# Tea saponin reduces the damage of looper caterpillar (*Ectropis obliqua*) to tea crops and exerts no significant harm to spiders (#21151)



First submission

## Editor guidance

Please submit by **18 Nov 2017** for the benefit of the authors (and your $200 publishing discount).

**Structure and Criteria**
Please read the 'Structure and Criteria' page for general guidance.

**Raw data check**
Review the raw data. Download from the materials page.

**Image check**
Check that figures and images have not been inappropriately manipulated.

Privacy reminder: If uploading an annotated PDF, remove identifiable information to remain anonymous.

**Files**
Download and review all files from the materials page.

4 Figure file(s)
4 Table file(s)

# Structure and Criteria

## Structure your review

The review form is divided into 5 sections.
Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

You can also annotate this PDF and upload it as part of your review

When ready submit online.

## Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your guidance page.

### BASIC REPORTING

- Clear, unambiguous, professional English language used throughout.
- Intro & background to show context. Literature well referenced & relevant.
- Structure conforms to PeerJ standards, discipline norm, or improved for clarity.
- Figures are relevant, high quality, well labelled & described.
- Raw data supplied (see PeerJ policy).

### EXPERIMENTAL DESIGN

- Original primary research within Scope of the journal.
- Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
- Rigorous investigation performed to a high technical & ethical standard.
- Methods described with sufficient detail & information to replicate.

### VALIDITY OF THE FINDINGS

- Impact and novelty not assessed. Negative/inconclusive results accepted. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
- Data is robust, statistically sound, & controlled.
- Conclusions are well stated, linked to original research question & limited to supporting results.
- Speculation is welcome, but should be identified as such.

# Standout reviewing tips



The best reviewers use these techniques

| Tip | *Example* |
| --- | --- |
| **Support criticisms with evidence from the text or from other sources** | *Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.* |
| **Give specific suggestions on how to improve the manuscript** | *Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).* |
| **Comment on language and grammar issues** | *The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult.* |
| **Organize by importance of the issues, and number your points** | *1. Your most important issue*<br>*2. The next most important item*<br>*3. …*<br>*4. The least important points* |
| **Please provide constructive criticism, and avoid personal opinions** | *I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC* |
| **Comment on strengths (as well as weaknesses) of the manuscript** | *I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.* |

# Tea saponin reduces the damage of looper caterpillar (*Ectropis obliqua*) to tea crops and exerts no significant harm to spiders

**Chi Zeng** [1] , **Lingbing Wu** [1] , **Yao Zhao** [1] , **Yueli Yue** [1] , **Yu Peng** [Corresp. 1]

[1] College of Life Science, Hubei University, Wuhan, Hubei, People's Republic of China

Corresponding Author: Yu Peng
Email address: pengyu@hubu.edu.cn

**Background.** Tea is one of the most economically important crops in China. However, the looper caterpillar (*Ectropis obliqua*), a serious leaf-feeding pest causes significant damage to tea crops, reduces tea yield and quality. This highlights the need for alternative pest control measures. Our previous studies have shown that tea saponin (TS) exerted insecticidal activity to lepidopteran pests. However, the insecticidal mechanism and the controlling efficacy in field of TS against pests were poorly known.

**Methods.** We investigated indoor bioactivities and field controlling role of TS solution against *E. obliqua*. (i) Leaf-dip bioassay method was used to evaluate the toxicity of TS to 3rd-instar larvae of *E. obliqua* and effects of TS on the activities of enzymes (glutathione-S-transferase (GST), acetylcholinesterase (AChE), carboxylesterase (CES) and peroxidase (POD) of 3rd-instar larvae of *E. obliqua* in the lab, (ii) topical application was used to compare the toxicity of 30% TS and insecticides to *E. tricuspidata* and *E. albaria*, and (iii) field trial was used to investigate the controlling efficacy of 30% (w/v) TS against the larvae of *E. obliqua* and classify the effect of TS on spiders in the tea garden.

**Results.** The toxicity of TS to 3rd-instar *E. obliqua* larvae was in a dose-dependent manner and $LC_{50}$ was 164.32 mg/mL. Activities of the detoxifying-related enzymes: GST and POD increased in 3rd-instar *E. obliqua* larvae, whereas AChE, CES wereinhibited through time as a result of treatment with TS. The mortality of *E. tricuspidata* and *E. albaria* after 48 h with 30% TS (16.67%, 20.00%) treatment were significant lower than Bi 10% EC (80.00%, 73.33%) and Di 50% SC (43.33%, 36.67%). The field trials indicated that 30% TS lacked acute controlling efficacy on the larvae of *E. obliqua*. The highest controlling efficacy of TS 30% WG was 77.02% at 5 d after treatment, which had no difference with bifenthrin 10% EC, diafenthiuron 50% SC. TS 30% WG was classified in the class N (harmless and slightly harmful) of IOBC categories for natural enemies, viz., spiders.

**Conclusions.** Our experimental results indicate that TS as a botanical insecticide, has a good controlling efficacy on *E. obliqua* larvae, which spurs it to be promising applications in the IPM envisaged for tea.

# Tea saponin reduces the damage of looper caterpillar (*Ectropis obliqua*) to tea crops and exerts no significant harm to spiders

Chi Zeng, Lingbing Wu, Yao Zhao, Yueli Yun, Yu Peng

Hubei Collaborative Innovation Center for Green Transformation of Bio-Resources, College of Life Sciences, Hubei University, Wuhan 430062, P. R. China

Corresponding author

E-mail address: pengyu@hubu.edu.cn

# Abstract

**Background.** Tea is one of the most economically important crops in China. However, the looper caterpillar (*Ectropis obliqua*), a serious leaf-feeding pest causes significant damage to tea crops, reduces tea yield and quality. This highlights the need for alternative pest control measures. Our previous studies had shown that tea saponin (TS) exerted insecticidal activity to lepidopteran pest. However, the insecticidal mechanism and the controlling efficacy in field of TS against pests were poorly known.

**Methods.** We investigated indoor bioactivities and field controlling role of TS solution against *E. obliqua*. (i) Leaf-dip bioassay method was used to evaluate the toxicity of TS to 3rd-instar larvae of *E. obliqua* and effects of TS on the activities of enzymes (glutathione-S-transferase (GST), acetylcholinesterase (AChE), carboxylesterase (CES) and peroxidase (POD) of 3rd-instar larvae of *E. obliqua* in the lab, (ii) topical application was used to measure the toxicity of 30% TS to two species of spiders, *Ebrechtella tricuspidata* and *Evarcha albaria*, and (iii) field trial was used to investigate the controlling efficacy of 30% (w/v) TS against the larvae of *E. obliqua* and classify the effect of TS on spiders in the tea garden.

**Results.** The toxicity of TS to 3rd-instar *E. obliqua* larvae was in a dose-dependent manner and $LC_{50}$ was 164.32 mg/mL. Activities of the detoxifying-related enzymes: GST and POD increased in 3rd-instar *E. obliqua* larvae, whereas AChE, CES wereinhibited through time as a result of treatment with TS. The mortality of *E. tricuspidata* and *E. albaria* after 48 h with 30% TS (16.67%, 20.00%) treatment were significant lower than Bi 10% EC (80.00%, 73.33%) and Di 50% SC (43.33%, 36.67%). The field trials indicated that 30% TS lacked acute controlling efficacy on the larvae of *E. obliqua*. The highest controlling efficacy of TS 30% WG was 77.02% at 5 d after treatment, which had no difference with bifenthrin 10% EC, diafenthiuron 50% SC. TS 30% WG was classified in the class N (harmless and slightly harmful) of IOBC categories for natural enemies, viz., spiders.

**Conclusions.** Our experimental results indicate that TS as a botanical insecticide, has a good controlling efficacy on *E. obliqua* larvae, which spurs it to be promising applications in the IPM envisaged for tea.

*Keywords: Tea saponin*, *E. obliqua*, *Toxicity*, *Enzyme activities*, *Controlling efficacy*

# Introduction

*Camellia sinensis* Kuntze (Theales: Theaceae) is one of the most economically important crops in China, cultivated in vast areas spreading over from north-south (37°N – 18°S) and east-west (122°E – 97°W), totaling more than 20 provinces across tropical, subtropical and temperate regions (Ye et al., 2014).

It was estimated that 808 species of insect and mite pests, belonging to 109 families from 13 orders of 2 classes were recorded in tea gardens in China (Zhang & Han, 1999). Among which, most are hemipterans (284 species) and lepidopterans (273 species), and only 6 insect species and 2 mite species have been confirmed as a major challenge to the China tea industry (Ye et al., 2014).

This small number of pests species often account for 10 – 20% yield loss, even total crop losses in some catastrophic cases (Chen & Chen, 1989).

*Ectropis obliqua* Prout (Lepidoptera: Geometridae) is a voracious leaf-feeding pest that severely reduces tea yield and quality in summer and autumn (Ye et al., 2014). The most effective measure for controlling this pest is the use of chemical pesticides (Harmatha et al., 1987; Hazarika, Puzari & Wahab, 2001; Xin et al., 2016). However, indiscriminate uses of chemicals in tea gardens have given rise to a large number of problems including resurgence of primary pests (Harmatha et al., 1987), resistance development (Gurusubramanian et al., 2008), undesirable residues on made tea (Feng et al., 2013) and environment contamination (Saha & Mukhopadhyay, 2013; Ye et al., 2014). Therefore, these problems have necessitated the study for alternative and effective biodegradable insecticides, which has greater acceptability (Roy, Mukhopadhyay & Gurusubramanian, 2010).

Compared with traditional chemical pesticides, botanical insecticides often exert favorable eco-toxicological properties, i.e. low human toxicity, rapid degradation and reduced environmental impact (Bourguet, Genissel & Raymond, 2000; Isman, 2006; Chermenskaya et al., 2010) and multiple bioactivities. They can act as repellents with unpleasant odors or irritants and growth regulators resulted from deterrence on oviposition, feeding and biocide activity (Isman, 2006; Chermenskaya et al., 2010; Martínez et al., 2015). These advantages spurred botanical insecticides to be an ideal candidate in pest management in an eco-friendly and economical way (Abou-Fakhr, Zournajian & Talhouk, 2001; Isman, 2006; Roy, Mukhopadhyay & Gurusubramanian, 2010; Martínez et al., 2015).

Tea saponin (TS), amphipathic glycoside, is extracted from the seed of plant species in *Camellia* of Theaceae that can enhance efficiency, solubilization and attenuated poison of pesticide as a wettable agent of powder pesticide (Chen, Zhang & Yang, 2012). Therefore, it has been widely used in the area of pesticides as the main component of environment-friendly pesticide additives (De Geyter, Geelen & Smagghe, 2007). TS has a potency to be used as a natural insecticide because it exerts a strong insecticidal activity against a broad range of insect types and stages (Potter et al., 2010; Cai et al., 2016) and no harm to the environment.

Previous studies reported that TS exerted negative effects on the biological activity of crop pests, acting as a feeding inhibitor (Nawrot et al., 1991), and toxicant on insect larvae (Harmatha et al., 1987). However, far few studies focus on the insecticidal mechanism and the controlling efficacy in the field of TS against pests. Our aims are to (1) evaluate the toxicity of TS to 3rd-instar larvae of *E. obliqua* and effects of TS on the activities of enzymes (GST, AChE, CES and POD) of 3rd-instar larvae of *E. obliqua* in the lab, and (2) investigate the controlling efficacy of 30% TS (w/v) against the larvae of *E. obliqua* in the tea garden. We hope to gain useful information to extend the application of TS.

# Material and Methods

## Test insects and spiders

Larvae of *E. obliqua* and two dominant species of spiders (*Ebrechtella tricuspidata* and *Evarcha albaria*) were originally collected from tea bushes in Wangdazhen Tea Garden (30°0′38.71″ N, 114°21′46.63″ E) at Xianning, Hubei Province, China during May to October

2014. Larvae of *E. obliqua* were fed on fresh tea leaves and reared for 5 generations in self-made plastic chambers (diameter × height = 10 cm × 10 cm) at 28 ± 1 ℃ and 75 ± 5% RH under a 14:10 LD photoperiod in Centre for Behavioral Ecology and Evolution (College of Life Sciences, Hubei University). A chamber was used for rearing 10 larvae, and 3rd-instar larvae of *E. obliqua* were used for following experiments.

Spiders were kept individually in glass tubes (diameter × length = 1.5 cm × 10 cm), which were closed with a plug of cotton and included a 1 cm bottom of moist sponge to maintain high humidity, in an illumination incubator (25 ± 1 ℃ and 75 ± 5% RH under 14:10 LD photoperiod). Wild-type fruit flies (*Drosophila melanogaster*) were provided twice a week as food. Adult spiders with similar size range were used for the toxicity tests.

## Toxicity assay of TS to the larvae of *E. obliqua*

The leaf-dip bioassay method described by Beloti et al. (2015) and Liang et al. (2003) was adopted for the toxicity assay of TS to 3rd-instar larvae of *E. obliqua*. A purity of 98% TS powder (purchased from Wuhan Bai Ming Technology Co., Ltd, Hubei province, China) was diluted to six concentrations (0, 18.75, 37.5, 75, 150 and 300 mg/mL) with distilled water. Tea leaf discs (4 cm in diameter) were dipped for 20 s in one of the 5 concentrations of TS solution. Then, the leaf discs were dried by placing them in a glass petri dishes (9 cm in diameter). Controlled leaf discs were dipped in distilled water as described above. Each leaf was placed inside a glass petri dishes (9 cm in diameter) in which 30 3rd-instar larvae of *E. obliqua* were confined. Third-instar *E. obliqua* larvae were fed only tea leaves treated with TS solution for 1 d, then they were fed fresh, non-contaminated tea leaves. Mortality was recorded at 48 h posttreatment. Three replicates were made for each concentration. Larvae were recorded as dead if they did not move when probed with a hair brush. Surviving larvae were used for the following enzyme activity assays.

## The activities assays of enzymes

We randomly took 3 survival 3rd-instar larvae of *E. obliqua* per concentration of TS solution with 4 replicates. GST, CES, AChE and POD activities were monitored using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu China) according to the manufacturer's instructions. GST, CES, AChE and POD activities were measured in units of U/mg. Sample protein concentrations were estimated by using the method described by Bradford (Bradford, 1976). Bovine serum albumin was used for the calibration curve. Measurements were performed at 595 nm using a microplate reader with SoftMax Pro 6.3 software (Molecular Devices Corporation, Sunnyvale, CA, USA).

## Toxicity assay of insecticides to spiders

For the insecticide treatment, 98% TS powder, bifenthrin (Bi) 10% EC (Jiangsu Dongbao Chemical Corporation Ltd., Jiangsu, China) and diafenthiuron (Di) 50% SC (same corporation as Bi 10% EC) diluted with distilled water to dose of 300, 0.01 and 0.05 mg/mL, respectively. Prior to insecticide treatment, spiders were individually anaesthetized with carbon dioxide. Toxicity assay was operated according to Deng et al. (2006). Two droplets (0.5 μL each) of insecticide solution were applied to the dorsal abdomen of spiders using a 5-μL microsyringe, and distilled water as control was employed. To reduce possible variation in response to treatments caused by differences in sex, spiders were randomly selected. After insecticide application, spiders were kept in petri dishes with one or two pieces of moist sponge to maintain humidity. Percentage spider mortality was recorded after 48 h.

## Comparative the controlling efficacy of TS 30% WG and chemical insecticides against the larvae of *E. obliqua* in tea garden

Field trials was conducted in Wangdazhen Tea Garden (30°0′38.71″ N, 114°21′46.63″ E) at Xianning, Hubei Province, China in dry days during June to July 2015 to evaluate the controlling efficacy of TS 30% WG along with Bi 10% EC (at recommended dose of 7.5 g a.i. $ha^{-1}$), Di 50% SC (at recommended dose of 45 g a.i. $ha^{-1}$) against *E. obliqua.* An untreated control (clean water) was simultaneously maintained during the study. It is following a randomized block design (Roy, Mukhopadhyay & Gurusubramanian, 2010; Kawada et al., 2014) with 3 treatments and replicated thrice. Each plot (20 $m^2$) with no difference was separated by two buffer rows of non-treatment tea bushes. Two rounds of foliar spray were applied by using a 16 L capacity knapsack sprayer equipped with a hallow cone nozzle (droplet diameter in 1.2 mm, the distance between nozzle and tea leaves was 30 – 40 cm) at 750 L $ha^{-1}$. Moreover, a pretreatment count was taken in the respective plots at random five tea bushes. After spraying, posttreatment count was made at 1, 3, 5, 7 d in each plot of the treatment during PM 4:00 – 5:00 (Beijing time) in the respective plots at random five tea bushes. For investigating the safety of TS 30% WG to natural enemies, spiders, the number of spiders was counted before and 7 days after insecticides application at five tagged plants in each plot. The mean populations of spiders were calculated. According to the IOBC (International Organization of Biological Control) classes of toxicity, the insecticides tested under the field conditions were classified as N, harmless or slightly harmful (0–50% reduction); M, moderately harmful (51–75% reduction), and T, harmful (75% reduction), respectively (Boller et al., 2005).

Mean population reduction of pest per treatment was calculated using the following formula:

Population reduction (PR) = [(Pre-treatment count – Posttreatment count) / Pretreatment population count] × 100%

Controlling efficacy (CE) = [(PR of reagents treatment – PR of clean water treatment) / (1 – PR of clean water treatment)] × 100%

## Statistics analysis

The $LC_{50}$ and their fiducial limits were determined by logistic regression based on the concentration probit-mortality (Finney, 1971). Mortality variables were summarized in percentages and the data transformed to arcsine square root. The differences in mortality of larvae and adult spiders, controlling efficacy and number of spiders were compared by using the least-significant difference (LSD) test at the 5% level of significance. The differences in enzymes activities of larvae were compared by using the unpaired Student's t-test at the 5% level of significance. Statistical analyzes were performed with SPSS 20.0 (IBM Corp Version 20.0. IBM SPSS Statistics for Windows. Armonk, NY, USA) and Prism 5 (GraphPad Software, La Jolla, CA, USA) software.

# Results

## The toxicity of TS solution to 3rd-instar larvae of *E. obliqua*

Mortality of 3rd-instar *E. obliqua* larvae were directly proportional to the TS concentrations with values of 13.33%, 27.78%, 30.0%, 43.33% and 66.67% with the five concentrations at 48 h (Table 1). The $LC_{50}$ value of TS solution to the 3rd-instar larvae of *E. obliqua* was 164.32 mg/mL.

## Effects of 30% (w/v) TS on enzyme activities in 3rd-instar larvae of *E. obliqua*

The activities of GST in 3rd-instar larvae of *E. obliqua* after treated by 30% TS showed that GST activities were highly significant increased at 6 h (t-test, t = 24.84, df = 6, $P < 0.001$), 12 h (t-test, t = 35.89, df = 6, $P < 0.001$) and 24 h (t-test, t = 25.01, df = 6, $P < 0.001$), then reduced in the later period (Fig. 1). There was no significant (t-test, t = - 2.18, df = 6, $P = 0.072$) between 30% TS treatment and distilled water treatment at 48 h. The activities of GST was highly lower (t-test, t = - 12.07, df = 6, $P < 0.001$) than distilled water treatment at 96 h.

The 30% TS solution significantly inhibited (t-test, $P < 0.001$) the activities of CES in 3rd-instar larvae of *E. obliqua* (Fig. 2), and the activities of CES maintain at a low-level over the experiment period

As shown in Fig. 3, the activities of AChE in 3rd-instar larvae of *E. obliqua* were highly significant inhibited (t-test, $P < 0.001$) by 30% TS during the whole experiment time.

After treated by 30% TS, the activities of POD in 3rd-instar larvae of *E. obliqua* were significant increased (t-test, $P < 0.01$) in the whole experiment time except 48 h, which had no significant (t-test, t = 0.363, df = 6, $P = 0.729$) with control (Fig. 4).

## Toxicity assay of insecticides to spiders

Because no individuals died in the control test within 48 h of distilled water treatment, no adjustment for control mortality was necessary. The results of toxicity assay were shown in Table 2. The mortality of *E. tricuspidata* adults with 300 mg/mL TS solution was significant lower than Bi 10% EC ($F_{1,4} = 23.63$, $P < 0.01$) and Di 50% SC ($F_{1,4} = 62.74$, $P < 0.01$). The mortalities of *E. albaria* adults were similar to *E. tricuspidata*, which was significant lower than Bi 10% EC ($F_{1,4} = 23.49$, $P = 0.01$) and Di 50% SC ($F_{1,4} = 46.83$, $P < 0.01$).

## Comparative the controlling efficacy of TS 30% WG and chemical insecticides against the larvae of *E. obliqua* in the tea garden

The controlling efficacy of TS 30% WG, Bi 10% EC and Di 50% SC against the larvae *E. obliqua* under field conditions were shown in Table 3. CR was highly significant lower ($P < 0.01$) on plots sprayed with 30% TS than Bi 10% EC and Di 50% SC during the first 3 d period posttreatment. Further, the CR of TS 30% WG was equivalent with chemical pesticides in 5 d ($P = 0.72$) and 7d ($P = 0.61$), respectively.

After all, we investigated the number of spiders in different trial plots (Table 4). The number of spiders in the plots treated by TS 30% WG were higher than Bi 10% EC ($F_{1,4} = 18.00$, $P = 0.013$)

and Di 50% SC ($F_{1,4}$ = 16.00, $P$ = 0.016), respectively. Both the treatments of clean water and TS 30% WG were classified in the class N (harmless or slightly harmful) of IOBC categories for spiders, whereas, Bi 10% EC and Di 50% SC treatments were classified in the class M (moderately harmful).

# Discussion

Control of the larvae of *E. obliqua* is mainly achieved by using synthetic chemical insecticides; however, these insecticides are extremely toxic to non-target organisms and the environment (Potter et al., 2010). In this study, we investigated the toxicity and controlling efficacy of 30% TS against *E. obliqua* larvae in the field in order to evaluate its use as a new and natural insecticide.

In our experiment, 30% TS showed insecticidal activities, causing mortality (66.67%) in 3rd-instar larvae of *E. obliqua* with a dose-dependent manner. Our result was similar to De Geyter et al. (2007), who found that *Quillaja* bark saponins showed high mortality (≥ 70%) on pea aphids (*Spodoptera littoralis*) and cotton leafworm caterpillars (*Acyrthosiphon pisum*). Chen et al. (1996) demonstrated that 25% active ingredient of TS-D solution significantly increased larval mortality (84%) in the cabbage butterfly (*Pieris rapae*). A similar result was demonstrated by Bandeira et al. (2013), they reported that ethanolic extracts of the flowers and fruits of *Muntingia calabura* were toxic to diamondback moth (*Plutella xylostella*) larvae.

Our findings also indicated that 30% TS exerted highly effects on the activities of detoxifying enzymes. Insect resistance is determined by the activities of detoxifying enzymes and decreased target sensitivity to chemical pesticides (Felton & Summers, 1995; Potter et al., 2010). GST, CES and POD are commonly involved in defense mechanisms (Lumjuan, 2005). GST plays a key role in detoxifying and cellular antioxidant defenses against natural and synthetic exogenous xenobiotics (Terriere, 1984; Serebrov et al., 2006; Li, Schuler & Berenbaum, 2007). CES perform important functions in detoxification of insects by the metabolism and degradation of various xenobiotics (Singh & Singh, 2000; Gopalakrishnan et al., 2011). POD is the key antioxidant enzyme that can be quickly up-regulated in response to natural penetrating xenobiotics (Wu et al., 2011), and the increases of POD activities are related to pesticide resistance and melanization in insects (Terriere, 1984; Potter et al., 2010). AChE is a key enzyme in the nervous system of various organisms, which can terminate nerve impulses by catalyzing the hydrolysis of the neurotransmitter acetylcholine (Wang et al., 2004; Senthil et al., 2008). Moreover, the herbivore insects are adapted to host secondary substances through physiological changes (Karban & Agrawa, 2002). Therefore, the multiple metabolic enzyme systems of plant secondary substances and chemical pesticides are usually considered to be identical or similar ( Brattsten, 1988; Snyder & Glendining, 1996). Rizwan-ul-Haq et al. (2009) evaluated the bioactivities of TS solution on *Spodoptera exigua* (Lepidoptera: Noctuidae). The authors provided some helpful information about activities of antioxidant enzymes against TS, which involved in the resistance mechanism of insects. In our study, 30% TS exerted highly significant promoted the activities of GST and POD in 3rd-instar *E. obliqua* larvae, whereas the activities of AChE and CES were inhibited, our results suggested that GST, CES, AChE and POD appear to participate in the defensive reaction of *E. obliqua* to the treatments with 30% TS during the different experimental time. Our results may provide a scientific basis for TS solution on 3rd-instar larvae of *E. obliqua*. However, the metabolic and defensive reactions in 3rd-instar larvae of *E. obliqua* were probably attributed to complex modes of action involving multiple mechanisms. We were unable to determine the definite role of detoxification enzymes in TS ingestion. Therefore, further studies were needed for characterization of active compounds from TS that possess complex modes of action.

As predators of the larvae of *E. obliqua*, *E. tricuspidata* and *E. albaria* are the important non-target, beneficial species affected by insecticides. Susceptibility of these species to the 30% TS, Bi 10% EC and Di 50% SC were assessed in the present study. Both of *E. tricuspidata* and *E. albaria* adults exerted significant lower mortalities after 48 h of 30% TS treatment compared with Bi 10% EC and Di 50% SC (Table 2). The similar result was reported by Peng *et al.* (2017), which shown that 30% TS exerted significant lower repellent rate to spiders compared with chemical insecticides. These results indicated that 30% TS is harmless to the spiders in lab condition.

The effectiveness of the TS 30% WG and two kinds of chemical insecticides against *E. obliqua* larvae in the field was investigated in this study. As a botanical production, the controlling efficacy of TS 30% WG is little exceeded to the Bi 10% EC, Di 50% SC at 5 d and 7 d, although there were no significant difference between them (Table 2). The previous study proposed that natural enemies should be the first consideration in any pest management intervention (Koul & Dhaliwal, 2003). Any integrated approach to pest management must be compatible with natural enemy conservation (Amoabeng et al, 2013). Yang *et al.* (2017) employed comprehensive indices for evaluating the predation of *E. obliqua* by nine common spider species in Chinese tea plantations. Though after 7 d of reagents application, the pooled mean population of spiders of the clean water treatment was significantly higher than other treatments (Table 4), our results indicated that TS 30% WG was classified in the class N (harmless and slightly harmful) of IOBC categories for natural enemies, viz., spiders. Thus, the distribution of spiders can indicate the potential usefulness of TS 30% WG in integrated pest management (IPM) of the tea garden. In addition, the procedure for preparation of TS 30% WG is simply method by using only water, and the TS production is cheap and readily available. This finding could be a point of view of controlling *E. obliqua* larvae without the use of chemical insecticides. This approach would help the tea industry in many ways (residues free on made tea, reduction in pesticide load, cost effectiveness and customer satisfaction) (Roy, Mukhopadhyay & Gurusubramanian, 2010) and remain important in controlling the larvae of *E. obliqua*.

# Conclusion

In conclusion, our results indicated that 30% TS has significant potential as a new alternative biocontrol insecticide against the larvae of *E. obliqua* and exerts no significant harm to the natural enemies viz. spiders in field applications. As the chosen native production that is abundant in tea plantations, thus, 30% TS could be effectively utilized in the IPM envisaged for tea.

# References

Abou-Fakhr H, Zournajian EMH, Talhouk S. 2001. Efficacy of extracts of *Melia azedarach* L. callus, leaves and fruits against adults of the sweetpotato whitefly *Bemisia tabaci* (Hom., Aleyrodidae). *Journal of Applied Entomology* 125(8):483–488. DOI: 10.1046/j.1439-0418.2001.00577.x.

Amoabeng BW, Gurr GM, Gitau CW, Nicol HI, Munyakazi L, Stevenson PC. 2013. Tri-Trophic Insecticidal Effects of African Plants against Cabbage Pests. PLoS One 8(10):e78651. DOI: 10.1371/journal.pone.0078651.

Beloti VH, Alves GR, Araújo DFD, Picoli MM, Moral RA, Demétrio CGB, Yamamoto PT. 2015. Lethal and sublethal effects of insecticides used on Critus, on the Ectoparasitoid *Tamarixia radiata*. *PLoS One* 10(7):e0132128. DOI: 10.1371/journal.pone.0132128.

Boller EF, Vogt H, Ternes P, Malavolta C. 2005. Working document on selectivity of pesticides (2005). IOBCwprs: Commission on IP Guidelines. Available at http://www.iobc-wprs.org/ip_ipm/03021_IOBC_WorkingDocumentPesticides_Explanations.pdf (accessed on 4 October 2017).

Bourguet D, Genissel A, Raymond M. 2000. Insecticide resistance and dominance levels. *Journal of Economic Entomology* 93(6):1588–1595. DOI: 10.1603/0022-0493-93.6.1588.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72:248–254. DOI: 10.1016/0003-2697(76)90527-3.

Brattsten LB. 1988. Potential role of plant allelochemicals in the development of insecticide resistance. Bell System Technical Journal 6(2):187–216.

Cai H, Bai Y, Wei H, Lin S, Chen YX, Tian HJ, Gu XJ, Murugan K. 2016. Effects of tea saponin on growth and development, nutritional indicators, and hormone titers in diamondback moths feeding on different host plant species. *Pesticide Biochemistry and Physiology* 131:53–59. DOI: 10.1016/j.pestbp.2015.12.010.

Chen J, Zhang S, Yang X. 2012. Control of brown rot on nectarines by tea polyphenol combined with tea saponin. *Crop Protection* 45(3):29–35. DOI: 10.1016/j.cropro.2012.11.006.

Chen SR, Li GT, Lai JH, Li X, Zhang YL. 1996. Study of Tea saponin TS-D insecticidal effects on cabbage butterfly (in Chinese with English abstract). *Plant Protection* 22(3):27–28.

Chen ZM, Chen XF. 1989. An analysis on the world tea pest fauna (in Chinese with English abstract). *Journal of Tea Science* 9(1):13–22. DOI:10.13305/j.cnki.jts.1989.01.003.

Chermenskaya TD, Stepanycheva EA, Shchenikova AV, Chakaeva AS. 2010. Insectoacaricidal and deterrent activities of extracts of Kyrgyzstan plants against three agricultural pests. *Industrial Crops and Products* 32(2):157–163. DOI:10.1016/j/indcrop.2010.04.009.

De Geyter E, Geelen D, Smagghe G. 2007. First results on the insecticidal action of saponins. *Comumunications in Agricultural & Applied Biological Science* 72(3):645–648.

De Geyter E, Lambert E, Geelen D, Smagghe G. 2007. Novel advances with plant saponins as natural insecticides to control pest insects. *Pest Technology* 1(2):96–105.

Deng LL, Dai JY, Cao H, Xu MQ. 2006. Effects of an organophosphorous insecticide o survival, fecundity and develepment of *Hylyphantes Graminicola* (Sundevall) (Araneae: Linypgiidae). *Enviromental Toxicology and Chemistry* 25(11):3073–3077. DOI: 10.1897/06-194R.1.

Felton GW, Summers CB. 1995. Antioxidant Systems in insects. *Archives of Insect Biochemistry and Physiology* 29(2):187–197. DOI: 10.1002/arch.940290208.

Feng J, Tang H, Chen DZ, Li L. 2013. *Monitoring and risk assessment of pesticide residues in tea samples from China. Human and Ecological Risk Assessment 21(1):169*–183. DOI: 10.1080/10807039.2014.894443.

Finney DJ. 1971. *Probit Analysis: 3rd ed*. Cambridge: Cambridge University Press.

Gopalakrishnan S, Chen FY, Thilagam H, Qiao K, Xu WF, Wang KJ. 2011. Modulation and interaction of immune-associated parameters with antioxidant in the immunocytes of crab Scylla paramamosain challenged with lipopolysaccharides. *Evidence-based Complementary and Alternative Medicine* (1741-427X):824962. DOI: 10.1155/2011/824962.

Gurusubramanian G, Rahman A, Sarmah M, Roy S, Bora S. 2008. Pesticide usage pattern in tea ecosystem, their retrospects and alternative measures. *Journal of Environmental Biology* 29(6):813–*826.*

Harmatha J, Mauchamp B, Arnault C, Sláma K. 1987. Identification of a spirostane-type saponin in the flowers of leek with inhibitory effects on growth of leek-moth larvae. *Biochemical Systematics and Ecology* 15(1):113–116. DOI: 10.1016/03051978(87)90089-5.

Hazarika LK, Puzari KC, Wahab S. 2001. *Biological Control of Tea Pests.* New York: Plenum Press.

Isman MB. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entomology* 51(1):45–66. DOI: 10.1146/annurev.ento.51.110104.151146.

Karban R, Agrawal AA. 2002. Herbivore offense. *Annual Review of Ecology and Systematics* 33(2):641–664. DOI: 10.1146/annurev.ecolsys.33.010802.150443.

Kawada H, Dida GO, Ohashi K, Kawashima E, Sonye G, Njenga SM, Mwandawiro C, Minakawa N. 2014. A small-scale field trial of pyriproxyfen-impregnated bed nets against pyrethroid-resistant Anopheles gambiae s.s. in western Kenya. *PLoS One* 9(10):e111195. DOI:

10.1371/journal.pone.0111195.

Koul O, Dhaliwal G. 2003. *Predators and Parasitoids.* New York: Taylor & Francis Press.

Liang P, Gao XW, Zheng BZ. 2003. Genetic basis of resistance and studies on cross-resistance in a population of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). *Pest Management Science* 59(11):1232–1236. DOI: 10.1002/ps.760.

Li X, Schuler MA, Berenbaum MR. 2007. Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. *Annual Review of Entomology* 52(1):231–253. DOI: 10.1146/annurev.ento.51.110104.151104.

Lumjuan N, McCarroll L, Prapanthadara L, Hemingway J, Ranson H. 2005. Elevated activity of an Epsilon class glutathione transferase confers DDT resistance in the dengue vector, *Aedes aegypti*. *Insect Biochemistry and Molecular Biology* 35(8):861–871. DOI: 10.1016/j.ibmb.2005.03.008.

Martínez LC, Plata-Rueda A, Zanuncio JC, Serrão JE. 2015. Bioactivity of six plant extracts on adults of *Demotispa neivai* (Coleoptera: Chrysomelidae). *Journal of Insect Science* 15(34):2015. DOI: 10.1093/jisesa/iev021.

Nawrot J, Koul O, Isman MB, Harmatha J. 1991. Naturally occurring antifeedants: effects on two polyphagous lepidopterans. *Journal of Applied Entomology* 112(1-5):194–201. DOI: 10.1111/j.1439-0418.1991.tb01046.x.

Neto Bandeira G, Camara CAG, Moraes MM, Barros R, Muhammad S, Akhtar Y. 2013. Insecticidal activity of *Muntingia calabura*extracts against larvae and pupae of diamondback, *Plutella xylostella* (Lepidoptera, Plutellidae). *Journal of King Saud University*

– *Science* 25(1):83–89. DOI: 10.1016/j.jksus.2012.08.002.

Peng JT, Peng Y, Zeng C. 2017. Determination of the bioactivities of tea saponin solution to 3rd-instar larvae of *Ectropis obliqua* and two species of spider (in Chinese with English abstract). *Acta Arachnologica Sinica* 26(2):21–25.

Potter DA, Redmond CT, Meepagala KM, Williams DW. 2010. Managing earthworm casts (Oligochaeta: Lumbricidae) in turfgrass using a natural byproduct of tea oil (*Camellia* sp.) manufacture. *Pest Management Science* 66(4):439–446. DOI: 10.1002/ps.1896.

Rizwan-ul-Haq M, Hu QB, Hu MY, Zhong G, Weng Q. 2009. Study of destruxin B and tea saponin, their interaction and synergism activities with *Bacillus thuringiensis kurstaki* against *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). *Applied Entomology and Zoology* 44(3):419–428. DOI: 10.1303/aez.2009.419.

Roy S, Mukhopadhyay A, Gurusubramanian G. 2010. Field efficacy of a biopesticide prepared from *Clerodendrum viscosum* Vent. (Verbenaceae) against two major tea pests in the sub Himalayan tea plantation of North Bengal, India. *Journal of Pest Science* 83(4):371–377. DOI: 10.1007/s10340-010-0306-5.

Saha D, Mukhopadhyay A. 2013. Insecticide resistance mechanisms in three sucking insect pests of tea in reference to North-East India; an appraisal. *International Journal of Tropical Insect Science* 33(1):46–70. DOI: 10.1017/S1742758412000380.

Senthil NS, Young CM, Yul SH, Hoon PC, Kalaivani K, Duk KJ. 2008. Effect of azadirachtin on acetylcholinesterase (AChE) activity and histology of the brown planthopper Nilaparvata lugens (Stål). *Ecotoxicology and Environmental Safety* 70(2):244–250. DOI:

10.1016/j.ecoenv.2007.07.005.

Serebrov VV, Gerber ON, Malyarchuk AA, Martemyanov VV, Alekseev AA, Glupov VV. 2006. Effect of entomopathogenic fungi on detoxification enzyme activity in greater wax moth *Galleria mellonella* L. (Lepidoptera, Pyralidae) and role of detoxification enzymes in development of insect resistance to entomopathogenic fungi. *Biology Bulletin* 33(6):581–586. DOI: 10.1134/S1062359006060082.

Singh K, Singh DK. 2000. Toxicity to the snail *Limnaea acuminata* of plant-derived molluscicides in combination with synergists. *Pest Management Science* 56(10):889–898. DOI: 10.1002/1526-4998(200010)56:10<889::AID-PS221>3.0.CO;2-0.

Snyder MJ, Glendining JI. 1996. Causal connection between detoxification enzyme activity and consumption of a toxic plant compound. *Journal of Comparative Physiology A* 179(2):255–261.

Terriere LC. 1984. Induction of detoxication enzymes in insects. *Annual Review of Entomology* 29:771–788. DOI: 10.1146/annurev.en.29.010184.000443.

Wang JJ, Cheng WX, Ding W, Zhao ZM. 2004. The effect of the insecticide dichlorvos on esterase activity extracted from the psocids, *Liposcelis bostrvchophila* and *L. entomophila*. *Journal of Insect Science* 4(1):23–27.

Wu HH, Liu JY, Zhang R, Zhang JZ, Gao YP, Ma EB. 2011. Biochemical effects of acute phoxim administration on antioxidant system and acetylcholinesterase in *Oxya chinensis* (Tunberg) (Orthoptera: Acrididae). *Pesticide Biochemistry and Physiology* 100(1):23–26. DOI: 10.1016/j.pestbp.2011.01.011.

Xin ZJ, Li XW, Li JC, C ZM, Sun XL. 2016. Application of chemical elicitor (*Z*)-3-hexenol enhances direct and indirect plant defenses against tea geometrid *Ectropis obliqua*. *Biocontrol* 61(1):1–12. DOI: 10.1007/s10526-015-9692-1.

Yang T, Liu J, Yuan L, Zhang Y, Peng Y, Li D, Chen J. 2017. Main predators of insect pests: screening and evaluation through comprehensive indices. *Pest Management Science* 73(11):2302–2309. DOI: 10.1002/ps.4613.

Ye GY, Xiao Q, Chen M, Chen XX, Yuan ZJ, Stanly DW, Hu C. 2014. Tea: Biological control of insect and mite pests in China. *Biological Control* 68(1):73–91. DOI: 10.1016/j.biocontrol.2013.06.013.

Zhang HG, Han BY. 1999. The analysis on the fauna of tea insect pests in China and their regional occurrence (in Chinese with English abstract). *Journal of Tea Science* 19(2):81–86. DOI: 1000-369X(1990)02-0081-06.

## Table 1(on next page)

Toxicity of TS solution to 3rd-instar *Ectropis obliqua* larvae.

TS, tea saponin; $LC_{50}$, Lethal concentration 50, the concentrations causing 50% mortality; FL, fiducial limits (mg/mL); SE, standard errors of the means. Mortalities (% ± SE) followed by the same letters represented no significant difference (Least-significant difference test at the 5% level of significance).

**Toxicity of TS solution to 3rd-instar *Ectropis obliqua* larvae.**

| Concentration of TS (mg/mL) | Mortality of larvae (% ± SE) | LC-P line | $LC_{50}$ | 95% FL (mg/mL) | $r^2$ |
|---|---|---|---|---|---|
| 300 | 66.67 ± 3.85a | y=4.18x – 4.27 | 164.32 | 126.62 – 233.27 | 0.898 |
| 150 | 43.33 ± 3.85b | | | | |
| 75 | 30.0 ± 1.92c | | | | |
| 37.5 | 27.78 ± 1.11c | | | | |
| 18.5 | 13.33 ± 1.93d | | | | |
| 0 | 1.11 ± 1.11e | | | | |

TS, tea saponin; $LC_{50}$, Lethal concentration 50, the concentrations causing 50% mortality; FL, fiducial limits (mg/mL); SE, standard errors of the means.

Mortalities (% ± SE) followed by the same letters represented no significant difference (Least-significant difference test at the 5% level of significance).

## Table 2 (on next page)

Mortality of *Ebrechtella tricuspidata* and *Evarcha albaria* adults after 48 h of treatment caused by different reagents.

Bi, bifenthrin EC; Di, diafenthiuron SC; TS, tea saponin. SE, standard errors of the means. Mortality (% ± SE) followed by the same letters represented no significant difference (Least-significant difference test at the 5% level of significance).

**Mortality of *Ebrechtella tricuspidata* and *Evarcha albaria* adults after 48 h of treatment caused by different reagents.**

| Treatment | Concentration (mg/mL) | Mortality (Mean ± SE) (%) | |
|---|---|---|---|
| | | *E. tricuspidata* | *E. albaria* |
| Bi 10% EC | 0.01 | 80.00 ± 5.77a | 73.33 ± 3.33a |
| Di 50% SC | 1.2 | 43.33 ± 3.33b | 36.67 ± 6.67b |
| TS | 300 | 16.67 ± 3.33c | 20.00 ± 5.77c |

Bi, bifenthrin EC; Di, diafenthiuron SC; TS, tea saponin. SE, standard errors of the means.

Mortality (% ± SE) followed by the same letters represented no significant difference (Least-significant difference test at the 5% level of significance).

## Table 3(on next page)

The controlling efficacy of TS 30% WG and chemical insecticides against the larvae of *Ectropis obliqua*.

Bi, bifenthrin; Di, diafenthiuron; TS, tea saponin. SE, standard errors of the means. Controlling efficacy (% ± SE) followed by the same letters represented no significant difference (Least-significant difference test at the 5% level of significance).

**The controlling efficacy of TS 30% WG and chemical insecticides against the larvae of *Ectropis obliqua*.**

| Treatment | Dose | Controlling efficacy (CE) (Mean ± SE) (%) | | | |
|---|---|---|---|---|---|
| | (g a.i. $ha^{-1}$) | 1 d | 3 d | 5 d | 7 d |
| Bi 10% EC | 7.5 | 71.23 ± 8.77a | 85.87 ± 4.07a | 60.12 ± 4.56a | 49.65 ± 3.04a |
| Di 50% SC | 45 | 56.53 ± 3.30a | 83.35 ± 4.39a | 61.32 ± 5.24a | 52.45 ± 3.72a |
| TS 30% WG | 562.5 | 15.93 ± 2.58b | 52.19 ± 3.37b | 77.02 ± 3.93a | 58.87 ± 4.44a |

Bi, bifenthrin; Di, diafenthiuron; TS, tea saponin. SE, standard errors of the means.

Controlling efficacy (% ± SE) followed by the same letters represented no significant difference (Least-significant difference test at the 5% level of significance).

## Table 4(on next page)

The toxicity classes of different reagents on spiders in the treatment plots.

Bi, bifenthrin; Di, diafenthiuron; TS, tea saponin; Control, water spray. SE, standard errors of the means. PTC: pre-treatment count; PR: population reduction = [(PTC – 7 d count) / PTC] × 100%; TC, toxicity classes (N, harmless or slightly harmful at the 0 – 50% level of PR; M, moderately harmful at the 51% – 75% level of PR; T, harmful at over the 75% level of PR). In column, the same letters represented no significant difference (Least-significant difference test at the 5% level of significance).

**The toxicity classes of different reagents on spiders in the treatment plots.**

| Treatment | Number of spiders (Mean ± SE) | | PR (%) | TC |
|---|---|---|---|---|
| | PTC | 7 d | | |
| Bi 10% EC | 6.33 ± 0.33a | 2.67 ± 0.33cd | 57.94 ± 4.82 | M |
| Di 50% SC | 5.67 ± 0.67a | 2.00 ± 0.58d | 66.27 ± 5.16 | M |
| TS 30% WG | 6.67 ± 0.33a | 4.67 ± 0.33b | 29.17 ± 6.25 | N |
| Control | 6.00 ± 0.57a | 6.67 ± 0.67a | - 11.42 ± 5.95 | N |

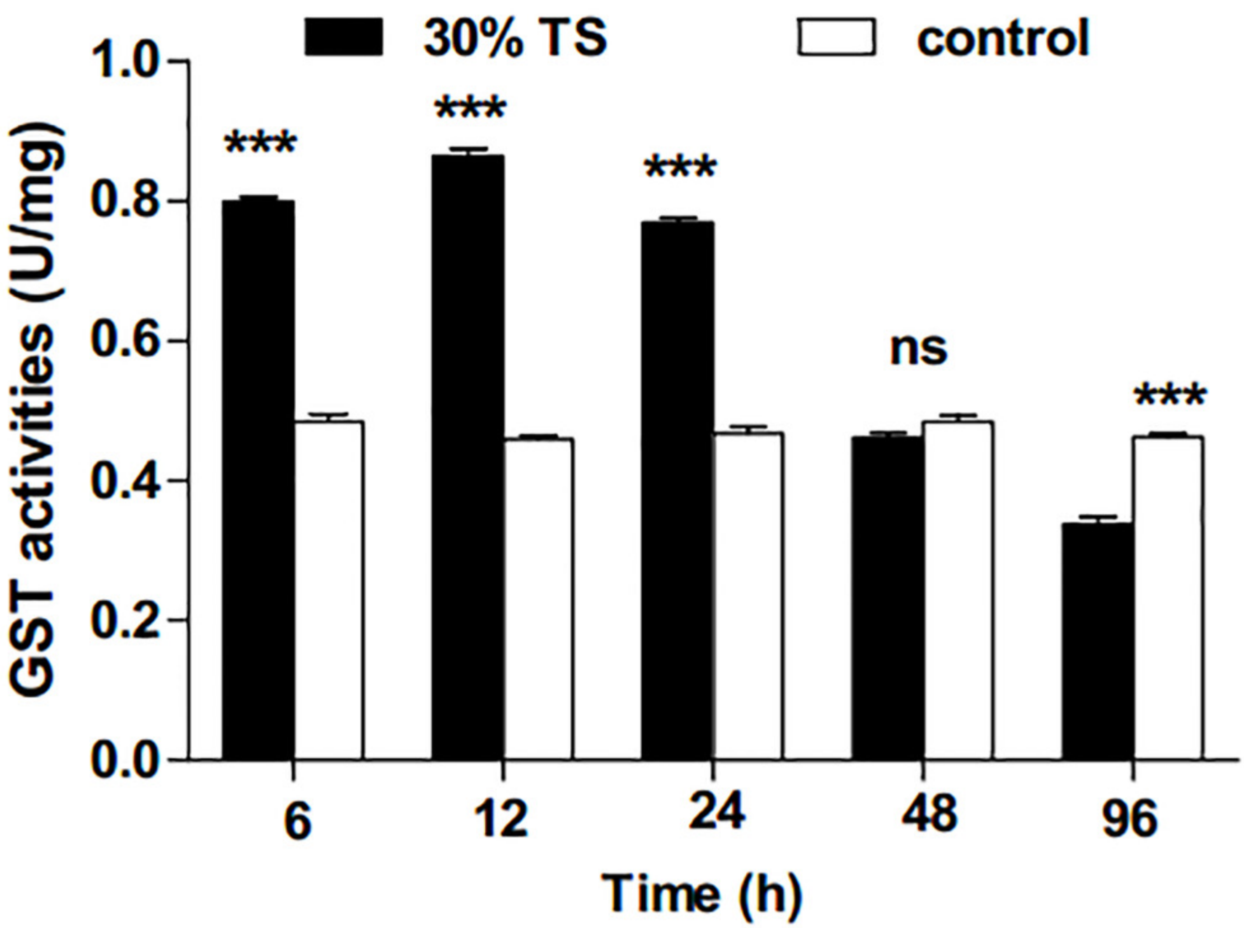
Bi, bifenthrin; Di, diafenthiuron; TS, tea saponin; Control, water spray. SE, standard errors of the means. PTC: pre-treatment count; PR: population reduction = [(PTC – 7 d count) / PTC] × 100%; TC, toxicity classes (N, harmless or slightly harmful at the 0 – 50% level of PR; M, moderately harmful at the 51% – 75% level of PR; T, harmful at over the 75% level of PR).

In column, the same letters represented no significant difference (Least-significant difference test at the 5% level of significance).

# Figure 1

Effects of 30% (w/v) TS on GST activities in 3rd-instar larvae of *Ectropis obliqua* at a different time.

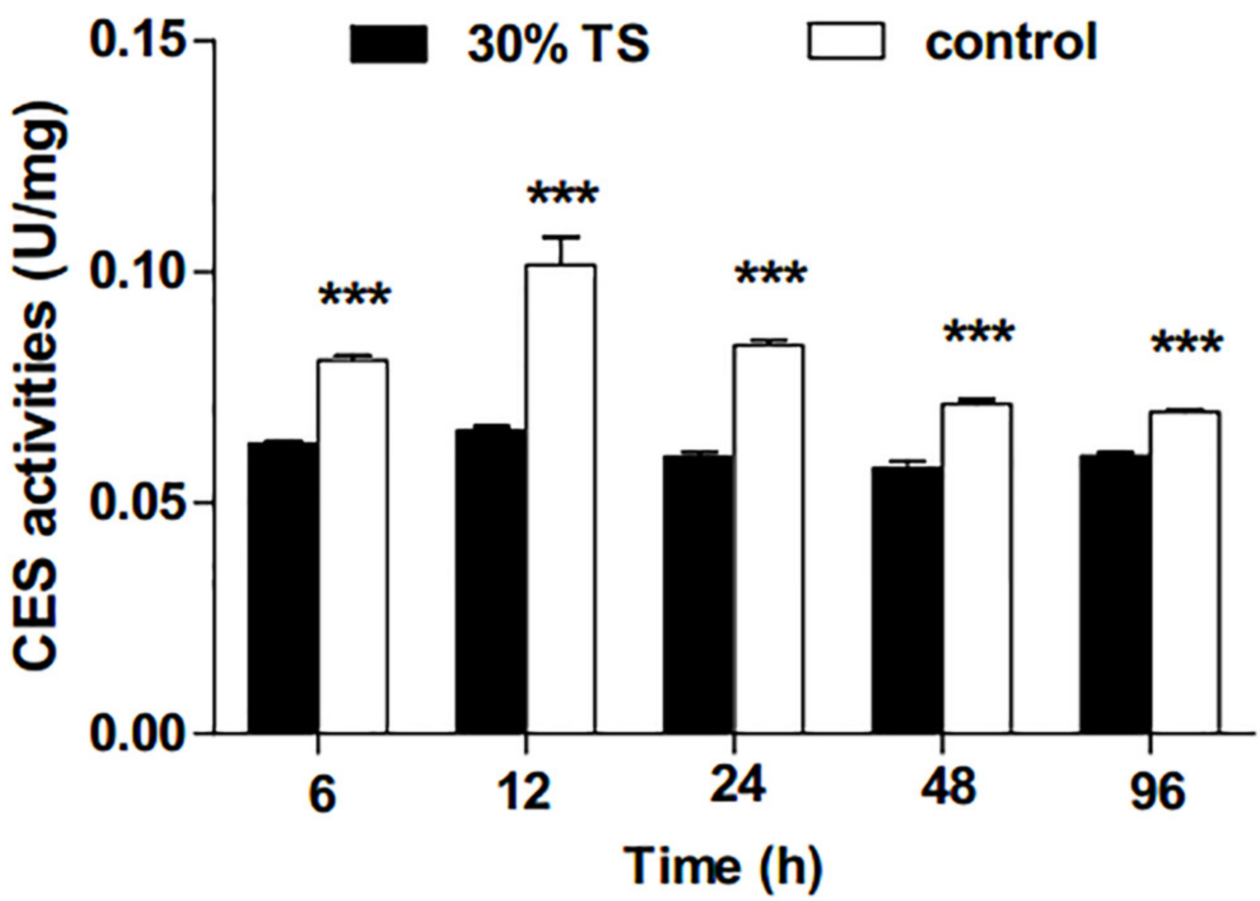
TS, tea saponin; control: distilled water. Each value represents the mean of three replicates from four paralleled experiments. Student's t-test, *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, no significant differences. The bars correspond to the standard error.



# Figure 2

Effects of 30% (w/v) TS on CES activities in 3rd-instar larvae of *Ectropis obliqua* at a different time.

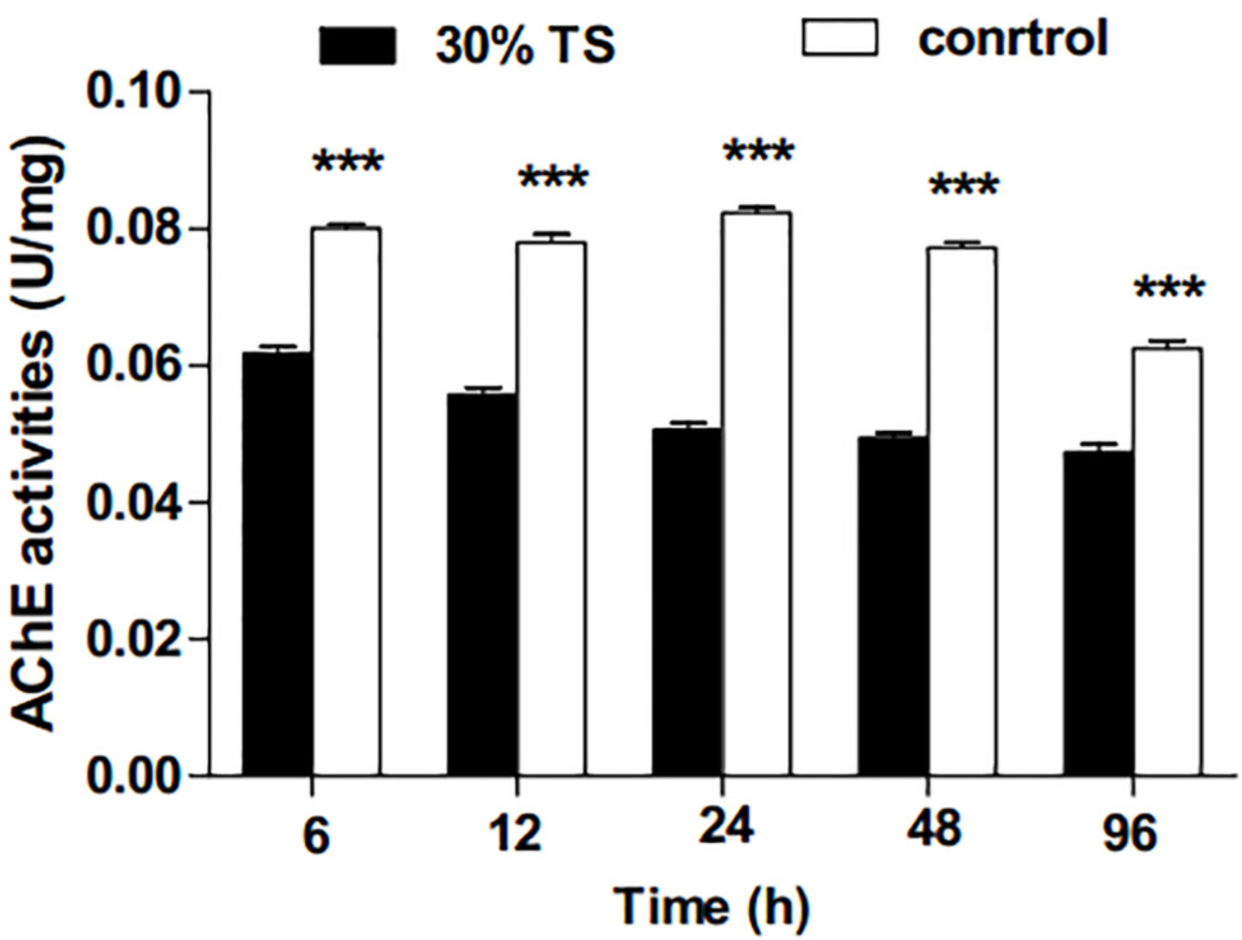
TS, tea saponin; control: distilled water. Each value represents the mean of three replicates from four paralleled experiments. Student's t-test, *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, no significant differences. The bars correspond to the standard error.



# Figure 3

Effects of 30% (w/v) TS on AChE activities in 3rd-instar larvae of *Ectropis obliqua* at a different time.

TS, tea saponin; control: distilled water. Each value represents the mean of three replicates from four paralleled experiments. Student's t-test, *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, no significant differences. The bars correspond to the standard error.



# Figure 4

Figure 4 Effects of 30% (w/v) TS on POD activities in 3rd-instar larvae of *Ectropis obliqua* at a different time.

TS, tea saponin; control: distilled water. Each value represents the mean of three replicates from four paralleled experiments. Student's t-test, *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, no significant differences. The bars correspond to the standard error.