studies, it has been found that light irradiation induced superoxide radicals in the Antarctic midge *Belgica antarctica* and *Helicoverpa armigera* adults (Lopez-Martinez et al. 2008; Meng et al., 2009). Moreover, SOD was reported to be stimulated by scavenging superoxide radicals, which protected insects from UV stress, as a response to increasing ROS (Krishnan and Kodrík 2006; Meng et al. 2009). Thus, the higher level of SOD activity in *A. corpulenta* indicated that *A. corpulenta* had a stronger response to light irradiation compared to *S. orientalis*.

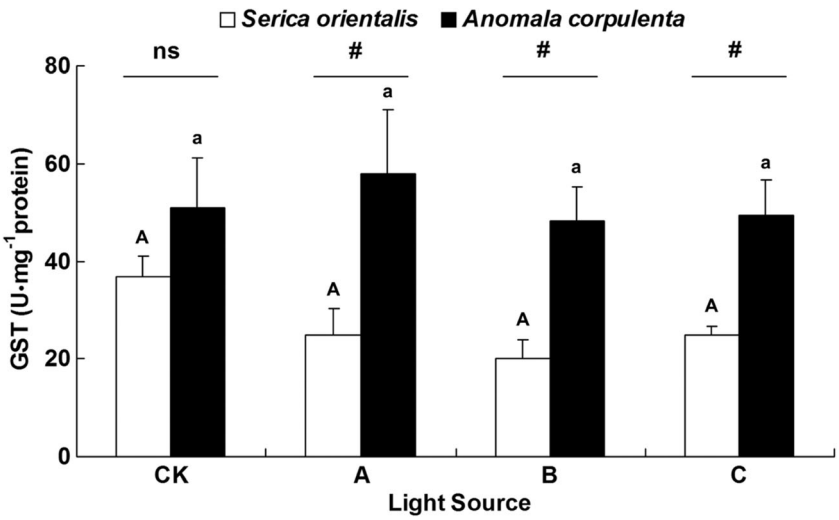

Fig. 5 Comparison of the GST activity between *S. orientalis* and *A. corpulenta* after irradiation by the three light sources. Values were expressed as the Mean ± SE ($n = 4$). Different letters over the bars denote significant differences ($p < 0.05$; capital letters: *S. orientalis*; lowercase letters: *A. corpulenta*). ns, not significant; #, $0.05 < p < 0.1$

GST is considered as a primary antioxidant enzyme, which is efficient in removing lipid peroxides in insects (Ahmad et al. 1991; Kono and Shishido 1992). In our study, GST activity in *A. corpulenta* was marginally significantly higher compared to *S. orientalis* when exposed to the three light sources. Thus, we proposed that GST might play an important role in response to oxidative stress induced by light stress in *S. orientalis* and *A. corpulenta*.

Collectively, we found that the catches of *S. orientalis* were significantly higher compared to *A. corpulenta*, but the MDA contents and activities of SOD and GST in *S. orientalis* were significantly and marginally significantly lower compared to *A. corpulenta* in response to light source A. These results suggested a weaker response of antioxidant enzyme activity to light stress and a larger quantity of trapping catches using light irradiation in cockchafers. Thus, we inferred that there was a potentially negative relationship between trapping effect and antioxidant enzyme activities in response to light stress in cockchafers.

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