RESEARCH ARTICLE



Influence of UV-A radiation on oxidative stress and antioxidant enzymes in *Mythimna separata* (Lepidoptera: Noctuidae)

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Abstract Abiotic stress factors, including ultraviolet (UV) radiation, significantly affect insect life. UV-A radiation (320-400 nm) has been widely used for insect control since it increases the production of ROS and causes oxidative cell damage. In the present study, we evaluated the effects of UV-A irradiation on an important pest in China, the ear-cutting caterpillar, Mythimna separata (Lepidoptera: Noctuidae). We exposed 3-day-old *M. separata* adults to UV-A radiation for different periods of time (0, 30, 60, 90, and 120 min) and evaluated the resulting total antioxidant capacity and the activity of the antioxidant enzymes superoxide dismutase, catalase, peroxidase, and glutathione-S-transferase. The total antioxidant capacity significantly increased after exposure to UV-A radiation for 60 min but decreased after 90 and 120 min of exposure, compared with the control. The antioxidant activity of glutathione-S-transferase, superoxide dismutase, catalase, and peroxidase increased after 60-min exposure, and it was decreased at the longest exposure period 120 min. The longest exposure time period relatively activates the xenobiotic detoxifying enzymes like glutathione-S-transferase, superoxide dismutase, catalase, and peroxidase enzymes. The longest duration of UV-A radiation may cooperate with pesticide detoxification mechanism in insects, making them more susceptible to insecticides. Our results demonstrated that UV irradiation causes oxidative stress, affects the activity of antioxidant enzymes, and disturbs the physiology of *M. separata* adults.

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Chao-Liang Lei ioir@mail.hzau.edu.cn **Keywords** Ultraviolet radiation · Oxidative stress · Antioxidant enzymes · *Mythimna separata*

Introduction

Abiotic stress factors, including ultraviolet (UV) radiation, significantly affect insect life because they increase the production and accumulation of reactive oxygen species (ROS). These free radicals of oxygen increase cell antioxidant capacity and oxidant production. At very low levels, they are not deleterious and play vital roles in cell signaling and defense (Kamata and Hirata 1999; Wang et al. 2001; Lijun et al. 2005). However, at higher levels, they are harmful to DNA and proteins. Other free radicals, superoxide (O₂⁻) and hydrogen peroxide (H₂O₂), are byproducts of cell metabolism that react indiscriminately with any molecule with which they come in contact, including proteins, membrane lipids, carbohydrates, nucleic acids, and other cellular components. These reactions lead to various cytotoxic effects and complications in chronic diseases (Cao et al. 2007; Graves et al. 2009; Zhao et al. 2013). Like other eukaryotes, insects have evolved a complex enzymatic and non-enzymatic defense system to combat oxidative stress. Antioxidant enzymes mitigate damage to DNA and proteins and also regulate the lipid peroxidation level (Felton and Summers 1995). The main antioxidant enzymes in insects are superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) (Felton and Summers 1995; Wang et al. 2001). SOD catalyzes the dismutation of superoxide radical into oxygen and H₂O₂, whereas both CAT and POX catalyze the dismutation of H₂O₂ into oxygen and water. Another important enzyme, glutathione-S-transferase (GST), eliminates lipid peroxidation products or hydroperoxides from the cells (Ahmad et al. 1991; Dubovskiy et al. 2008). UV-A (320-400 nm) has been used broadly in integrated pest

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management to monitor and control Lepidoptera (Antignus 2000; Kojima et al. 2005). UV radiation directly affects insect behavior, biochemistry, and developmental physiology (Gunn 1998; Mackerness et al. 1999; Mazza et al. 2002) as it significantly increases oxidative stress. The effects of UV-A radiation on cell biochemistry, adult longevity, and reproduction have been studied in various insect species, including cotton bollworm (*Helicoverpa armigera* [Hubner 1805]), red flour beetle (*Tribolium castaneum* [Herbst 1797]) (Meng et al. 2009, 2010; Zhang et al. 2011 Sang et al. 2002), and the butterfly species *Papilio xuthus* (L.) and *Pieris napi* (L.). Currently, it was found that UV-A radiation has effected on the biology of *M. separata* (Ali et al. 2016)

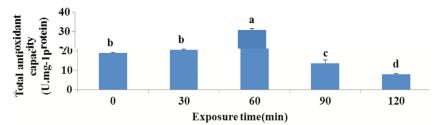


Fig. 1 Changes in the total antioxidant capacity of *Mythimna separata* adults after 0, 30, 60, 90, and 120 min of exposure to ultraviolet (UV-A) radiation. Data are presented as means (\pm SE) of the replicate experiments.

substrate to determine the activity of GST. One unit of GST activity was defined as the amount that catalyzes the conjugation of 1 μ mol l⁻¹ GSH with CDNB per minute per milligram of protein. GST activity was expressed as unit per milligram protein.

Statistical analysis

Data analyses were performed by SPSS 16.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) is conjunction with Tukey's post test was used to determine significant difference at p < 0.05.

Results

Total antioxidant capacity

No significant changes were observed in the T-AOC of *M. separata* adults after exposure to UV-A radiation for 30 min, but a significant increase (p < 0.05) was noted after 60 min of exposure, whereas a significant decrease occurred after 90 and 120 min of exposure compared with the control (Fig. 1).

Activity of antioxidant enzymes

No significant changes were found in the activity of SOD in *M. separata* adults after exposure to UV-A radiation for 30 and 90 min. A significant increase (p < 0.05) was observed in SOD activity after 60 min while a significant decrease was

Letter above bars indicate significant differences (P < 0.05) determined by ANOVA with Tukey's post test

seen at 120 min as compare to the control group as shown in (Fig. 2).

The activity of CAT in *M. separata* adults did not change after 30-min exposure to UV-A radiation. However, a significant increase (p < 0.05) was seen in the activity of CAT after 60 min of exposure. A significant decrease was observed in the activity of CAT after 90 and 120 min of exposure as compare to the control group (Fig. 3).

The activity of POX in *M. separata* did not show any difference among the controls, 30 and 90 min of exposure. A marked (p < 0.05) elevation was observed at 60-min exposure. However, a significant decrease was observed in enzyme activity after 120 min as compare to the control group (Fig. 4).

No changes were found in the activity of GST between control and 30 min of UV-A radiation exposure. A significant increase (p < 0.05) was observed in the activity of GST after 60 and 90 min of exposure, and a significant decrease was observed in the activity of GST after 120 min of exposure, compared with the control (Fig. 5).

Discussion

Solar UV radiation has three main components, UV-A, UV-B, and UV-C. Usually, UV-C is fully obstructed by the ozone layer, whereas both UV-A and UV-B have the capacity to reach the Earth's surface (Karentz 1994; Cockell 2001; Franco et al. 2009; Dahms and Lee 2010). UV radiation is usually considered a resilient ecological stress factor for living organisms (Urbach 1989; Schauen et al. 2007). It can cause

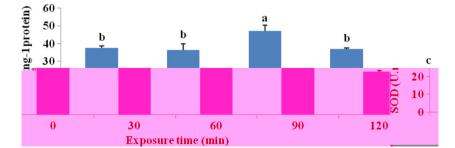


Fig. 2 Changes in the activity of superoxide dismutase (SOD) in *Mythimna separata* adults after 0, 30, 60, 90, and 120 min of exposure to ultraviolet (UV-A) radiation. Data are presented as means (\pm SE) of the

replicate experiments. *Letter above bars* indicate significant differences (P < 0.05) determined by ANOVA with Tukey's post test

damage to nucleic acids, membrane fatty acids, and amino acids (Jurkiewicz and Buettner 1994; Vile and Tyrrell 1995), leading to cell toxicity, genetic changes, and modifications in cell signaling pathways (McMillan et al. 2008). The absorption of weak UV radiation below 320 nm by nucleic acid bases can generate ROS due to photo-oxidation reactions produced by endogenous photosensitizers (Ravanat et al. 2001; Cadet et al. 2005).

T-AOC has been widely used as a tool to assess redox status in various organisms (Ghiselli et al. 2000; Meng et al. 2009; Sashidhara et al. 2011). Our results showed that the exposure of *M. separata* adults to UV-A radiation for 60 min significantly increased T-AOC compared with the control, revealing that the insects could effectively manage the oxidative stress and free radicals associated with this exposure. However, longer exposure periods of 90 or 120 min decreased their ability to defend against ROS (Meng et al. 2009). The antioxidant system of organisms may be unable to remove significant amounts of ROS produced under harsh environmental conditions (Foyer et al. 1994).

A significant increase in the activity of antioxidant enzymes, such as SOD, CAT, POX, and GST is a sign of oxidative stress since these four defensive enzymes function cooperatively to handle the relatively by high amounts of ROS inside the cell (Foyer et al. 1994). The main role of SOD is the reduction of superoxide radicals in the cells produced by the stimulation of extracellular factors such as UV radiation. In the present study, the activity of SOD increased in *M. separata* adults after 60 min of exposure to UV-A radiation, revealing increased production of superoxide radicals. However, a longer exposure (120 min) significantly reduced the activity of SOD compared with the control, probably because the elevated amount of UV radiation inhibits the defensive antioxidant system of the cells, including the effective reduction of accumulated superoxide radicals by SOD (Heck et al. 2003; Polte and Tyrrell 2004). These results were in agreement with previous studies that also reported variations in SOD activity in response to the increased production of ROS (John et al. 2001; Krishnan and Kodrík 2006; Karthi et al. 2014).

CAT is a crucial element in the insect antioxidant sys-

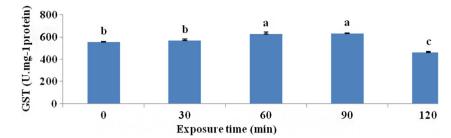


Fig. 5 Changes in the activity of glutathione-S-transferase (GST) in *Mythimna separata* adults after 0, 30, 60, 90, and 120 min of exposure to ultraviolet (UV-A) radiation. Data are presented as means (\pm SE) of the

1993; Orr and Sohal 1994). Therefore, the level of CAT may indicate oxidative stress in insects.

POX is also responsible for increasing stress tolerance in organisms (Clavaron-Mathews et al. 1997). The activity of POX in *M. separata* adults significantly increased after 60 min of exposure to UV-A radiation but decreased after 120 min of exposure, results that were in agreement with those reported in *H. armigera* (Meng et al. 2009). It is already known that negative feedback from an excess of substratum due to oxidative change can reduce the enzyme activity (Tabatabaie and Floyd 1994). We observed that the level of POX activity was lower than SOD, indicating that CAT may have a more important role in scavenging H_2O_2 than POX (Meng et al. 2009).

The GST plays a crucial role in the detoxification of broad spectrum of a toxic chemical that may provoke to mutagenic events and cytotoxicity (Coles et al. 1990; Hayes and Pulford 1995). Aside from the detoxification of the foreign compounds (pesticides and chemicals), there are various harmful endogenous compounds formed as a by-product of normal metabolism that is GST substrate. The aerobic respiration process can provoke the production of ROS (the superoxide anion O_2^{-} , hydrogen peroxide H₂O₂, and the hydroxal radical OH) (Finkel and Holbrook 2000). The production of the Reactive Oxygen Species (ROS) damages the membrane lipids which trigger the formation of lipid peroxidation products which can propagate a chain reaction in lipid peroxidation in an aerobic environment, which will ultimately end in membrane destruction (Slater 1984). So, GST is another important antioxidant enzyme in insects that effectively metabolizes lipid peroxides (Ahmad et al. 1991; Konno and Shishido 1992). In the present study, the activity of antioxidant enzymes (SOD, CAT, and POX) increased significantly only at 60 min. While the activity of GST increased significantly at both 60 and 90 min of UV-A radiation exposure, indicating that GST possibly has a greater role in the management of oxidative stress. It might be possible the ROS in *M. separata* due to UV-A radiation cause severe damage to membrane lipids that enhance the production of lipid peroxidation products, so GST effectively removed toxic lipid peroxidation for a longer duration (60 and 90) and protected the cells from potential oxidative damage.

replicate experiments. *Letter above bars* indicate significant differences (P < 0.05) determined by ANOVA with Tukey's post test

These results were in agreement with those reported in *H. armigera* (Meng et al. 2009). However, a longer exposure (120 min) decreased the activity of GST in *M. separata* adults, as in *S. litura* (Karthi et al. 2014).

Conclusion

The present study demonstrated that UV-A irradiation could cause oxidative stress in *M. separata* adults and disrupt the functional activity of antioxidant enzymes. A relatively short period of exposure (60 min) to UV-A radiation increased the activity of SOD, CAT, POX, and GST, which defend against oxidative damage due to the overproduction and accumulation of ROS. However, a longer exposure (90 and 120 min) reduced the T-AOC and antioxidant enzyme activity, leading to high levels of oxidative stress. UV-A radiation can cause irreversible oxidative damage to *M. separata* adults.

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