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ANTIOXIDANT ENZYMES IN COTTON MEALY BUG *PHENACOCCUS SOLENOPSIS* TINSLEY EXPOSED TO HIGH TEMPERATURE

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ABSTRACT

Effect of high temperature on the antioxidant enzymes catalase (CAT), superoxide dismutase (SOD) and peroxidases (POD) in the third instar of *Phenacoccus solenopsis* was studied under laboratory condition. Temperature influences the level of antioxidant enzymes with exposure to high temperature (40^{0}C). There was a marked rise in catalase activity and the maximum activity (0.399 nmol/ min/ mg) was observed after 6 hr in Bhatinda population. Irrespective of the population, the activity of peroxidase was positively correlated with time of exposure, and maximum activity was observed in Sri Ganganagar (0.218 nmol/ min/ mg) population at 6 hr of exposure. Within the 3 hr of exposure the maximum activity of SOD (0.127 µM/ min /mg) was observed in Rajkot and at 4 hr (0.060± 0.019 µM/ min /mg) in Sri Ganganagar populations. With further increase in the period of exposure, significant reduction in the activity of SOD was observed, and it was maximum in Guntur populations (0.218 µM/ min/ mg) at 6 hr of exposure. Thus, the findings suggest that the exposure of mealybug to high temperature induce oxidative stress in *P. solenopsis.* In all the population high temperature stress induces the activity of antioxidant enzymes to overcome the oxidative cell damage in mealy bug.

Key words: Thermal stress, *Phenacoccus solenopis,* reactive oxygen species, catalase, superoxide dismutase, peroxidases, exposure, population variations,Bhatinda, Sri ganganagar, Rajkot, Guntur

The cotton mealy bug *Phenacoccus solenopsis* Tinsley is an important sucking pest of cotton reported in all cotton growing states of India. This occurs throughout the year, with peak infestations observed during April- May in central India and June- August in north India (Fand et al., 2014), when the temperature is very high. Being an ectothermic pest, its various life processes are severely affected by temperature (Prasad et al., 2012; Waqas et al., 2020). During thermal stress it is very important to maintain the homeostatic balance by preventing the oxidative stress as well as damage by reactive oxygen species. To overcome this insects have their own defense system of non-enzymatic scavengers and a series of antioxidant enzymes. In insects, the most important antioxidant enzymes are superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and glutathione S-transferase (GST) (Wang et al., 2001; Dubovskiy et al., 2008; Yang et al., 2010). These antioxidant enzymes play a major role in insects to protect cells and thus keeping the homeostatic balance by removing the oxidative stress. Catalase and peroxidase breaks H_2O_2 into H_2O and O_2 and SOD removes the O_2 through the method of dismutation to O_2 and H_2O_2. The effects of varying temperature regimes on developmental biology of various insects including mealybug had been well documented (Vennila et al., 2010; Nikam et al., 2010; Rishi Kumar et al., 2010; Prasad et al., 2012; Kumar et al., 2013). However, the studies on thermal stress induced effect over the major antioxidant enzymes in *P. solenopsis* have been less studied. Keeping this in view, the present study was aimed to understand the effect of high temperature 40^{o}C on the level of antioxidant enzymes, when exposed to 1 to 6 hrs.

MATERIALS AND METHODS

Mealybugs from the farmers' fields of six cotton growing states viz., Sri Ganganagar (Rajasthan), Bhatinda (Punjab), Saoner (Maharashtra), Bharuch (Gujarat), Guntur (Andhra Pradesh) and Perambalur (Tamil Nadu) were collected. These were established on sprouted potato in a plastic container under laboratory condition (27± 2^{0}C; 65% RH) following standard protocol (Nagrare et al., 2011). Samples required for thermal stress experiments were drawn from this culture. Second instar (7 days old, n=30) was selected from each population and exposed to 40^{o}C and 65%RH for varying periods (1- 6 hr). After the exposure, the cultures were transferred into a 2 ml centrifuge tube, with samples kept at 27°C serving as a control. A temperature controller was used in all stress treatments.

In order to check mortality, populations were allowed to recover at 27°C for 30 min after shock. Then the surviving ones were frozen immediately stored at -80°C until analysis. The treatments were replicated thrice.

The activities of antioxidant enzymes, including catalase (CAT), peroxidases (POD), superoxide dismutase (SOD) was measured using commercially available assay kits. Enzyme estimation was carried out by following the manufacturer's protocol and the absorbance was read in a microplate reader (Multiskan EX, Labsystems Inc., Franklin, MA, USA). For determination of enzyme expression level, single mealybug which was subjected to thermal stress experiment was homogenized in respective sample buffer (100 µl of phosphate buffer; pH 7.5), centrifuged at 15,000 rpm for 10 min at 4°C and the resultant supernatant was used for protein content and enzymatic activity analysis. Supernatants obtained from these homogenates were used to determine the CAT, POD and SOD activities according to the Cai et al. (2019) with slight modifications.

The CAT activity was determined with a catalase assay kit (# 707002, Cayman Chemical Co., Ann Arbor, MI, USA) as per manufacturer's instruction. The CAT assay buffer (100mM potassium phosphate, pH 7.0) and sample buffer (25mM potassium phosphate pH 7.5, containing 1mM EDTA and 0.1% BSA) was used for measuring the absorbance at 540 nm due to H_2O_2 decomposition. Enzyme activity was calculated as concentration of hydrogen peroxide reduced/ min/ mg protein. One unit is defined as the amount of enzyme that will cause the formation of 1.0 nmol of formaldehyde/ min at 25°C. The specific activity peroxidase was expressed in nmol/ min/ mg protein. Peroxidase activity was assayed with commercially available assay kit (#MAK092, Sigma-Aldrich) by measuring the amount of H_2O_2 reduced during the assay at a wavelength of 570 nm (homogenization buffer consist of 100 µl of phosphate buffer; pH 7.5) and assay buffer 100 µl (50mM Tris-HCl, pH-7.6 containing 5 mM EDTA). The specific activity peroxidase was expressed in nmol/ min/ mg protein.

The SOD activity was determined using a SOD determination kit (#19160, Sigma-Aldrich, St. Louis, MO, USA). The activity of SOD was quantified by the sum of inhibition or the decrease in colour development at 450 nm. SOD activity was standardized using cytochrome c and xanthine oxidase coupled assay. The specific activity SOD was expressed in µM/ min/ mg protein. Protein concentrations of individual samples were determined kit (Himedia) using bovine serum albumin as a standard (Bradford, 1976) and absorbance was read at 595 nm (Labsystem Multiskan EX, Lab systems Inc., Franklin, MA, USA) after the incubation period of samples and reagents for 15 min (25°C) in micro titer plates.

RESULTS AND DISCUSSION

The activity of antioxidant enzyme (SOD, POD and CAT) in *P. solenopsis* was significantly affected due to high temperature (40°C) exposure. In Saoner population, maximum catalase activity was observed at 2 hr after exposure (0.172 nmol/ min/ mg) and further increase in the period of exposure resulted in reduction in the activity of the catalase. Gradual increase in the activity of catalase was noticed in Bhatinda, Rajkot and Guntur populations and the maximum activity (1.666 nmol/ min/ mg) was observed at 6 hr of exposure in Guntur and Perambalur population. In response to the period of exposure, peroxidase activity with 40°C exposed *P. solenopsis* was in increasing trend. The maximum activity was observed in Guntur population at 0. 218 nmole / min / mg at 6 hr of exposure followed by Bhatinda, Rajkot and Saoner populations. While, in Perambalur, the maximum peroxidase activity (0.127 nmol/ min/ mg) was observed at 5 hr after exposure. Exposure of 3rd instar of *P. solenopsis* to high temperature influenced the activity of superoxide dismutase (SOD). Increasing trend in the activity was observed in Bhatinda, Saoner and Guntur populations from 1- 6 hr after exposure. The maximum activity of SOD was observed in Guntur populations (0.218 µM/ min/ mg). In Sri Ganganagar the maximum activity of SOD reached at 4 hr but showed a significant reduction in the activity of SOD at 6 hr of exposure (Table 1).

Temperature is one of the most key environmental factors that bring out physiological changes in most of the organisms (Jia et al., 2011). As insects are ectotherms, suffers losses from physiological fitness and sometimes leads to death (Dillon et al., 2009). Catalase and SOD are the most important antioxidant enzymes against ROS. Superoxide dismutase catalyses the dismutation of superoxide radicals to H_2O_2 and O_2, and constitutes the most important enzyme in cellular defense because its activation directly modulates the amounts of O_2 and H_2O_2 (Foyer and Noctor 2000; Thannickal and Fanburg 2000). In the present study, temperature stress significantly influenced the activities of antioxidant enzymes in insects. The overall activity of

Table 1. Activity of antioxidant enzymes in field populations of *P. solenopsis* exposed to high temperature (40°C)

Period of exposure (Hrs)	Sriganganagar	Bhatinda	Rajkot	Saoner	Guntur	Perambalur
	Catalase (nmol/ min/ mg ± SEm)					
Control	0.093 ± 0.003	0.134 ± 0.001	0.184 ± 0.007	0.119 ± 0.002	0.311 ± 0.009	0.090 ± 0.016
1	0.111± 0.002	0.110 ± 0.006	0.203 ± 0.002	0.125 ± 0.002	0.407 ± 0.009	0.203 ± 0.003
2	0.125 ± 0.001	0.119 ± 0.004	0.213 ± 0.124	0.172 ± 0.007	1.073 ± 0.008	0.252 ± 0.002
3	0.159 ± 0.003	0.108 ± 0.033	0.243 ± 0.002	0.141 ± 0.001	1.081 ± 0.001	0.247 ± 0.006
4	0.180 ± 0.006	0.179 ± 0.002	0.267 ± 0.004	0.134 ± 0.001	1.106 ± 0.006	0.199 ± 0.024
5	0.243 ± 0.002	0.246 ± 0.002	0.290 ± 0.002	0.125 ± 0.003	1.250 ± 0.005	0.283 ± 0.009
6	0.161± 0.006	0.399 ± 0.005	0.295 ± 0.004	0.126 ± 0.002	1.666 ± 0.403	0.235 ± 0.019
	Peroxidase (nmol/ min/ mg ± SEm)					
Control	0.023 ± 0.016	0.034 ± 0.002	0.089 ± 0.009	0.066 ± 0.002	0.173 ± 0.038	0.081 ± 0.001
1	0.034 ± 0.006	0.012 ± 0.052	0.084 ± 0.044	0.060 ± 0.003	0.177 ± 0.003	0.086 ± 0.024
2	0.050 ± 0.006	0.051 ± 0.075	0.087 ± 0.028	0.069 ± 0.005	0.185 ± 0.036	0.094 ± 0.043
3	0.057 ± 0.013	0.052 ± 0.029	0.103 ± 0.031	0.072 ± 0.003	0.175 ± 0.088	0.104 ± 0.005
4	0.060 ± 0.019	0.058 ± 0.007	0.112 ± 0.024	0.075 ± 0.141	0.192 ± 0.033	0.126 ± 0.022
5	0.028 ± 0.006	0.062 ± 0.032	0.096 ± 0.007	0.077 ± 0.061	0.183 ± 0.038	0.127 ± 0.006
6	0.023 ± 0.001	0.063 ±0.004	0.097 ± 0.005	0.124 ± 0.001	0.218 ± 0.088	0.124 ± 0.046
	Superoxide dismutase (µM/ mg/ min ± SEm)					
Control	0.050 ± 0.006	0.034 ± 0.002	0.089± 0.009	0.066 ± 0.002	0.173 ± 0.038	0.081± 0.001
1	0.034 ± 0.006	0.012 ± 0.052	0.084 ± 0.044	0.060 ± 0.003	0.177 ± 0.003	0.086 ± 0.024
2	0.023 ± 0.016	0.051 ± 0.075	0.087± 0.028	0.069 ± 0.005	0.185 ± 0.036	0.094 ± 0.043
3	0.057 ± 0.013	0.052 ± 0.029	0.103± 0.031	0.072 ± 0.003	0.192 ± 0.033	0.104 ± 0.005
4	0.060 ± 0.019	0.058 ± 0.007	0.112± 0.024	0.075 ± 0.141	0.183 ± 0.038	0.126 ± 0.022
5	0.028 ± 0.006	0.062 ± 0.032	0.096± 0.007	0.077 ± 0.061	0.175 ± 0.088	0.127 ± 0.006
6	0.023 ± 0.001	0.063 ± 0.004	0.097± 0.005	0.124 ± 0.001	0.218 ± 0.088	0.124 ± 0.046

CAT, POD and SOD increased with period of exposure to ambient temperature in all the populations of *P. solenopsis* (Waqas et al., 2020b; Shankarganesh et al., 2020). The CAT activity increased at 40°C as compared to control. Under thermal stress condition the CAT activity increased in parasitized Oriental fruit fly larvae (Cai et al., 2019). Similarly, the activity of POD was significantly higher in all the populations. Depending upon the period of thermal stress the SOD activity in the *P. solenopsis* was significantly influenced. Early or initial-stage of exposure to temperature changes resulted in oxidative stress regulated by antioxidant enzymes, but continued stress caused by varied temperature exposure resulted in decreased SOD activity.

Jia et al. (2011) showed that the activities of CAT, peroxidase (POX) and SOD significantly increased in *Bacterocera dorsalis* (Hendel) in response to thermal stress. However, prolonged exposure to heat or cold shock resulted in decreased activities of CAT, GST and SOD accompanied by impaired antioxidant capacity and high levels of oxidative stress. In *Panonychus citri* and *Propylaea japonica*, the high temperature exposure increased the levels of SOD and GST (Yang et al., 2010; Zhang et al., 2015). Similar results were observed in the aphid parasitoid *Aphidius gifuensis* when the pupae and adults were exposed to temperature above 30°C; activities of GST, SOD, CAT and POD significantly increased (Kang et al., 2017). The effects of temperature on the activity of antioxidant enzymes in larvae of *B. dorsalis* parasitized by *Diachasmimorpha longicaudata* (Wang et al., 2013; Cai et al., 2019) and *Paracoccus marginatus* parasitized by *Acerophagus papayae* (Shankarganesh et al., 2020) indicated that CAT, POD and SOD together, have an important role in preventing the insect suffering from oxidative damage with ROS induced by heat stress. This basic information will certainly help in predicting the direct or indirect effect temperature stress on population dynamics of *P. solenopsis* under varied environmental conditions and thereby to frame management strategies against this mealy bug.

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