Tea saponin reduces the damage of *Ectropis obliqua* to tea crops and exerts reduced effects on *Ebrechtella tricuspidata* and *Evarcha albaria* (#21151)

First revision

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Tea saponin reduces the damage of *Ectropis obliqua* to tea crops and exerts reduced effects on *Ebrechtella tricuspidata* and *Evarcha albaria*

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Background. Tea is one of the most economically important crops in China. However, the tea geometrid (*Ectropis obliqua*), a serious leaf-feeding pest, causes significant damage to tea crops and reduces tea yield and quality. Spiders are the most dominant predatory enemies in the tea plantation ecosystem, which makes them a potentially useful biological control agent of *E. obliqua*. These highlight the need for alternative pest control measures. Our previous studies have shown that tea saponin (TS) exerts insecticidal activity against lepidopteran pests. Here, we investigate whether TS represents a potential new alternative insecticide and causes no harm to spiders. **Methods.** We investigated laboratory bioactivities and the field control properties of TS solution against *E. obliqua*. (i) A leaf-dip bioassay was used to evaluate the toxicity of TS in 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 laboratory. (ii) Topical application was used to measure the toxicity of 30% TS and two chemical insecticides (bifenthrin 10% EC and diafenthiuron 50% SC) in two species of spider, *Ebrechtella tricuspidata* and *Evarcha albaria* . (iii) Field trials were used to investigate the controlling efficacy of 30% (w/v) TS against *E. obliqua* larvae and to classify the effect of TS in spiders in the tea plantation. **Results.** The toxicity of TS in 3rd-instar *E. obliqua* larvae occurred in a dose-dependent manner and the LC_{50} was 164.32 mg/mL. Activities of the detoxifying-related enzymes, GST and POD, increased in 3rd-instar larvae of *E. obliqua*, whereas AChE and CES were inhibited with time by treatment with TS. Mortality of *E. tricuspidata* and *E. albaria* after 48 h with 30% TS treatment (16.67% and 20%, respectively) were significant lower than with Bi 10% EC (80% and 73.33%, respectively) and Di 50% SC (43.33% and 36.67%, respectively). The highest controlling efficacy of TS 30% WG was 77.02% at 5 d after treatment, which showed no difference to bifenthrin 10% EC or diafenthiuron 50% SC . TS 30% WG was placed in the class N (harmless and slightly harmful) of IOBC categories for natural enemies, namely spiders. **Conclusions.** Our results indicate that TS is a botanical insecticide that has a good controlling efficacy in *E. obliqua* larvae, which suggests it has promise as application in the integrated pest management (IPM) envisaged for tea crops.

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Abstract

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Methods. We investigated laboratory bioactivities and the field control properties of TS solution against *E. obliqua*. (i) A leaf-dip bioassay was used to evaluate the toxicity of TS in 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 laboratory. (ii) Topical application was used to measure the toxicity of 30% TS and two chemical insecticides (bifenthrin 10% EC and diafenthiuron 50% SC) in two species of spider, *Ebrechtella tricuspidata* and *Evarcha albaria*. (iii) Field trials were used to investigate the controlling efficacy of 30% (w/v) TS against *E. obliqua* larvae and to classify the effect of TS in spiders in the tea plantation.

Results. The toxicity of TS in 3rd-instar *E. obliqua* larvae occurred in a dose-dependent manner and the LC_{50} was 164.32 mg/mL. Activities of the detoxifying-related enzymes, GST and POD, increased in 3rd-instar larvae of *E. obliqua*, whereas AChE and CES were inhibited with time by treatment with TS. Mortality of *E. tricuspidata* and *E. albaria* after 48 h with 30% TS treatment (16.67% and 20%, respectively) were significant lower than with Bi 10% EC (80% and 73.33%, respectively) and Di 50% SC (43.33% and 36.67%, respectively). The highest controlling efficacy of TS 30% WG was 77.02% at 5 d after treatment, which showed no difference to bifenthrin 10% EC or diafenthiuron 50% SC. TS 30% WG was placed in the class N (harmless or slightly harmful) of IOBC (International Organization of Biological Control) categories for natural enemies, namely spiders.

Conclusions. Our results indicate that TS is a botanical insecticide that has a good controlling efficacy in *E. obliqua* larvae, which suggests it has promise as application in the integrated pest management (IPM) envisaged for tea crops.

Introduction

Tea, *Camellia sinensis* Kuntze (Theales: Theaceae), is one of the most economically important crops in China, cultivated in vast areas spreading from 37° N – 18° S and 122° E – 97° W, totaling more than 20 provinces across tropical, subtropical and temperate regions (Ye et al., 2014). The tea geometrid, *Ectropis obliqua* (Lepidoptera: Geometridae), is a major pest throughout tea plantations in China (Zhang et al., 2014). Its larvae, a type of voracious worm, exclusively feed on tea leaves and tender buds, causing the severe yield loss in yield and deterioration in commercial tea quality (Ma et al., 2016). The therapeutic approach of killing this pest with chemicals has been the prevailing control strategy (Hazarika, Puzari & Wahab, 2001; Ehi-Eromosele, Nwinyi & Ajani, 2013; 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 in tea products (Feng et al., 2013) and environmental contamination (Saha & Mukhopadhyay, 2013; Ye et al., 2014).

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 have multiple bioactivities. They represent an alternative for pest control as repellents, deterrents of oviposition and feeding, growth regulators, and toxicity to larvae and adults (Isman, 2006; Chermenskaya et al., 2010; Martínez et al., 2015). These advantages indicated that botanical insecticides can be ideal candidates for 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) is extracted from the seed of plant species belonging to the genus *Camellia*, of the family Theaceae, that can enhance efficiency and solubilization of pesticide as a wetting powder pesticide (Chen, Zhang & Yang, 2012). TS has been widely used in pesticides as the main component of environmently-friendly pesticide additives (De Geyter, Geelen & Smagghe, 2007). Chaieb (2010) concluded that the insecticidal activity of saponins is due to their properties, causing a disturbance of the synthesis of ecdysteroids, protease inhibitors or cytotoxic to certain insects. Therefore, TS has the potential for use 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 presents no harm to the environment. In our previous studies, we found that

30% (w/v) TS exerted a strong toxic effect in *E. obliqua* larvae.

To date, biological control has gained recognition as an essential component of successful integrated pest management (IPM) (Murphy & Briscoe, 1999; Jacobsen, Zidack & Larson, 2004; Yang et al., 2017). Predatory natural enemies play key functional roles in the biological control of IPM (Rutledge, Fox & Landis, 2004), and spiders are the most dominant predatory natural enemies in the tea plantation ecosystem (Chen et al., 2004; Das, Roy & Mukhopadhyay, 2010). Hu et al. (1994) used feeding trails in the laboratory to show that *Ebrechtella tricuspidata* and *Evarcha albaria* preyed on the larvae of *E.obliqua*. And Yang et al. (2017) demonstrated the maximum potential of these two species of spider take control of *E. obliqua* larvae. Their role in pest control, however, can be disturbed if chemicals with adverse effects are applied. Therefore, these problems have necessitated the study of alternative and effective biodegradable insecticides which have greater acceptability (Roy, Mukhopadhyay & Gurusubramanian, 2010).

The physiological and metabolic functions of insects have frequently been reported to be influenced by chemicals or host plant variety (Cai et al., 2016). Likewise, insects defend against insecticides assault via multiple enzyme systems (Terriere, 1984; Serebrov et al., 2006). Karban et al. (2002) suggested that herbivore insects are adapted to host secondary substances through physiological changes. 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). Various detoxification enzymes such as glutathione-S-transferase (GST) and carboxylesterase (CES) are most commonly involved in insects defense against insecticides (Serebrov et al., 2006). Acetylcholinesterase is a key enzyme catalyzing the hydrolysis of the neurotransmitter, acetylcholine, in the nervous system in various organisms

(Oehmichen & Besserer, 1982; Wang et al., 2004). It is well-known that altered AChE is one of the main mechanisms of resistance in many insect pests that is affected by chemical and botanical insecticides (Miao et al., 2016). Peroxidase (POD) is an antioxidant enzyme that can provide defense against pathogens and insecticides (Felton & Summers, 1995; Miao et al., 2016). Previous studies have demonstrated that POD can be quickly up-regulated in response to xenobiotic threats and that increased activity of this enzyme is related to pesticide resistance (Miao et al., 2016).

Therefore, the biological traits of TS prompt us to test if: (i) TS exerts a strong lethal effect, but is not toxic to predators, such as *E. tricuspidata* and *E. albaria*, in the laboratory; (ii) the multiple enzyme system in 3rd-instar *E. obliqua* participates in defense against TS assault; (iii) 30% TS (w/v) shows effective control of *E. obliqua* larvae and dose not harm mainly natural enemies in the tea plantation.

Material and Methods

Test insects and spiders

Larvae of *E. obliqua* and two *E. tricuspidata* and *E. albaria* were originally collected from tea bushes at the Wang Dazhen tea plantation (30.011° N, 114.363° E), Xianning, Hubei Province, China, during the period May to October 2014. The total area of the collection site is about 6.5 ha with parallel rows of tea plants about 100 m long and 1 m apart. The site is an organic tea plantation in which no insecticides have been applied. Larvae of *E. obliqua* were fed on fresh tea leaves and reared for 5 generations in self-made plastic chambers (10 cm diameter × 10 cm height) at 28 ± 1 °C and 75 ± 5% relative humidity under a 14-h light:10-h dark photoperiod in the 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 in the following experiments.

Spiders were kept individually in glass tubes (1.5 cm diameter × 10 cm length), which were blocked with a plug of cotton and included 1 cm of moist sponge at the bottom of the tube to maintain high humidity. The tubes were kept in an illumination incubator (25 ± 1 °C, 75 ± 5% relative humidity and under a 14-h light:10-h dark photoperiod). Wild-type fruit flies (*Drosophila melanogaster*) were provided twice a week as food. Adult spiders with similar sizes were used for the toxicity tests.

Reagents

Tea saponin (98% purity) was purchased from Wuhan Bai Ming Technology Co., Ltd, Hubei province, China. Bifenthrin (Bi) 10% EC and diafenthiuron (Di) 50% SC were purchased from Jiangsu Dongbao Chemical Corporation Ltd., Jiangsu, China. Both of the low toxicity chemical insecticides are widely applied in the tea area of China to control leaf-feeding insects (Wu et al., 2013; Liu 2014).

Toxicity of TS in *E. obliqua* larvae

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*. We evaluated five TS concentrations (18.75, 37.5, 75, 150 and 300 mg/mL). The dilutions were prepared using distilled water. Tea leaf discs (diameter 4 cm) were dipped for 20 s in one of the five concentrations of TS. Then, the leaf discs were dried by placing them in a glass Petri dish (diameter 9 cm). Control leaf discs were dipped in distilled water as described above. Thirty 3rd-instar *E. obliqua* were starved for 24 h and then transferred to the glass Petri dish (two leaves per Petri dish). Three replicates were made for each concentration. Larvae were considered to be dead if they did not respond when lightly prodded with a hair brush. Surviving larvae were used for the following enzyme activity assays. Larvae mortality (%) was quantified after 48 h of treatment.

Toxicity of insecticides in spiders

For the insecticide treatment, TS powder was diluted with distilled water to a concentration of 300 mg/mL; Bi 10% EC and Di 50% SC were diluted with distilled water to concentration of 0.01 mg/mL and 0.05 mg/mL (advised by the manufacturer of the chemicals), respectively. Prior to insecticide treatment, spiders were individually anaesthetized using carbon dioxide. The toxicity assay was conducted according to Deng et al. (2006). Two droplets (0.5 μL each) of insecticide solution were applied to the dorsal abdomen of each spider using a 5-μL microsyringe; distilled water was employed as the control. 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. Twenty individuals were used for each treatment with three replications. Spiders mortality was recorded after 48 h.

Assays of enzyme activity

We randomly collected three surviving 3rd-instar larvae of *E. obliqua* per concentration of TS solution with four replicates. Larvae were weighed and placed in a glass homogenizr with physiological saline (w/v = 1:9) for homogenization. Samples were centrifuged at 10,000 × *g* for 10 min at 4 °C. The supernatant from this final centrifugation was used to determine enzyme activities and protein concentration for each sample.

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 assayed in units of U/mg. Sample protein concentrations were estimated 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).

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

To evaluate the controlling efficacy of TS 30% WG along with Bi 10% EC (at the recommended dose of 7.5 g a.i. ha^{-1}), Di 50% SC (at the recommended dose of 45 g a.i. ha^{-1}) against *E. obliqua*, field trials were conducted on dry days during the period June to July 2015 in

Wang Dazhen tea plantation (30.011° N, 114.363° E) Xianning, Hubei Province, China. The experiment as a randomized block design (Roy, Mukhopadhyay & Gurusubramanian, 2010; Kawada et al., 2014) with three treatments and three replicates. Each identical plot (20 m^2) was separated by two buffer rows of non-treated tea bushes. Two rounds of foliar spray were applied by using a 16 L capacity knapsack sprayer equipped with a hollow cone nozzle (droplet diameter 1.2 mm, distance between nozzle and tea leaves was 30 – 40 cm) at 750 L ha^{-1}. An untreated control plot, involving application of clean water was simultaneously proceed during the study. A pre-treatment count was carried out in the respective plots on five randomly selected tea bushes. After spraying, post-treatment counting took place at 1, 3, 5 and 7 d in each treatment plot during 4:00 – 5:00 pm (Beijing time) in the respective plots using five randomly selected tea bushes. *E. obliqua* larvae and spiders were randomly sampled using a sweep-net (diameter 40 cm) by beating the tea canopies 10 times with a stick. To investigate the safety of TS 30% WG to natural enemies (spiders), the number of spiders was counted before and 7 days after insecticide 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); or T, harmful (75% reduction) (Boller et al., 2005).

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

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

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

Statistical analysis

The LC_{50} and their fiducial limits were determined by logistic regression based on the concentration probit-mortality (Finney, 1971). Mortality variables were expressed as percentages and the data transformed to arcsine square root. The differences in mortality of larvae and adult spiders, and 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 analyses were performed using 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 in 3rd-instar *E. obliqua* larvae

Mortality of 3rd-instar *E. obliqua* larvae was 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), respectively. The LC_{50} value of TS solution to the 3rd-instar larvae of *E. obliqua* was

164.32 mg/mL.

Toxicity of insecticides in spiders

As 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 are shown in Table 2. The mortality of *E. tricuspidata* adults with 300 mg/mL TS solution was 16.67%, which was significantly 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 mortality of *E. albaria* adults was 20%, which was significantly lower than Bi 10% EC ($F_{1,4} = 23.49$, $p = 0.01$) and Di 50% SC ($F_{1,4} = 46.83$, $p < 0.01$).

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

The activities of GST in 3rd-instar larvae of *E. obliqua* after treatment with 30% TS showed significant increase 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, $t = 25.01$, $df = 6$, $p < 0.001$); this activity was then reduced in the later period (Fig. 1). There was no significant difference (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 were significantly lower (*t*-test, $t = -12.07$, $df = 6$, $p < 0.001$) than distilled water 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 were maintained 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 significantly inhibited (*t*-test, $p < 0.001$) by 30% TS during the whole experimental period.

After treatment with 30% TS, the activities of POD in 3rd-instar *E. obliqua* larvae were significantly increased (*t*-test, $p < 0.01$) in the whole experimental period, except at 48 h when there, no significant difference (*t*-test, $t = 0.363$, $df = 6$, $p > 0.05$) to the control (Fig. 4).

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

The controlling efficacies of TS 30% WG, Bi 10% EC and Di 50% SC against the larvae of *E. obliqua* under field conditions are shown in Table 3. Controlling efficacy (CE) was significantly lower ($p < 0.01$) in plots sprayed with 30% TS than Bi 10% EC and Di 50% SC during the first 3 d period posttreatment. Further, the CE of TS 30% WG was equivalent to chemical pesticides at 5 d ($p > 0.05$) and 7 d ($p > 0.05$), respectively.

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 with Bi 10% EC ($F_{1,4} = 18.00$, $p < 0.05$) and Di 50% SC ($F_{1,4} = 16.00$, $p < 0.05$). Treatments of clean water and with TS 30% WG were both classified as N (harmless or slightly harmful) of IOBC categories for spiders, whereas, Bi 10% EC and Di 50% SC treatments were classified M (moderately harmful).

Discussion

Control of *E. obliqua* larvae has been mainly achieved 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 larvae of *E. obliqua* in the field in order to evaluate its use as a new and natural insecticide.

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

As predators of the larvae of *E. obliqua*, *E. tricuspidata* and *E. albaria* are easily affected by insecticides and are the important non-target and beneficial species in tea plantation. Susceptibility of these species to the 30% TS, Bi 10% EC and Di 50% SC was assessed in the present study. Although 30% TS caused 16.67% and 20.0% mortality of *E. tricuspidata* and *E. albaria*, respectively, both of *E. tricuspidata* and *E. albaria* adults showed significantly lower mortalities after 48 h of 30% TS treatment compared with Bi 10% EC and Di 50% SC (Table 2). These results indicated that 30% TS had a reduced effect to clean water on the two types of spiders in laboratory. It is clear that acute toxicity tests do not reflect the full range of effects of a compound on an organism. Our study mainly focused on acute toxicity tests, which can reveal details of intoxication, but often underestimate mortality in comparison with field studies, as they only take into account one route of uptake (Wiles & Jepson, 1992; Pekár, 2012). The effects that an insecticide has on a beneficial species in the field are complex processes involving both susceptibility and exposure, with exposure being a multidimensional process (John, Paul & Daniel, 1995). Vânia et al. (2015) demonstrated that the acute toxicity of botanical insecticides might involve delayed effects. In addition, insecticides affect virtually all life-history trails of spiders (Pekár, 2012), whereas the long-term effects of TS on these spiders are currently unknown.

Our findings also indicated that 30% TS exerted remarkable effects on the activities of detoxification 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). The changes usually involve increased detoxification enzyme activities and introduction of additional isoforms (Miao et al., 2016). Increased activity of detoxifying enzymes in insects represents a response to intoxication with insecticides or xenobiotics (Singh & Singh, 2000; Serebrov et al., 2006; Gopalakrishnan et al., 2011). Rizwan-ul-Haq et al. (2009) evaluated the bioactivities of TS solution in *Spodoptera exigua* (Lepidoptera: Noctuidae), which provides some helpful information about activities of enzymes against TS which involved in the resistance mechanism in insects. In this study, we found that the activities of GST significantly increased during the initial period following TS treatment, which suggests that this enzyme may act to detoxify TS. Whereas the activities of CES decreased significantly, indicating that TS inhibited

CES which may increase susceptibility to the insecticide. Based on these results, GST and CES appear to participate in the defensive reaction of TS to *E. abliqua* larvae. 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). It is well known that altered AChE is one of the main mechanisms of resistance in many insect pests (Serebrov et al., 2006). Our results showed that AChE activities decreased. Inhibition of AChE causes accumulation of ACh at the synapses, so the post-synaptic membrane is in a state of permanent stimulation. These results in paralysis, ataxia, general lack of co-ordination in the neuromuscular system, and eventual death (Singh & Singh, 2000). POD is the key antioxidant enzyme that can be quickly up-regulated in response to natural penetrating xenobiotics (Wu et al., 2011), and the increase of POD activities is related to pesticide resistance and melanization in insects (Terriere, 1984; Potter et al., 2010). It is shown that POD activities were shown to increase over the whole experimental period, except 48 h. We assumed that the enhanced activities of POD are associated with eliminating ROS. Large quantities of generated ROS can rapidly denature a wide range of biomolecules, thereby threatening virtually all cellular processes and leading to insect death (Felton & Summers, 1995).

We found that the enzymatic defense against TS assault in 3rd-instar *E. obliqua* larvae was generally activated. Our results reveal that underlying the perturbation of enzyme activities by TS seems to be one of the modes of action. In addition, several studies documented another probable mode of action of saponins involving interaction with membrane cholesterol, which causes membrane destabilization and provokes cell death (Sung et al., 1995; Hu, Konoki & Tachibana, 1996; Chaieb et al., 2007). This interaction structurally modified the phospholipid double layer which would be at the origin of disturbances of cellular exchanges leading to cytotoxicity. Chaieb et al. (2007) demonstrated a cytotoxic effect of crude saponic extract on the fat body of *spodoptera littoralis* larvae, and cell destruction of the foregut and gastric caeca of *schistocerca gregaria* using histological methods. The same results were reported by Gögelein et al. (1984) and Hu et al. (1996). Therefore, TS exerted multiple modes of action involving enzymatic and physiological perturbation on the 3rd-instar larvae of *E. obliqua*.

The effectiveness of TS 30% WG and two types 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 was exceeded by Bi 10% EC, Di 50% SC at 5 d and 7 d, although the difference was not significant (Table 2). A 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. Although after 7 d of reagent application in this study, the pooled mean population of spiders was significantly lower with TS treatment than with clean water application (Table 4), we supposed that this lower abundance of spiders in experimental plots may be due to reduce prey availability (Sunderland, 1992; Markó et al., 2009). Peng et al. (2017) have reported that 30% TS exerted a significantly lower repellent rate to spiders compared with chemical insecticides, which could be partly support our results. As TS exerts strong fungicidal activity, TS might reduce the abundance of fungi. The reduction in levels of fungi may reduce the abundance mycetophagous pests such as springtails and some beetles, changing the prey availability for spiders (Sunderland, 1992). Direct evidence of this has not been reported so far. However, pyrazophos applied in the field decreased spider abundance (Volkmar & Wetzel, 1993), although laboratory tests showed that this fungicide is harmless to spiders (Mansour, Heimbach & Wehling, 1992), which could partly support our supposition. Our results indicated that TS 30% WG should be placed in the class N (harmless or slightly harmful) of IOBC categories for natural enemies, namely spiders. Thus, the distribution of spiders can indicate that TS was relatively friendlier than chemical insecticides. In addition, the procedure for preparation of TS 30% WG is simple by using only water, TS production is cheap and TS is readily available. This finding could be a point of view of controlling larvae of *E. obliqua* without the use of chemical insecticides. This approach would help the tea industry in many ways, such as prepared tea that is free of residues, reduced 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 *E. obliqua* larvae involving by multiple modes of action, and exerts only slightly harmful effects on the natural enemies suchas spiders, in field applications. As a natural product that is abundant in tea plantations, thus, 30% TS could be effectively utilized in the IPM envisaged for tea.

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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* 125:483–488. 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:e78651. DOI: 10.1371/journal.pone.0078651.

Bandeira GN, Da Camara CAG, De 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 25:83–89. DOI: 10.1016/j.jksus.2012.08.002.

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: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* 93:1588–1595. 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* 6:187–216.

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.

Chaieb I, Trabelsi M, Ben HKM, Ben HMH. 2007. Histological effects of *cestrum parqui* saponins on *schistocerca gregaria* and *spodoptera littoralis*. *Journal of Biological Sciences* 7:95–101.

Chaieb I. 2010. Saponins as insecticides: a review. *Tunisian Journal of Plant Protection* 5:39–50.

Chen J, Zhang S, Yang X. 2012. Control of brown rot on nectarines by tea polyphenol combined with tea saponin. *Crop Protection* 45:29–35. 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* 22:27–28.

Chen YF, Chen ZH, Song CQ, Xu HZ. 2004. Review on the investigation and protection measurement of spiders in Chinese tea gardens (in Chinese with English abstract). *Acta Arachnologica Sinica* 13:125–128.

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* 32:157–163. DOI:10.1016/j/indcrop.2010.04.009.

Das S, Roy S, Mukhopadhyay A. 2010. Diversity of arthropod natural enemies in the tea plantations of North Bengal with emphasis on their association with tea pests. *Current Science* 99:1457–1463.

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

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

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

Ehi-Eromosele CO, Nwinyi O, Ajani OO. 2013. Integrated pest management. In: Soloneski S, Larramendy M ed. *Weed and Pest Control – Conventional and New Challenges*. Rijeka: InTech, 105–116.

Felton GW, Summers CB. 1995. Antioxidant Systems in insects. *Archives of Insect Biochemistry and Physiology* 29:187–197. 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* 21:169–183. DOI: 10.1080/10807039.2014.894443.

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

Gögelein H, Hüby A. 1984. Interaction of saponin and digitonin with black lipid membranes and lipid monolayers. *Biochimica et Biophysica Acta* 773:32–38.

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: 10.1146/annurev.en.29.010184.000443

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* 29:813–826.

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* 15:113–116. DOI: 10.1016/03051978(87)90089-5.

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

Hu C, Zhu JQ, Ye GY, Hong J. 1994. *Ectropis obliqua Prout, a serious geometrid pest of tea bush in east china*. Shanghai: Shanghai Scientific and Technical Publishers.

Hu M, Konoki K, Tachibana K. 1996. Cholesterol independent membrane disruption caused by triterpenoid saponins. *Biochimica et Biophysica Acta* 1299:252–258.

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

Jacobsen BJ, Zidack NK, Larson BJ. 2004. The role of Bacillus-based biological control agents in integrated pest management systems: plant diseases. *Phytopathology* 94:1272–1275. DOI: 10.1094/PHYTO.2004.94.11.1272.

John DS, Paul CJ, Daniel FM. 1995. Limitations to use of topical toxicity data for predictions of pesticide side effects in the field. *Journal of Economic Entomology* 88:1081–1088. DOI: 10.1093/ee/26.4.763.

Karban R, Agrawal AA. 2002. Herbivore offense. *Annual Review of Ecology and Systematics* 33:641–664. 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: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* 59:1232–1236. DOI: 10.1002/ps.760.Markó V, Keresztes B, Fountain MT, Cross JV. 2009. Prey availability, pesticides and the abundance of orchard spider communities. *Biological Control* 48:115–124. DOI: 10.1016/j.biocontrol.2008.10.002.

Liu CY. 2014. Control efficacy of 25% thiamethoxam and 25% diafenthiuron on green leafhopper (*Empoasca pirisuga*) (in Chinese). *Modern Horticulture* 17:115–116.

Ma L, Li ZQ, Bian L, Cai XM, Luo ZX, Zhang YJ, Zong MC. 2016. Identification and comparative study of chemosensory genes related to host selection by legs transcriptome analysis in the tea geometrid *Ectropis obliqua*. *Plos ONE* 11:e0149591. DOI:10.1371/journal.pone.0149591.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* 15:2015. DOI: 10.1093/jisesa/iev021.

Mansour F. 1987. Effect of pesticides on spiders occurring on apple and citrus in Israel. *Phytoparasitica* 15:43–50

Miao J, Cao GC, Li YB, Tu XB, Wang GJ, Nong XQ, Whitman DW, Zhang ZH. 2016. Biochemical basis of synergism between pathogenic fungus *Metarhizium anisopliae* and insecticide chlorantraniliprole in *Locusta migratoria* (Meyen). *Scientific Reports* 22:28424. DOI: 10.1038/srep28424.

Murphy ST, Briscoe BR. 1999. The red palm weevil as an alien invasive: biology and the prospects for biological control as a component of IPM. *Biocontrol News and Information* 20:35N–46N.

Oehmichen M, Besserer K. 1982. Forensic significance of acetylcholine esterase histochemistry in organophosphate intoxication. *Zeitschrift Für Rechtsmedizin Journal of Legal Medicine* 83:149–165.

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* 26:114–118.

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* 66:439–446. 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* 44:419–428. 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* 83:371–377. DOI: 10.1007/s10340-010-0306-5.

Rutledge CE, Fox TB, Landis DA. 2004. Soybean aphid predators and their use in integrated pest management. *Annals of the Entomological Society of America* 97:240–248.

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* 33:46–70. 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* 70:244–250. 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* 33:581–586. 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* 56:889–898. 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* 179:255–261.

Sung MK, Kendall WC, Rao AV. 1995. Effect of soybean saponins and *Gypsophilla* saponins on morphology of carcinoma cells in culture. *Food and chemical toxicology* 33:357–363.

Pekár S. 2012. Spiders (Araneae) in the pesticide world: an ecotoxicological review. *Pest Management Science* 68:1438–1446. DOI 10.1002/ps.3397.

Sunderland KD. 1992. Effects of pesticides on the population ecology of polyphagous predators. *Aspects of Applied Biology* 31:19–28.

Terriere LC. 1984. Induction of detoxication enzymes in insects. *Annual Review of Entomology*

29:771–788. DOI: 10.1146/annurev.en.29.010184.000443.

Vânia MX, Message D, Picanc MC, Chediak M, Paulo AJS, Ramos RS, Martins JC. 2015. Acute toxicity and sublethal effects of botanical insecticides to honey bees. *Journal of Insect Science* 15:137. DOI: 10.1093/jisesa/iev110.

Volkmar C and Wetzel T. 1993. On the occurrence of insect pests and soil surface spiders (Araneae) in cereal fields and the side effects of some fungicides. *Nachrichtenblatt Des Deutschen Pflanzenschutzdienstes* 45:233–239 (1993).

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* 4:23–27.

Wiles JA, Jepson PC. 1992. In situ bioassay techniques to evaluate the toxicity of pesticides to beneficial invertebrates in cereals. *Aspects of Applied Biology* 31:61–68.

Wu GY, Zeng MS, Xia HL, Ma XJ, Wang QS, Wang WJ, Chen ZL. 2013. Security analysis of residual bifenthrin tea plantation. *Fujian Journal of Agriculture Sciences* 28:366–371.

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* 100:23–26. 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* 61:1–12. 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* 73:2302–2309. 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* 68:73–91. DOI: 10.1016/j.biocontrol.2013.06.013.

Zhang GH, Yuan ZJ, Zhang CX, Yin KS, Tang MJ, Guo HW, Fu JY, Xiao Q. 2014. Detecting deep divergence in seventeen populations of tea geometrid (*Ectropis obliqua* Prout) in China by COI. *PloS ONE* 9:e99373. DOI: 10.1371/journal.pone.0099373.

Table 1(on next page)

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

TS, tea saponin; LC_{50}, Lethal concentration 50, the concentration causing 50% mortality; FL, fiducial limits (mg/mL); SE, standard error 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 in 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 concentration causing 50% mortality; FL, fiducial limits (mg/mL); SE, standard error 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 using different reagents

Bi, bifenthrin EC; Di, diafenthiuron SC; TS, tea saponin. SE, standard error 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 using 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
Control		0d	0d

Bi, bifenthrin EC; Di, diafenthiuron SC; TS, tea saponin. SE, standard error 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. Within columns, data (% ± 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 (g a.i. ha^{-1})	Controlling efficacy (CE) (Mean ± SE) (%)			
		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. Within columns, data (% ± 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 in 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). Within columns, the same letters represented no significant difference (Least-significant difference test at the 5% level of significance).

The toxicity classes of different reagents in 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).

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

Figure 1

The effects of 30% (w/v) TS on GST activity in 3rd-instar larvae of *Ectropis obliqua* at different times

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

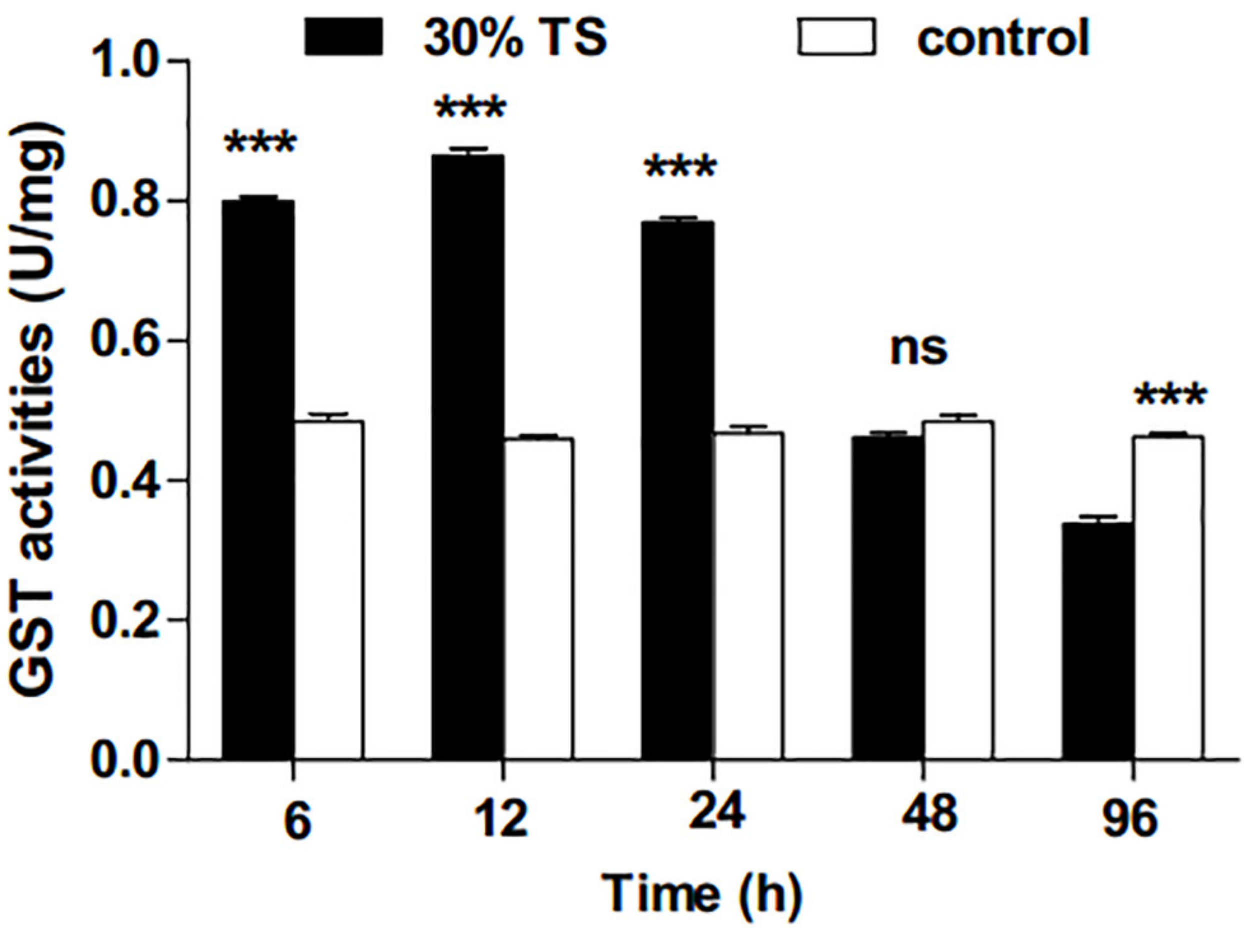

Figure 2

The effects of 30% (w/v) TS on CES activity in 3rd-instar larvae of *Ectropis obliqua* at different times

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

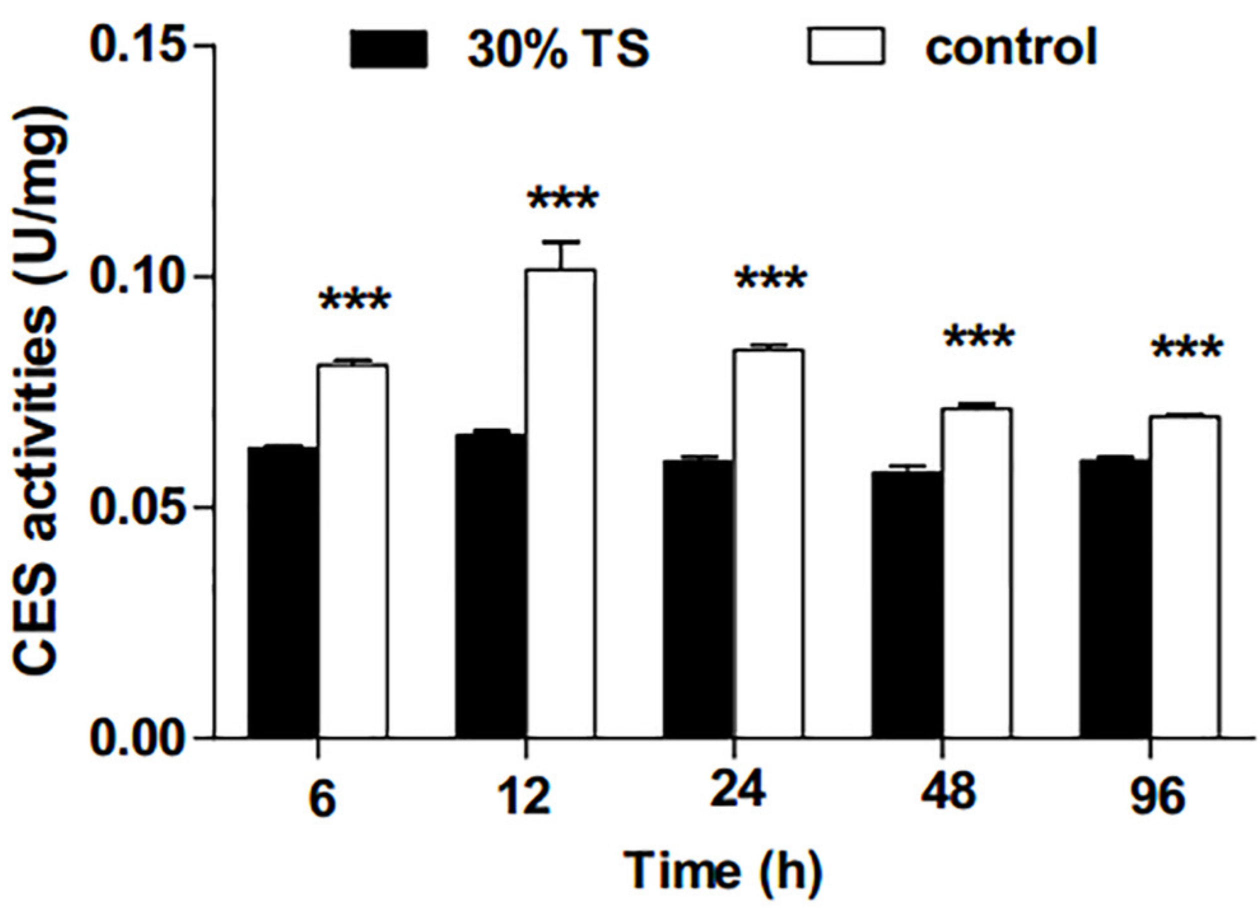

Figure 3

The effects of 30% (w/v) TS on AC activity in 3rd-instar larvae of *Ectropis obliqua* at different times

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

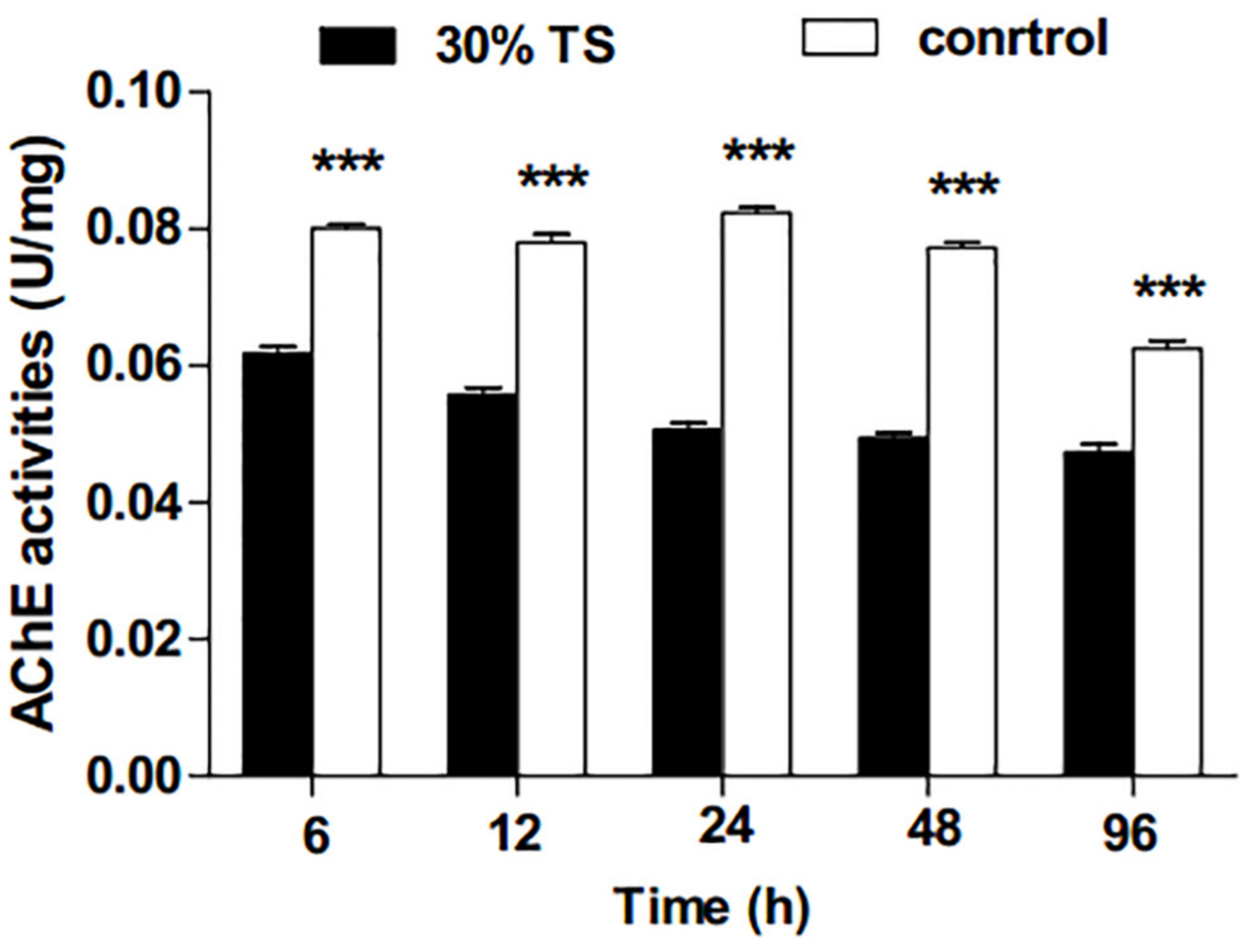

Figure 4

The effects of 30% (w/v) TS on POD activity in 3rd-instar larvae of *Ectropis obliqua* at different times

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