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ORIGINAL RESEARCH ARTICLE

Effects of thermal stress on the antioxidant defenses in *Paracoccus marginatus* Williams and Granara de Willink parasitized by *Acerophagus papayae* Noyes & Schauff

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Abstract

Acerophagus papayae (Encyrtidae: Hymenoptera) is a potential parasitoid successfully used to control papaya mealybug, *Paracoccus marginatus* (Homoptera: Pseudococcidae) on different horticultural crops such as papaya, mulberry, cotton, cassava, citrus, sweet potato, peas, beans, okra, eggplant, guava, *Hibiscus Jatropha, Allamanda*. However, the parasitic efficiency is mostly affected by prevailing weather parameter in the crop canopy. Understanding the effect of thermal stress on the parasitizing efficiency of natural enemy and the role of antioxidant mechanisms for temperature stress is important for improving efficiency under stressed condition. In this study, we investigated the effect of constant temperatures 25, 28, 30, 32 and 34ºC on percent parasitism, developmental periods and adult emergence of *A. papayae* under laboratory condition. Increase in temperature negatively affected the performance of *A. papayae* in terms of developmental duration, parasitism efficiency and adult emergence. Exposure of *A. papayae* to different temperature led to overall increase in activities of superoxide dismutase, catalase, peroxidases and glutathione-S-transferases. Our results indicated that thermal tolerance in *A. papayae* could be mainly associated with changes in activity of antioxidant enzymes in scavenging of reactive oxygen species (ROS) under stress.

Keywords *Acerophagus papayae* · Thermal stress · Catalase · Peroxidases · Superoxide dismutase · Glutathione-S-transferases

Introduction

Papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink is a major sucking pest infesting more than 80 plant species of horticultural crops such as tropical fruits, vegetables, ornamentals, weeds and agricultural crops (Lalitha et al. 2015). In India during 2009, it was a major threat to economically important agricultural and horticultural crops. To curb the menace of *P. marginatus*, three exotic encyrtid parasitoids viz.., *Acerophagus papayae*, *Anagyrus loecki* and *Pseudleptomastix mexicana* were introduced from Puerto Rico. Among these, *A. papayae* was the most effective parasitoid based on its parasitizing efficiency, proliferation and field activity (Sakthivel 2013). Due to its high parasitic efficiency, it has already been successfully used to control *P. marginatus* on different crops and has been considered a potential biological-control agent for effective Integrative Pest Management (IPM) programs on field and in greenhouse. However, under natural conditions, prevailing weather parameters such as high temperature and rainfall are restricting the application of *A. papayae*. High temperature is an important environmental factor which influences the field performance of parasitoids by reducing their longevity and fecundity (Jervis and Copland 1996; Joodaki et al. 2018). Adaptation of parsitoids to high temperature plays a vital role in biological control of pests which ultimately increases the parasitoid's efficiency in controlling pests. Therefore, the development of temperature tolerant strain of *A.papayae* is of great importance for the management of *P. marginatus* infesting crops in high temperature regions.

Insects have evolved complex protective mechanism to protect themselves against the high temperature. Understanding the bases of tolerance to temperature stress would help in designing the strategies for sustainable pest management under adverse environmental conditions. Antioxidant enzymes present in insect including superoxide dismutase (SOD), catalase (CAT), peroxidases (POD) and

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glutathione-S-transferases (GST) are the major immune system involved in the oxidative damage response (Lopez-Martinez et al. 2008; Yang et al. 2010). These antioxidant enzymes can scavenge the thermal stress, UV, xenobiotic exposures and parasitoid infestation induced surplus reactive oxygen species (ROS) (Lopez-Martinez et al. 2008; Yang et al. 2010; Lopez-Martinez and Hahn 2012; Zhang et al. 2015; Ali et al. 2016). The SOD is one of the most important antioxidant enzymes in the defense system against ROS. Superoxide dismutase (SOD) catalyses the dis-mutation of superoxide radicals into oxygen (O_2) and hydrogen peroxide (H_2O_2); then H_2O_2 is converted by both CAT and POD into oxygen and water (H_2O) (Kang et al. 2017).

Several studies have been based on the developmental period and natural parasitism by *A. papayae* under field condition and also parasitism behavior of parasitoid and host relationship of *A. papayae* on *P. marginatus* under laboratory conditions (Amarasekare et al. 2009, 2012; Sakthivel 2013; Mastoi et al. 2018; Arshad et al. 2019). However, little is known about the effect of extreme temperature on efficiency of the parasitoid, *A. papayae*. Further, there has not been any research focusing on elucidating the effect of different temperatures on the developmental biology and the antioxidant enzymes of *A. papayae*. From this perspective, the study was undertaken to evaluate the response of different temperature regimes on the development, parasitism and parasitoid emergence of *A. papayae.* The impact of different temperature regimes on the level of antioxidant enzymes was also investigated.

Materials and methods

Establishment of insect cultures Initially the field collected *P. marginatus* was mass cultured on sprouted potatoes (Amarasekare et al. 2008). The pure culture of *A. papayae* was obtained from the mummified mealybugs collected from papaya and 70% honey solution was given as food for parasitoids.

Thermal stress experiment: Effect of constant temperatures on *A. papayae* development and parasitizing potential

Thermal stress experiment was conducted according to the methodology described by Laneesha (2016) and Prasad et al. (2012) with slight modifications. For each experiment the leaves were collected from 30 to 45 day old potted plants (30 cm diameter) raised under screen house. The fully expanded and healthy cotton leaves with petiole were collected and wiped with cotton and a slant cut was given to its petiole and it was inserted into a 2.0 ml eppendorf tube containing 10% sucrose solution, wrapped with parafilm and kept in an insect breeding dish. The eppendorf tubes were kept in the insect breeding dishes and 100 second instar *P. marginatus* nymphs were released per dish. Thereafter, 10 pairs of one day old (24 h of emergence) *A. papayae* collected from the pure culture were released into the insect breeding dishes. The dishes were sealed with parafilm to avoid the escape of either the mealybug or parasitoid kept in the insect growth chamber set at constant temperatures of 25, 28, 30, 32 and 34ºC ± 1ºC and 70 ± 5% relative humidity with 10 replicates. One week after exposure to treatment temperatures i.e. when the parasitized mealybug changed to brown colour (mummies), all mummified were checked daily until the emergence of all parasitoids. Number of parasitized mealybugs and number of adults that emerged from mummified mealybugs were also counted. Observations on adult emergence (%), parasitism (%) and developmental period (days) at each temperature were recorded. For estimating the level of enzymes such as catalase (CAT), peroxidases (POD), superoxide dismutase (SOD) and glutathione-S-transferases (GST) in the mummified mealybug, samples were kept in the vial and stored at -20°C immediately until further analyses.

Enzyme activity assay

The activities of antioxidant enzymes, including catalase (CAT), peroxidases (POD), superoxide dismutase (SOD) and glutathione-S-transferases (GST) were 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).

Homogenate preparation for enzyme and protein assays This was done for the determination of enzyme expression level in the parasitized mealybugs (*P. marginatus*) by *A. papayae* which were subjected to thermal stress experiment (25°C, 28°C 30°C, 32 and 34°C), a single parasitized mealybug was homogenized in respective sample buffer, centrifuged at 15,000 rpm for 10 min at 4°C and the resultant supernatant was used for protein content and enzymatic activity analyses.

Protein estimation

Protein concentrations were determined according to the method developed by Bradford (1976) Kit (# ML178, HiMedia Laboratories Pvt. Ltd, LBS Marg Mumbai, India) using bovine serum albumin as a standard. Absorbance was read at 595 nm (Multiskan EX, Labsystems Inc., Franklin, MA, USA) after the 15-minute incubation period of samples and reagents under room temperature in microtiter plates.

Enzyme activity determination Supernatants obtained from the parasitized mealybug homogenates were used to determine the CAT, POD, GST and SOD activities according to the Cai et al. (2019) method with slight modifications. The CAT activity was determined with a Catalase assay kit (# 707,002, Cayman Chemical Co., Ann Arbor, MI, USA) according to the manufacturer's instruction. The CAT assay was performed by measuring the absorbance at 540 nm due to H_2O_2 decomposition (Luck 1971). Peroxidase activity was assayed with commercially available Catalase assay kit (#MAK092, Sigma-Aldrich, USA) by measuring the amount of H_2O_2 reduced during the assay at a wavelength of 570 nm. The SOD activity was determined using a SOD determination kit (#19,160, Sigma-Aldrich, St. Louis,MO, USA). The activity of SOD was quantified by the sum of inhibition or the decrease in the color development at 450 nm. For GST activity determination, GST assay kit (#CS0410), Sigma-Aldrich, St. Louis,MO, USA) was used with 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate). The increase in absorbance due to the conjugation of the glutathione thiol group to CDNB was monitored at 340 nm for 6 min.

Statistical analysis

All the data were subjected to Analysis of variance' using OPSTAT online statistical programme developed by CCS HAU Hisar (Sheoran et al. 1998). Means were separated using Tukey's honestly significant difference (HSD) test when significant differences were found at $P < 0.05$ and were denoted as means ± SE (standard error of the mean).

Results

Thermal stress experiment

The developmental period, parasitism efficiency (%), percentages of adult emergence of *A. papayae* in response to thermal stress are shown in Table 1.The parasitoid, *A. papayae*, was able to develop and emerge at all the five temperatures studied. Further, there was a significant effect of temperatures on time required to complete its development to the adult stage. At 25°C, *A. papayae* took significantly longer time (15.90 ± 0.23 d) to reach the adult stage in comparison to the *A. papayae* exposed to higher temperature at 34°C (8.20 ± 0.24 d). At 28°C, developmental period of *A. papayae* was 14.10 ± 0.76 d, percent parasitism and adult emergence was 38.10 ± 0.90 and 76.87 ± 2.89%, (respectively). At 30°C and 32°C, the developmental periods were 13.60 ± 0.30 and 10.20 ± 0.32 days, respectively (Table 1). Variation in temperatures had a significant effect on percentage of parasitism and adult emergence in *A. papayae* and was higher at 25°C (43.15 ± 0.26% and 55.23 ± 0.94%, respectively). Significantly lower percent parasitism and adult emergence of *A. papayae* was recorded at 32°C and 34°C.

Enzyme activity assay The antioxidant enzyme activities (SOD, CAT, POD and GST) of *A. papayae* in response to thermal stress are presented in Table 2. The CAT activity was (1.169 nmol/min/ml) at 25°C and 28°C and it increased and the highest activity was recorded at 34°C (1.248 nmol/min/ml). The CAT activity was close to the control (1.154 nmole/min/ml) mummies incubated at 30°C. The marked increase in POD activity (0.0090 nmole/min/ml) was observed in parasitized *P. marginatus* exposed to 25°C. The POD activities of mummified *P. marginatus* declined significantly when incubated at high temperatures (32 and 34°C), compared to the control and it was totally nil at 30°C. SOD activity was increased from lower to higher temperature. The activity of GST did not differ between treatment and control temperature but was significantly higher at 25°C (0.025 µmol/ml/mi).

Discussion

Temperature is one of the key factors limiting the ability of natural enemies to suppress the target pests. Extreme temperatures in the field affect the parasitoid's efficiency as biological control agent (Foerster et al. 2015). In the present work, we have not only evaluated the perspective of thermal stress on parasitoid's performance but also screened the activity of key biochemical enzymes involved in this process.

The obtained results suggested that *A. papayae* was able to develop at different temperature regimens (25–34ºC). On the other hand, developmental durations, parasitism efficiency and emergence rate differed significantly among different temperature treatments. In the thermal stress experiment, the duration of development from oviposition to adult emergence varied from 8.20 to 15.90 days. Increase in temperature negatively affected the performance of *A. papayae*. The findings of Baitha et al. (2003) also indicated that the parasitizing efficacy of *Trichogramma japonicum* on *Chilo partellus* was highest (60%) at 30°C. Similarly, temperature based differences in biological traits of species/strains of *Trichogramma* was found to be best at 26°C (Ghosh et al. 2017). High temperature (30°C) had a similar negative effect on the level of parasitism by *Aphidius ervi* on *Aphis pomi* (Malina and Praslicka 2008).

As the temperature increased from 25 to 34°C, a decrease in percent parasitism by *A. papayae* was observed. In contrast, parasitization of *Phenacoccus solenopsis* by *Aenasius arizonensis* was increased with increase in temperature and peaked at 36 and 35°C, respectively (Zhang et al. 2016;

Table 1 Developmental period (mean ± SE), percent parasitism, adult emergence of *Acerophagus papayae* a parasitoid of *Paracoccus marginatus* at different temperatures

Temperature (°C)	Developmental period (days)	Percent parasitism	Percent adult emergence
25	15.90 ± 0.23	43.15 ± 0.26	55.23 ± 0.94
28	14.10 ± 0.76	38.10 ± 0.90	76.87 ± 2.89
30	13.60 ± 0.30	39.85 ± 0.37	50.24 ± 0.93
32	10.20 ± 0.32	34.31 ± 0.30	39.22 ± 0.80
34	8.20 ± 0.24	26.52 ± 0.50	28.80 ± 1.10

Each temperature treatments were replicated 10 times, n = 100 mealybugs per each replication; Means were separated using Tukey's honestly significant difference (HSD) test when significant differences were found at $P < 0.05$ and were denoted as means ± SE (standard error of the mean)

Joodaki et al. 2018). Further, highest parasitism and emergence rates of *A. arizonensis* were recorded at 31°C. The parasitism and emergence rates of the parasitoid significantly increased with increase in temperature from 19°C to 31°C, but dropped when the temperature exceeded 31°C (He et al. 2018), while in our study there was decrease in percent parasitism as temperature increased above 28°C. In the present study, the percentage adult emergence was highest (76.87%) at 28°C similar to *T. chilonis* with highest fecundity and emergence observed at 27°C (Singh and Ram 2006). In another study, adult emergence of *A. papayae* decreased when the mummified mealybug was exposed to the temperature above 30°C (Srivastava and Singh 2015). Temperature shocks at >33 ± 1°C resulted in decrease in adult emergence of *T. chilonis* (Singh and Ram 2006) indicating the effects of high temperature on parasitoid's performances.

Among the antioxidant enzymes, SOD and catalase were 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 defence because its activation directly modulates the amounts of O_2 and H_2O_2 (Foyer and Noctor 2000). Catalase is another strong antioxidant enzyme that reduces H_2O_2 to H_2O (Thannickal and Fanburg 2000). In the present study, when temperature increased from 25 to 34°C, increased activity of SOD and catalase enzymes were observed which was consistent with temperature exposure study. The POD activity was involved in protection of parasitoid against the oxidative damage caused by H_2O_2. 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, the activities of GST, SOD, CAT and POD were significantly increased and played an important role in the antioxidant response under high temperature (Kang et al. 2017).

The effects of temperature on the activity of antioxidant enzymes in larvae of *Bactrocera dorsalis* parasitized by *Diachasmimorpha longicaudata* indicated that CAT, POD and SOD together, have an important role in preventing the larvae of *B. dorsalis* suffering from oxidative damage induced by parasitization by *D. longicaudata* (Wang et al. 2013; Cai et al. 2019). This study represents the first report on the role of antioxidant enzymes in tolerating the thermal stress by *A. papayae*. Temperature fluctuations have strong influence on the performance of laboratory reared *A. papayae.* Therefore, releases of these temperature tolerant parasitoids under varied environmental conditions can be useful for sustained mealybug management in different crops.

Table 2 Specific activity of different stress enzymes in parasitized *Paracoccus marginatus* by *Acerophagus papayae* exposed to thermal stresses

Temperatures(°C)	Catalase (nmol/min/ml)	Peroxidases (nmole/min/ml)	Superoxide dismutase (%inhibition)	Glutathione-S-transferases (μmol/ml/min)
25°C	1.169	0.0090	71.783	0.025
28°C	1.169	0.0060	77.53	ND
30°C	1.154	-	86.110	0.008
32°C	1.196	0.0020	102.777	0.005
34°C	1.248	0.0023	146.297	0.010
Unparasitised *Paracoccus marginatus*(control)	1.155	0.0027	95.1775	0.016

Values mentioned in each treatment were an average of three replications

Conclusion

The present study not only highlighted the significant effects of thermal stress on *A. papayae* to specific temperature regime performance but also explored the potential mechanism of antioxidant response in *A. papayae*. We found that the protection of thermal stress damage is regulated by antioxidant enzymes. This study provides the basic information for developing high temperature tolerant strain of *A. papayae* for the management of papaya mealybug in high temperature regions. The natural enemies having these qualities can overtake their hosts quickly whenever the host begins to increase in numbers. The developed *A. papayae* strain can consequently be used as a component of bio-intensive IPM in various crops, when temperature goes higher.

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Compliance with ethical standards Hereby, I Dr. K. Shankarganesh consciously assure that for the manuscript "Effects of thermal stress on the antioxidant defenses in *Paracoccus marginatus* Williams and Granara de Willink parasitized by *Acerophagus papayae* Noyes & Schauff" following is fulfilled:

- This material is the OUR own original work, which has not been previously published elsewhere.
- This is to certify that there is no potential conflict of interest in the research work articles.
- This is to certify that there is no human or animal were harmed in this research work.
- This is to certify that pre-informed consent were obtained from all co authors.

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