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Gene Expression of Detoxification Enzymes in *Tenebrio molitor* after Fungicide Captan Exposure

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살진균제인 캡탄 처리 후 갈색거저리의 해독효소 유전자 발현

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ABSTRACT: The application of fungicides is indispensable to global food security, and their use has increased in recent times. Fungicides, directly or indirectly, have impacted insects, leading to genetic and molecular-level changes. Various detoxification mechanisms allow insects to eliminate reactive oxygen species (ROS) toxicity induced by agrochemicals including fungicides. In the present study, we analyzed the mRNA expression levels of detoxifying enzymes in *Tenebrio molitor* larvae following exposure to non-lethal doses (0.2, 2, and 20 μg/μL) of a fungicide captan. Transcripts of peroxidases (POXs), catalases (CATs), superoxide dismutases (SODs), and glutathione-s-transferases (GSTs) were screened from the *T. molitor* transcriptome database. RT-qPCR analysis showed that *TmPOX5* mRNA increased significantly 24 h post-captan exposure. A similar increase was noticed for *TmSOD4* mRNA 3 h post-captan exposure. Moreover, the expression of *TmCAT2* mRNA increased significantly 24 h post-treatment with 2 μg/μL captan. *TmGST1* and *TmGST3* mRNA expression also increased noticeably after captan exposure. Taken together, these results suggest that *TmPOX5* and *TmSOD4* mRNA can be used as biomarkers or xenobiotics sensors for captan exposure in *T. molitor*, while other detoxifying enzymes showed differential expression.

Key words: Detoxification, Fungicide, mRNA expression

초 록: 최근 살진균제는 세계 식량 안보에 없어서는 안될 필수 요소이며, 그 사용량은 증가하고 있다. 살진균제는 직접적 또는 간접적으로 곤충에 영향을 미쳐 유전자 및 분자 수준의 변화를 일으킨다. 곤충은 다양한 해독 매커니즘을 통해 살진균제를 포함한 농약으로부터 유발되는 활성산소(ROS) 독성을 제거한다. 본 연구는 살진균제 캡탄의 비치명적 투여량(0.2, 2, and 20 μg/μL)을 주입 후 갈색거저리의 유충에서 해독효소의 mRNA 발현량을 분석했다. 갈색거저리의 전사체 분석을 통해 해독 매커니즘 관련 유전자인 퍼옥시다제(POX), 카탈라제(CAT), 슈퍼옥사이드 디스뮤타제(SOD) 및 글루타티온-S-트랜스퍼라제(GST)를 발굴하였다. 처리 24시간 후 *TmPOX5* mRNA가 유의하게 증가한 것으로 나타났다. 처리 3 시간 후 *TmSOD4*의 mRNA가 유사하게 증가하였다. 또한 2 μg/μL 처리 24시간 후 *TmCAT2*의 mRNA 가 유의하게 증가하였다. 캡탄 노출 후 *TmGST1* 및 *TmGST3*의 mRNA 발현량도 증가하였다. 결론적으로, *TmPOX5* 및 *TmSOD4* 유전자는 갈색거저리에서 캡탄 노출에 대한 바이오마커 또는 생체이물 센서로 작용할 수 있음을 시사한다.

검색어: 해독작용, 살진균제, mRNA 발현

Insects have adapted to a variety of habitats, making them the most successful organisms to inhabit the Earth. Given the diverse environments that they inhabit, insects encounter various biotic and abiotic stresses. In the absence of an adaptive immune system, insects have developed a most versatile innate immune repertoire to handle the stressors and create suitable niches in various habitats (Brucker et al., 2012). Among environ-

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mental threats, agrochemicals in particular are the most toxic and also a common hazard that insects share with humans (Xin and Zhang, 2020). Agrochemicals are classified into distinct categories based on their hazard, target pest species, and chemical properties. Based on their target pest species, agrochemicals can be further classified into insecticides, herbicides, rodenticides, nematicides, fungicides, and acaricides (Abdollahi et al., 2004).

Previous studies have investigated oxidative stress induced by agrochemicals (Jia and Misra, 2007; Radhakrishnan et al., 2018; Semren et al., 2018; Shakir et al., 2018; Caverzan et al., 2019; Lu et al., 2019; He et al., 2021). Reactive oxygen species (ROS) such as superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-) are highly reactive chemicals that induce oxidative stress (Freyre et al., 2021). All species produce ROS excessively under stress conditions, and scavenge these ROS through antioxidant production (oxidative stress response). Insects possess a variety of antioxidant enzymes and small molecular weight antioxidants that provide a coordinated response against exogenous and endogenous oxidants (Felton and Summers, 1995). Antioxidant defense mechanisms in insects can be classified in to enzymatic antioxidants such as glutathione-S-transferases (GSTs), peroxidases (POXs), catalases (CATs), and superoxide dismutases (SODs) (Barbehenn, 2002), and non-enzymatic antioxidants such as ascorbic acid, thiols, and alpha-tocopherol (Irato and Santovito, 2021; Kolawole et al., 2014).

GSTs confer resistance to all main classes of insecticides via direct metabolism, or sequestration of chemicals, or indirectly by protecting against oxidative stress induced by exposure to insecticides (Pita-Oliveira and Rodrigues-Soares, 2021). CAT is an antioxidant enzyme that alleviates the toxic effects of H_2O_2 (Yu et al., 2006) by converting it to H_2O and O_2 (Thannickal and Fanburg, 2000). RNAi-mediated knockdown of *Spodoptera litura* CAT was reported to induce ROS generation, cell cycle arrest, and apoptosis in SL-1 cells (Zhao et al., 2013). SOD reduces ROS by converting superoxide into O_2 and H_2O_2. H_2O_2 is subsequently converted to water by CAT or glutathione peroxidase (GPx) (Fridovich, 1975). Three types of SOD proteins have been previously reported in insects; SOD1 is a cytoplasmic protein, which provides a defense against O_2 toxicity, while SOD2 is a mitochondrial matrix enzyme, which scavenges O_2 radicals in the mitochondria. Meanwhile, SOD3 is found mainly in hemolymph and the molting fluid of insects (Zhang et al., 2014). Additional SOD proteins have been discovered recently, but their functions remain unknown. Seven types of SODs, each of which plays differential roles in resistance to oxidative stress have been identified in *Bombyx mori* (Kobayashi et al., 2019). The antioxidant functions of these enzymes in insects under insecticidal and bio-insecticidal stresses have been investigated in cowpea storage bruchid (*Callosobruchus maculatus*) [Coleoptera: Chrysomelidae]. The levels of GPx and glutathione synthetase in *C. maculatus*, have been reported to increase in a dose-dependent manner in response to insecticide and bio-insecticide exposure (Kolawole and Kolawole, 2014).

Captan [N-(trichloromethylthio)-cyclohex-4-ene-1,2-dicarboximide], a member of the phthalimides, was introduced commercially in 1951 and has been used ever since as an agrochemical fungicide (He et al., 2022). This broad-spectrum non-systemic fungicide has been extensively used in agriculture to control diseases affecting fruit, vegetable, and ornamental crops (Zhou et al., 2019). Captan inhibits respiratory and metabolic processes of numerous fungal and bacterial species. Additionally, degradation of captan leads to production of transient thiophosgene, which reacts greatly with thiols and other functional groups (Barreda et al., 2006). Captan causes cytotoxic effects, disrupts emergence, and significantly affects reproduction of transgenic *Drosophila melanogaster* (hsp70-lacZ) Bg9 (Nazir et al., 2003).

The yellow mealworm beetle, *Tenebrio molitor* is a convenient model organism, due to ease of rearing under experimental conditions, and the availability of genetic, biochemical, and molecular data (Tindwa et al., 2015; Seo et al., 2016; Kim et al., 2017; Seong et al., 2018; Jo et al., 2019; Edosa et al., 2020; Jang et al., 2021). This study investigated the toxic effects of captan on *T. molitor* to identify the potential antioxidant response of the host. Accordingly, we treated *T. molitor* with different concentrations of captan (0.2, 2, and 20 μg/μL), and analyzed the mRNA expression patterns of detoxification genes including GSTs, POXs, CATs, and SODs.

Materials and Methods

Insect rearing and captan injection

Larvae of *T. molitor* were reared at 27 ± 1°C and 60 ± 5%

relative humidity in an environmental chamber under dark conditions. The reared insects (healthy 10^{th} to 12^{th} instar larvae) were fed with a diet consisting of 170 g wheat flour, 20 g roasted soy flour, 10 g protein, and 100 g wheat bran in 200 mL of distilled water, pre-autoclaved at 121°C for 15 min before feeding. We prepared 1000-fold serial dilutions of 200 mg/mL captan in PBS (Nonghyup, Seongnam, South Korea), and injected 1 μL of captan solutions with concentrations of 20 mg/mL, 2 mg/mL, and 0.2 mg/mL into *T. molitor* larvae. The injected larvae were fed with artificial diet in the mentioned condition. Subsequently, time-course sampling (n = 10) was performed 3, 6, 9, 12, and 24 h post-injection of captan. Collected larvae were ground with liquid nitrogen using mortar and pestle. Samples were stored immediately at -80°C until further analysis.

RNA extraction and cDNA synthesis

The isolated samples (200 μL) were mixed with 800 μL of fresh RNA lysis buffer (17.72 g guanidine thiocyanate, 0.58 g sodium chloride, 2 ml 5M EDTA, 1 ml 1M MES buffer, 25 μL Triton X, 250 μL acetic acid, 500 μL isoamyl alcohol, 0.7 mg phenol red in 50 ml of distilled water) in a 1.5 mL Eppendorf tube. The mixture was incubated at room temperature for 5 min and subsequently centrifuged at 13,000 rpm for 1 min. The supernatant was collected, mixed with an equal volume of 99% ethanol, and incubated for 1 min at room temperature. Subsequently, the sample was transferred to an RNA binding column (Hyundai Micro, Seoul, South Korea), centrifuged at 4°C at 13,000 rpm for 1 min, and then the flow-through was discarded. Next, a mixture of DNase and DNase buffer was added to the column, and the column was incubated at 37°C for 15 min. Next, 500 μL of sodium acetate was added to the column, and the column was centrifuged at 4°C at 13,000 rpm for 1 min and the flow-through was discarded. Then, 500 μL of 80% ethanol was added, followed by centrifugation at 4°C at 13,000 rpm for 1 min (this step was repeated twice). Another round of centrifugation was performed to dry the column completely. Then, RNA was eluted in 30 μL of distilled water. We checked the RNA quality using Epoch (BioTek, Santa Clara, CA, USA). Next, cDNA was synthesized from extracted RNA using AccuPower RT-PreMix (Bioneer, Daejeon, South Korea) according to the manufacturer's instruction. cDNA synthesis was performed using 2 μg total RNA as the template with Oligo$(dT)_{12-18}$ primers at 72°C for 5 min, 42°C for 1 h, and 94°C for 5 min on a MyGenie96 Thermal Block (Bioneer). cDNA was stored at

Table 1. Primers used in this study

Name	Primer sequences (5′-3′)	Name	Primer sequences (5′-3′)
TmL27a-qPCR-Fw	TCATCCTGAAGGCAAAGCTCCAGT	TmPx-5-qPCR -Fw	TCCGCCATATTTGTCCAACG
TmL27a-qPCR-Rv	AGGTTGGTTAGGCAGGCACCTTTA	TmPx-5-qPCR -Rv	TCGGGTGTTCCAAATTCGTG
TmGST-1-qPCR -Fw	AAGAATGCCGTTCGGAATGC	TmCat-1-qPCR-Fw	GTTGTACAAGGATGTGCTCACG
TmGST-1-qPCR -Rv	ATATCTCGCCACTGCATTGC	TmCat-1-qPCR-Rv	TGCAAAAACTCCGACGCTTG
TmGST-2-qPCR -Fw	TCGCCGATTTTCAACTGGTC	TmCat-2-qPCR-Fw	ACGCCATAGCTTCAGGAAAC
TmGST-2-qPCR -Rv	TTCCGCCTTCAACAATTGCC	TmCat-2-qPCR-Rv	ACACTTTGGTCACGTCGAAG
TmGST-3-qPCR -Fw	ATGTTTTATCGGCGGTTCGG	TmCat-3-qPCR-Fw	TCACACCCAAAAACGCAACC
TmGST-3-qPCR -Rv	CTTTTGCAGTGCACTTGACG	TmCat-3-qPCR-Rv	CGGGCCTCAGAGAAATGAAATC
TmPx-1-qPCR -Fw	TGCTCCATTCTGTGTTTGCG	TmSOD-1-qPCR-Fw	TCAAGGGTGCGAAAAGTTGG
TmPx-1-qPCR -Rv	ACACATTGTCCGGAACATCC	TmSOD-1-qPCR-Fw	ACTTCCACATTGCCCAAGTC
TmPx-2-qPCR -Fw	ATTGTCAGTTGAGCGACGAC	TmSOD-2-qPCR-Fw	TTGCGGTGCAAATGACATGC
TmPx-2-qPCR -Rv	ATGCATCACAGTCAGTTGCG	TmSOD-2-qPCR-Fw	TCGACGGTTTTGATGTGTGG
TmPx-3-qPCR -Fw	AATCAAGGGGTGGATTGCAG	TmSOD-3-qPCR-Fw	TGCCGACAAAAGCTGTTTGC
TmPx-3-qPCR -Rv	TATTGTGTTCGCGCATCCAC	TmSOD-3-qPCR-Fw	TGCGAACCCTTCTTCAAACC
TmPx-4-qPCR -Fw	TGGGACGACGAGAGACTTTTTC	TmSOD-4-qPCR-Fw	TTGTAGCCGCATTGAGTTGC
TmPx-4-qPCR -Rv	ACAAGACCGGCAAAAACTCG	TmSOD-4-qPCR-Fw	TCGGTTTTGGTGAACGTCAC

-20°C until further use.

qRT-PCR

Quantitative real-time polymerase chain reaction (qRT-PCR) was performed to determine the mRNA expression patterns of *T. molitor* detoxification related genes including *TmGSTs* (*TmGST1-3*), *TmCATs* (*TmCAT1-3*), *TmPOXs* (*TmPOX1-5*), and *TmSODs* (*TmSOD1-4*) using AriaMx Real-time PCR (Agilent Technologies, Santa Clara, CA USA). *T. molitor ribosomal protein L27a* (*TmL27a*) was used as an internal control. Information regarding the primers used is shown in Table 1. The qRT-PCR reaction mixture consisted of 5 μL cDNA template, 2 μL forward and reverse primers (10 pmol), 3 μL water, and 10 μL 2x SYBR green mix (Bioneer). The PCR reaction conditions were as follows: initial denaturation at 95°C for 5 min; 40 cycles of denaturation at 95°C for 15 s; annealing and extension at 60°C for 30 s. The relative mRNA expression levels normalized to *TmL27a* were calculated using the $2^{-(\Delta\Delta Ct)}$ method.

Statistical analysis

The datasets were analyzed using one-way analysis of variance (ANOVA) using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). Cumulative expression levels were compared using Tukey's multiple range tests. Differences were considered significant at $p < 0.05$. All experiments were performed in triplicate.

Results

Expression of *TmGSTs* mRNA in *T. molitor*

The effect of different concentrations of captan on *TmGST* mRNA expression was investigated (Fig. 1). The results showed a significant increase in *TmGST* expression in the captan-treated group compared to the negative control group ($p \leq 0.05$). *TmGST1* and *TmGST3* mRNA expression in *T. molitor* was found to be 2.5 fold and 3.5 fold higher 3 h post-treatment with 20 μg/μL captan, respectively, compared to the negative control group. The increase in levels of *TmGST1* and *TmGST3* mRNA was in a dose-dependent manner 3h post injection, with 20 μg/μL captan treated group showing higher expression levels.

Expression of *TmPOXs* mRNA in *T. molitor*

To examine the effect of captan on *TmPOXs* expression, qPCR was conducted (Fig. 2). In the captan-treated group, the expression of *TmPOX1*, *TmPOX2*, and *TmPOX4* showed a significant concentration-dependent increase compared to the negative control group ($p \leq 0.05$). *TmPOX1, TmPOX2,* and *TmPOX4* mRNA expression showed a 5-fold, 5-fold, and 3-fold increase, respectively, 3 h post-treatment with 20 μg/μL of captan compared to the control group. Remarkably, 24 h post-treatment with 0.2 μg/μL of captan, a 25-fold increase was observed in the expression of *TmPOX5* mRNA compared to the negative control group. In fact, the mRNA expression of *TmPOX5* was significantly higher at all concentrations of captan treatment 24h post captan treatment compared to the negative control group.

Expression of *TmCATs* mRNA in *T. molitor*

To test the effect of captan on the mRNA expression of *TmCATs* (*TmCAT1-3*) in *T. molitor* larvae, qPCR was performed (Fig. 3). *TmCAT1* mRNA expression significantly increased at 3 h post injection ($p \leq 0.05$), followed by a decline at 6 and 9 h, and subsequent increase at 12 and 24 h, in the 2 μg/μL captan treated group compared to the control. On the other hand, *TmCAT2* mRNA expression increased significantly, an 8-fold increase, 24 h post-2 μg/μL injection compared to the negative control group.

TmCAT3 mRNA expression in the 20 μg/μL captan-treated group was more than 5 fold higher 3 h post-injection compared to the negative control group. *TmCAT3* showed highest mRNA expression in 20 μg/μL captan treated group at all time courses. *TmCAT2* mRNA expression increased drastically 24 h post-2 μg/μL captan treatments.

Expression of *TmSODs* mRNA expression in *T. molitor*

The levels of mRNA expression of *TmSODs* in response to captan treatment was examined (Fig. 4). *TmSOD1* mRNA

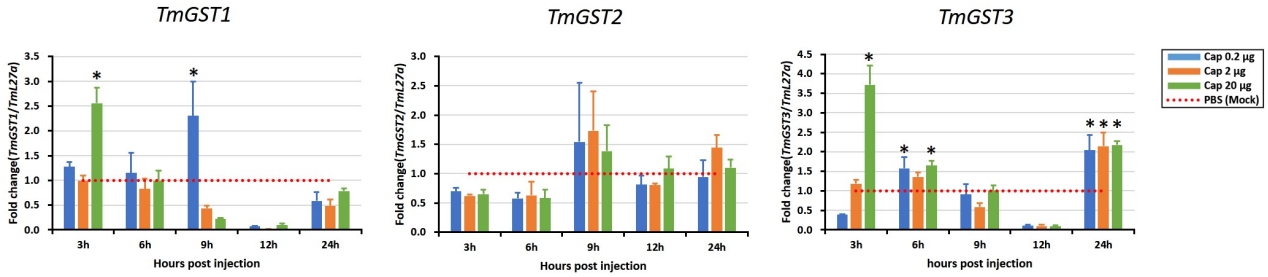

Fig. 1. Effect of captan (at 0.2, 2, and 20 μg/μL) on the relative mRNA expression of glutathione-S-transferases (GSTs) in the whole body of *Tenebrio molitor* larvae. Expression levels of *TmGSTs* mRNA in the negative control group were normalized to 1. Data are shown as the mean ± standard error (three biological replicates). Significant differences between the experimental and control groups are indicated by asterisks (* $p < 0.05$).

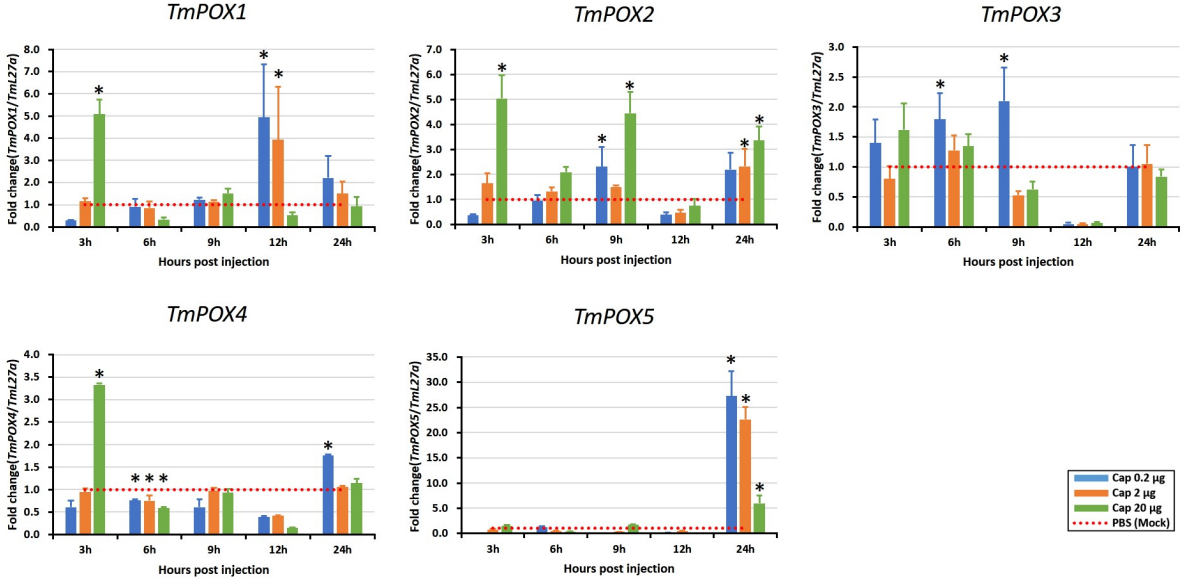

Fig. 2. Effect of captan (at 0.2, 2, and 20 μg/μL) on the relative mRNA expression of peroxidases (POXs) in the whole body of *Tenebrio molitor*. Expression levels of *TmPOXs* mRNA in the negative control group were normalized to 1. Data are shown as the mean ±standard error (three biological replicates). Significant differences between the experimental and control groups are indicated by asterisks (* $p < 0.05$).

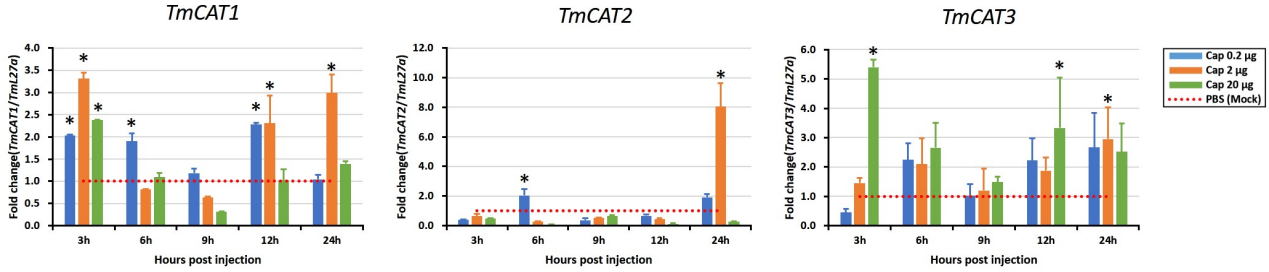

Fig. 3. Effect of captan (at 0.2, 2, and 20 μg/μL) on the relative mRNA expression of catalases (CATs) in the whole body of *Tenebrio molitor*. Expression levels of *TmCATs* mRNA in the negative control group were normalized to 1. Data are shown as the mean ± standard error (three biological replicates). Significant differences between the experimental and control groups are indicated by asterisks (* $p < 0.05$).

expression increased significantly (10-fold) 6 h post-20 μg/μL captan treatment compared to the negative control group ($p <$ 0.05). The increase in *TmSOD1* mRNA expression was dose-dependent. The expression of *TmSOD2* and *TmSOD3* mRNA

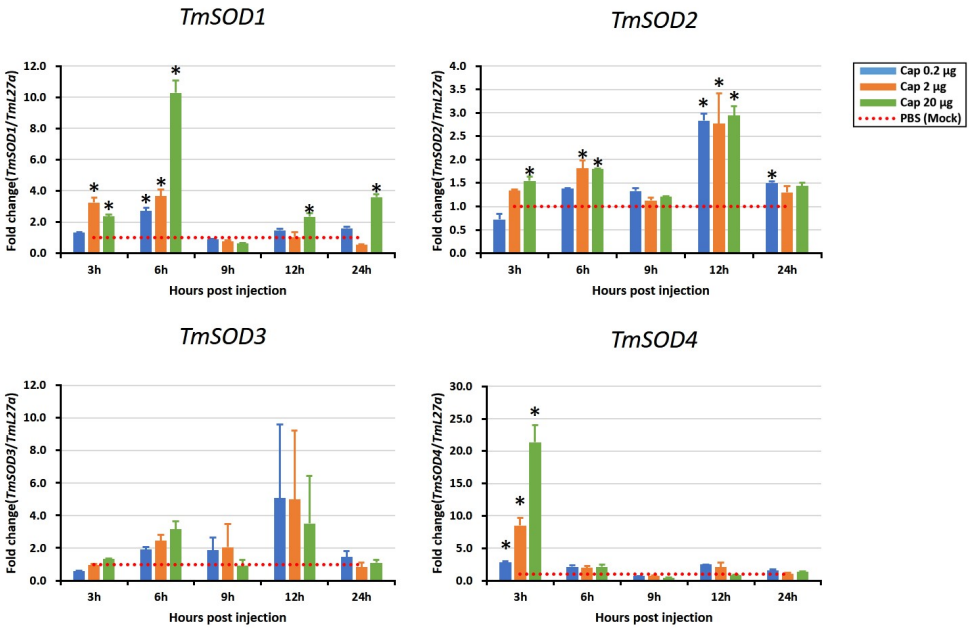

Fig. 4. Effect of captan (at 0.2, 2, and 20 μg/μL) on the relative mRNA expression of superoxide dismutases (SODs) in the whole body of *Tenebrio molitor*. Expression levels of *TmSODs* mRNA in the negative control group were normalized to 1. Data are shown as the mean ± standard error (three biological replicates). Significant differences between the experimental and control groups are indicated by asterisks (* $p < 0.05$).

peaked 12 h post-captan treatment at all concentrations. The expression of *TmSOD4* mRNA increased in dose-dependent manner at 3 h, but decreased with increased duration of exposure and was found to be the highest at 3 h (more than 20-fold increase).

Discussion

While using fungicides are considered to be benign to insects, number of fungicides can have harmful effects on insects. On the other hand, combination of these fungicides with other chemicals and antimicrobial drugs, apply on fields, can cause significant toxic outcome on insects (Johnson et al., 2013). The effects of fungicide treatment on non-target insects as well as fungicidal resistance mechanisms in insects can be attributed primarily to detoxification performance by qualitative or quantitative alteration in enzymes. In the present study, we investigated the expression of enzymes involved in the detoxification process in *T. molitor* larvae exposed to different concentrations of the fungicide captan. GSTs (*TmGST1-3*), POXs (*TmPOX1-5*), CATs (*TmCAT1-3*), and SODs (*TmSOD1-4*) were indentified from the *T. molitor* transcriptome database. Accordingly, we analyzed the mRNA expression patterns of GSTs, POXs, CATs, and SODs in *T. molitor* larvae following exposure to the oxidative stress induced by captan treatment. In previous studies, fungicidal treatment resulted in weaker expression of Hsp70 in third-instar *D. melanogaster* larvae, while no harmful effect was observed on brood development in *Apis mellitera* (Nazir et al., 2003; Everich et al., 2009). However, in another study, significant levels of captan were detected in the brood, worker bees, and honey samples posing a threat to honey consumers (Piechowicz et al., 2021). Furthermore, in other studies, the exposure of common pesticides, their mixtures, and a formulation solvent triggered high oral toxicity in honey bee larvae (Zhu et al., 2014). Pesticide contamination of *T. molitor* for human consumption has been reported with increased uptake rate of pesticides with higher K_{ow} values (concentration in octanol/concentration in water) (Houbraken et al., 2016).

Limited studies have investigated GST function in *T. molitor*. The expression of different isoenzymes of GST during developmental stages of *T. molitor* has been reported previously (Kostaropoulos et al., 1996). Other studies reported the detoxification function of GST following activation of the binding site and eventual conjugation with glutathione (Kostaropoulos

et al., 2001). In the present study, mRNA expression patterns of *TmGSTs* were confirmed following treatment with captan. We observed a concentration-dependent increase in expression of *TmGST1* and *TmGST3* mRNA 3 h post-treatment with captan. A previous study investigating the expression patterns of GST in *Leptinotarsa decemlineata* under the stress of three insecticides, such as cyhalothrin, fipronil, and endosulfan, reported differential expression of 20 candidate GST molecules (Han et al., 2016). Furthermore, consistent with our results, it was previously reported that the midgut detoxification enzyme expression (including GSTs) in *B. mori* increased 24 and 48 h post-exposure to low doses of acetamiprid (Wang et al., 2020). Our results indicate that GST mRNA expression increased rapidly in response to captan exposure.

Furthermore, *TmPOXs* mRNA were also rapidly affected in response to captan exposure, except for *TmPOX5* whose expression was the highest (nearly 30-fold increase) 24 h post-exposure. Other studies have shown POX expression in response to insecticidal stresses in different insect species. Both concentration and time-dependent responses were observed in the enzymatic activity of POX in *Sogatella furcifera* following exposure to multiple insecticides (Zhou et al., 2018). Similarly, the effects of bio-pesticides and chemical-pesticides on POX expression in whole bodies of beetle *C. maculatus* have also been reported (Kolawole et al., 2014).

The role of CATs as an antioxidant in insects is known. In *Tribolium castaneum*, survival during oxidative stress induced by insecticides and pathogens is thought to be related to CAT activity (Rauf and Wilkins, 2021) although the underlying mechanisms are yet to be clarified. *S. furcifera* and *C. maculatus* have been shown to express CAT enzymes against pesticides including thiamethoxam, and cypermethrin (Kolawole et al., 2014; Zhou et al., 2018). In the present study, *T. molitor* larvae also showed early mRNA expression of *TmCAT1* and *TmCAT3* (3 h post-captan treatment) and the late expression of *TmCAT2* mRNA (24 h post-captan treatment).

Moreover, expression levels of *TmSODs* mRNA after captan treatment were dose-dependent, with *TmSOD1* and *TmSOD4* showing the highest early response (6 and 3 h, respectively post-20 μg/μL captan treatment). This suggests that the expression of *TmSOD4* mRNA can be considered as an early-stage biomarker for captan exposure in *T. molitor* larvae. However, the relative mRNA expression of *TmSOD2* and *TmSOD3* showed a dose-independent increase, which peaked 12 h post-treatment of captan. In agreement with our results, the relative mRNA expression of *SOD4*, *5*, and *6* increased up to 4 folds in the fat body of *B. mori* after rotenone (insecticide) injection (Kobayashi et al., 2019).

Conclusions

To the best of our knowledge, this is the first study to report the mRNA expression patterns of detoxifying (oxidative stress counteracting) enzymes in *T. molitor* following exposure to different concentrations of the fungicide captan. We observed that higher concentration of captan (20 μg/μL) can elicit mRNA expression of some detoxifying enzymes, and a dose-dependent increase was also noticed in some cases. The expression of *TmPOX5* and *TmCAT2* mRNA was significantly higher 24 h post-captan injection, while *TmSOD4* expression was highest 3 h post-captan injection in *T. molitor* larvae.

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Statements for Authorship Position & Contribution

Jang, H.A: Chonnam national university, Post-doctoral researcher; Wrote the manuscript and conducted the experiments

Baek, H.S: Chonnam national university, BS student; Conducted the experiments

Kim, B.B: Chonnam national university, MS student; Conducted the experiments

Ali Mohammadie Kojour, M: Chonnam national university, PhD student; Wrote and edit the manuscript

Patnaik, B.B: Fakir Mohan university, Professor; Edit the manuscript

Jo, Y.H: Chonnam national university, Research Professor; Designed the research

Han, Y.S: Chonnam national university, Professor; Designed the research

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