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Insect spontaneous ultraweak photon emission as an indicator of insecticidal compounds

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ABSTRACT

The influence of beta-cypermethrin, a commercial insecticide, and *Cicuta virosa* L. var. *latisecta* Celak (Umbelliferae:Cicutal), an insecticidal plant, on the spontaneous ultraweak photon emissions from larvae of *Spodoptera litura* Fabricius and *Zophobas morio* Fabricius were studied. The increased percentages of spontaneous photon emission intensities from *S. litura* treated with 0.1 and 1 μg/ml beta-cypermethrin were both lower than those of the control in the 24 post-treatment hours, remarkable difference could also be observed during the same period from *Z. morio* treated with beta-cypermethrin at 0.156, 0.313 and 0.625 μg/ml. The increased percentages of spontaneous photon emission intensities from the two mentioned insects treated with 10,100 and 1000 μg/ml petroleum ether fraction of *C. virosa* L. var. *latisecta*, which displayed little activity against whole insects, could also be changed noticeably. The present study indicated that change in the intensity of spontaneous ultraweak photon emission from insect could be used as a novel method for screening insecticidal compounds with very low content in plant.

1. Introduction

Ultraweak photon emission is universal among many all living organisms, including fungi, plants, and mammals, which was called biophotons and radiate spontaneously in the spectral range from ultraviolet to near infrared with an intensity of 10^{-16} W/cm^2 from biochemical reactions in the cells [1–5]. The features of biological ultraweak illumination are closely related with many factors, including temperature [6,7], oxidative stress [8–11], exogenous toxins [12], etc., and could reflect physiological states of cells and tissues. Detection of biophoton emission provides methods to acquire biological information rapidly and non-invasively, thus the ultraweak illumination has been used in many fields, such as assessing cancer [7,13–15] and other health problems [16,17] and detection of food quality [18,19]. However, ultraweak photon emission from biological systems has not been widely used in agrochemical research, up to today only a few herbicides were studied about their influences on weeds. Scordino et al. [20] reported influence of atrazine in water on the delayed luminescence of *Acetabularia acetabulum*, a unicellular alga, and indicated that delayed luminescence from the alga could be used as a new methodology for monitoring water pollution. Inagaki et al. [21] found that characters of ultraweak photon emissions from sulfonylurea-resistant biotypes of *Monochoria vaginalis*, a hydrophilous weed, were different than those of the susceptible ones. As for the affect of toxic compounds on insect ultraweak biophotons, no data could be found so far. Inspired by the published results, we hypothesize that features of insect ultraweak biophotons might be changed by exogenous toxins and preliminarily studied the ultraweak photon emissions from larvae of *Spodoptera litura* Fabricius and *Zophobas morio* Fabricius, which belongs to lepidopterous and coleopterous insect respectively, after treatment with beta cypermethrin, a commercial insecticide most commonly used in China, and petroleum ether fraction of *Cicuta virosa* L. var. *latisecta* Celak. (Umbelliferae), an insecticidal plant. This research showed that photon emission intensity of insect could be changed remarkably by insecticidal or toxic compounds at under-lethal concentrations, which may be used as a new method for screening insecticidal compounds in plants.

2. Materials and methods

2.1. Plant extracts, insects and chemicals

The petroleum ether fraction of *C. virosa* L. var. *latisecta*. was prepared according to the author's published method [22]. The

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powdered dry plant material was extracted with methanol three times at room temperature and the methanol solutions were combined and concentrated in vacuo. The residue was suspended in water and then sequentially extracted three times each with petroleum ether, chloroform, and ethyl acetate. The resulting petroleum ether, chloroform, and ethyl acetate solutions, all yielded a deep brown syrup upon evaporation. In this research only the petroleum ether fraction was used, which was chosen according to the author's previous study [22]. That bioassay showed that the petroleum ether fraction could not cause death of 4th-instar larvae of the Asian tiger mosquito, *Aedes albopictus* (Skuse), however, obvious toxic symptoms appeared, the test larvae twisted their bodies painfully. This result proved that the petroleum ether fraction contained insecticidal compounds, but the content was too low to kill *A. albopictus* larvae.

The 5th-instar larvae of *S. litura* were from laboratory colonies maintained in the Biology Control Station of Guangzhou, Guangzhou, China. The 5th-instar larvae of *Z. morio* were bought from Guangzhou Yuehe Market.

Beta-cypermethrin was purchased from the Zhongshan Aestar Fine Chemical Inc. Ltd (Guangdong Province, China) and the purity was 98%.

2.2. Apparatus and measurement of ultraweak photon emission

2.2.1. Apparatus

A single sample photon counting system BPCL (model: BPCL-4; manufacturer: Institute of Biological Physics of Chinese Academy of Sciences, Beijing, China) was used to measure time-dependent intensity variation, its main construction was shown in Fig. 1. The device has a special dark box with two units: sample unit for containing samples and filter unit for settling band-pass filter. Filter unit is behind sample unit; filter unit could be pulled out to place filters and then pushed back. A photomultiplier tube (PMT) whose spectral response ranges from 400 to 620 nm was assembled under the dark box to detect photons emitting from samples. The peak wavelengths of nine band-pass filters (10 nm passband each) were 400, 425, 440, 460, 490, 535, 555, 575 and 620 nm, respectively. Since there was not temperature control system for the dark box, the room temperature was kept at 25 ± 1 °C using air conditioner. At the same time, to avoid the effect of heat from PMT on photon counts, the testing device was turned off for 15 min after each 60-min operation.

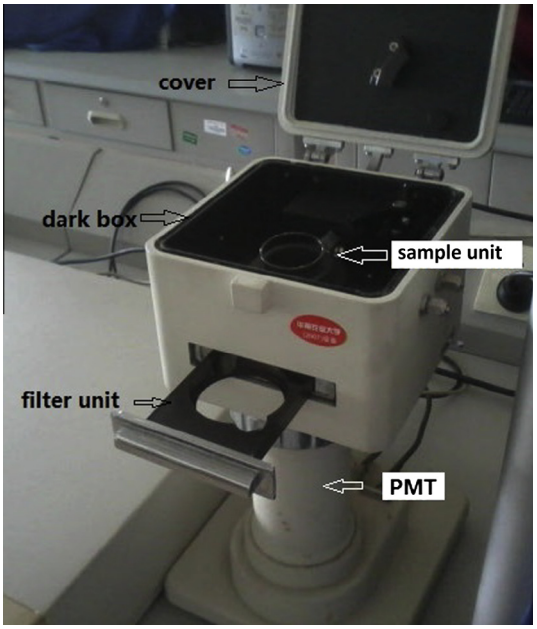

Fig. 1. Main construction of the photon counting system.

2.2.2. Measurement of ultraweak photon emission

Ultra weak photon emission includes spontaneous photon emission and delayed luminescence. Delayed luminescence is obvious for plant tissue and organ, especially those containing rich chlorophyll after being excited by experimental light. However, the preliminary experiments showed test insects only emitted spontaneous photon emission, which was different from plant materials. Even so, in order to eliminate the possible effect of environmental light on spontaneous photon emission of treated insects, the counting system was set to start counting two minutes later after the treated insects was put into the dark box. Therefore, only spontaneous ultraweak photon emission was measured in experiment. The time distribution of dark count noise of the PMT was shown in Fig. 2, where the typical dark count noise was 67 ± 10 count/s.

In our experiment, each sampling-data time interval was 1 s, and the testing time was 60 s. The system was used to measure the spontaneous ultraweak photon emission intensity without filters and spectral distribution of spontaneous ultraweak photon emission using the above filters.

2.3. Measurements of spontaneous ultraweak photon emission intensities from treated insects

The intensities of spontaneous ultraweak photon emissions from insects were measured at different times after treated with beta-cypermethrin and petroleum ether fraction of *C. virosa* L. var. *latisecta*. The beta-cypermethrin was dissolved by 0.4% (v/v) aqueous acetone and then prepared as emulsifiable concentrate (EC) using Tween-80 (0.0056%, m/m) as emulsifier, and the emulsion without beta-cypermethrin was used as the control. The concentrations of beta-cypermethrin in the emulsifiable concentrate were decided according to preliminary study to ensure not to cause insect death. Fifteen 5th-instar larvae of *S. litura* were dipped into the beta-cypermethrin emulsifiable concentrates at under-lethal concentrations of 1 and 0.1 μg/ml for 3 s, then were put immediately in the photon counter and spontaneous ultraweak photon emissions from the insects were measured for 60 s at different times in 24 h after treatment. The experiment towards 5th-instar larvae of *Z. morio* was conducted similarly except that the beta-cypermethrin concentrations were 0.156, 0.313 and 0.625 μg/ml. As for the petroleum ether fraction of *C. virosa* L. var. *latisecta*, the fraction was dissolved by 0.4% (v/v) aqueous acetone but did not use Tween-80, and the rest procedure was similar to those for beta-cypermethrin except that the fraction concentrations were 10,100 and 1000 μg/ml. All experiments were performed in triplicate. The average increased percentages of photon emission

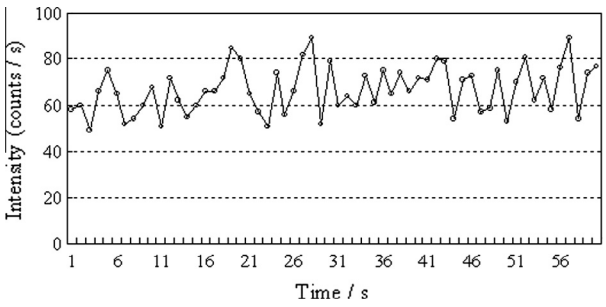

Fig. 2. Time distribution of dark count noise of the PMT.