



The botanical pesticide derived from *Sophora flavescens* for controlling insect pests can also improve growth and development of tomato plants



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ABSTRACT

Sophora flavescens alkaloids (SFA), a promising botanical pesticide, have been registered and commercialized in China. In this study, we attempted to investigate the effect of SFA on the growth and development of tomatoes. Tomato seedlings were exposed to SFA at three dosages: 333.0, 166.5 and 111.0 mg L⁻¹. The morphological and physiological responses were recorded after spraying during the vegetative period, and yield and fruit quality after spraying during the reproductive period. SFA treatment increased the height and stem diameter of the seedlings as well as having a positive effect on almost all physiological leaf characteristics. Early yield and fruit quality were also improved. These findings suggest that the application of SFA has a favorable effect on the growth and yield of tomatoes, improving overall quality and antioxidative performance.

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1. Introduction

With the increasing demand for organic food, botanical pesticides, due to their biodegradability, systemicity, capacity to alter the behavior of target pests and safe profile (Dubey et al., 2010; Isman, 2006), have become a hot topic. By early 2014, approximately 69 botanical pesticide products were registered and commercialized in the United States (Zhang et al., 2015); however, they are yet to be widely applied in agricultural production, and occupy only a small market share (for example <0.05% of all pesticides used in California in 2011). This is partly due to the limited information on application, efficacy and safety of many of these products (Foerester et al., 2001; Isman and Grieneisen, 2014; Sola et al., 2014).

The alkaloids extracted from the roots and stem of *Sophora flavescens* (Leguminosae, Sophora) include a number of water-soluble alkaloids such as matrine, sophoridine, sophocarpine and cytosine, which have a wide range of medicinal and pesticidal activity (Fu et al., 2005; Wang et al., 2012). SFA is currently the most extensively used pesticide product, its registration ranking first among botanical pesticides in China, with 70 manufac-

turers and 92 registration products according to the statistics for October 2015 (<http://www.chinapesticide.gov.cn/hysj/index.jhtml>). Furthermore, most products are registered for use on vegetables for the control of aphids, caterpillars and other pests. A few products are also used to control plant diseases such as venturia, downy mildew pathogen and powdery mildew (Zhou, 2009).

In addition to its insecticidal and antimicrobial activity, SFA has also been shown to be active in the regulation of plant growth. For example, application of SFA was found to promote the fresh and dry weights of cucumber cotyledon as well as the cane sugar content of wheat leaves (Wang et al., 2000; Zhao et al., 1987). Furthermore, the application of *S. alopecuroides* alkaloids, which include similar components to SFA, was found to stimulate the growth of tomato seedlings and increase tomato production (Xiong et al., 2015). However, despite these findings, no systemic report has yet to document the effect of SFA on crop growth.

Given the outstanding control efficiency and safe profile of SFA, understanding the effect on crop growth from both a physiological and biochemical perspective is important in determining the mechanism of regulation and providing a basis for further application of this botanical pesticide. In the present study, the effects of SFA treatment on tomato plant growth and development as well as its antioxidative effect were investigated by examining the morphological and physiological responses and effect on fruit quality and yield.

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2. Materials and methods

2.1. Plant materials

Seeds of tomato (*Solanum lycopersicum* L. cv. 'Sunshine 906') were purchased from Yangling market, Xianyang, Shaanxi Province, PR China. SFA (50% sophoridine, 30% matrine, and other alkaloids) were donated by Golden Camel Pharmaceutical Company Ltd., China.

2.2. Plant culture and treatment

The concentration of SFA stock solution used in the present study was 5.00 g in 100 mL according to the manufacturer's instructions. This was then diluted to 166.5 mg L⁻¹ (the recommended dose), 333.0 mg L⁻¹ (two-fold dose), and 111.0 mg L⁻¹ (two-thirds dose), respectively. As a result, five treatments were applied as follows: blank control, 25.0 mg L⁻¹ avermectin (pesticide control), 333.0 mg L⁻¹ SFA (T1), 166.5 mg L⁻¹ SFA (T2), and 111.0 mg L⁻¹ SFA (T3).

According to normal agronomic practice, tomatoes were grown in plastic pots in a greenhouse (Research & Development Center of Biorational Pesticide, Northwest A&F University, China). At the four-leaf stage, uniform seedlings were selected and divided into 15 groups (20 pots per group) following a randomized block design with three replications. SFA was then applied to the leaves every 7 days during the vegetative period (starting from the four-leaf stage), three times consecutively at a spray volume of 5, 7 and 10 mL/plant, respectively. During the reproductive period (starting at the nine-leaf stage), SFA was applied every 7 days, three times consecutively at a spray volume of 25, 35 and 50 mL/plant, respectively. Samples were collected 1, 3, 7, 11 and 15 days after the third application.

2.3. Morphological assessment

Measurements of seedling morphology were taken based on our previous research (Xiong et al., 2015). Plant height, stem diameter, leaf number, and the length and width of the largest leaf were assessed. Data were reported as the mean value of three replicates (five seedlings per replicate) for each treatment.

2.4. Physiological and biochemical measurements

For assessment of antioxidant enzymes, fresh leaf samples (0.5 g) were ground with 8 mL of pre-cooled phosphate buffer (50 mM, pH 7.8) and 1% (w/w) polyvinylpyrrolidone (PVP). The homogenates were then centrifuged at 8,000 × g for 15 min at 4 °C and the resulting supernatants used for enzyme assay. According to the methods proposed by Zhang et al. (2010), activities of superoxide dismutase (SOD), peroxidases (POD), and catalase (CAT) were detected.

The chlorophyll content of the tomato leaves was determined as described by Arnon (1949) and the content of malondialdehyde (MDA) using the TBA method (Zhao et al., 2007). Proline was determined using the method of Bates et al. (1973) and expressed as μg g⁻¹ dry matter. The contents of phenols and flavonoids were estimated according to the method proposed by Mirecki and Teramura (1984) with some modifications. Fresh leaf samples (1 g) were homogenized in 8 mL of acidified methanol (methanol:HCl=99:1, V/V) then incubated for 24 h at 4 °C. After incubation, the UV-B absorbing pigments were analyzed, with changes at 280 and 320 nm indicative of the relative concentration of phenols and flavonoids, respectively. Electrolyte leakage by the leaf samples was assayed as the relative conductivity following the method of

Ekmekci and Terzioglu (2005), and root vigor determined using the triphenyltetrazolium chloride (TTC) method (Wang, 2007).

2.5. Yield and yield component assessment

To assess yield, the total weight of tomatoes on the first three clusters was measured until full maturity. Overall yield was then determined as the sum of these weights, with data representing the mean of three replicates (five plants per replicate) per treatment.

To assess yield components, approximately 10 tomatoes were homogenized with distilled water. The content of soluble sugar was then detected using the anthrone method and the content of titratable acid measured using the alkali titration method. Vitamin C content was assayed using the 2,6-dichloro indophenol method (Wang, 2007) and the nitrate content measured based on the method of Luo and Cai (2004).

2.6. Statistical analysis

One-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test ($P < 0.05$) was used to determine the differences among means. All data represent the mean ± standard deviation (SD). All analyses were carried out using SPSS Software 13.0.

3. Results

3.1. Effect of SFA on seedling morphology

Effects of different concentrations of SFA on seedling morphology after three applications compared with the blank control are shown in Table 1. Relative growth rates increased by 62.89, 61.64, and 27.67% at 333.0, 166.5 and 111.0 mg L⁻¹ SFA, respectively. Net increments of stem diameter also increased with increasing SFA by 51.40, 53.73 and 61.12% at 111.0, 166.5 and 333.0 mg L⁻¹, respectively. Average increases in leaf number in individual tomato plants and the length-width ratio of the largest leaf showed no significant differences between SFA treatment and the blank control. SFA application during the vegetative period therefore promoted stem growth, but had no effect on the leaves. In avermectin treatment, the seedling morphology showed no significant differences compared with blank control, but relative growth rates and stem diameter were significant lower than SFA applications, the decrease ratio were 8.56–38.50% and 42.30–51.44%.

3.2. Effect of SFA on antioxidant enzyme activity

To determine the effects of SFA on antioxidant enzymes, SOD, POD and CAT activity in tomato leaves pre-treated with different concentrations of SFA were investigated (Table 2). Overall levels of SOD activity increased in SFA treatments compared to the blank and avermectin control, especially on day 3. However, the difference in SOD activity between the blank control and avermectin control was not significant except day 11. POD activity also increased with SFA treatment by 9.86–55.50% and 0.00–24.67% at 333.0 and 166.5 mg L⁻¹ SFA, respectively; however, only a minor increase was observed at 111.0 mg L⁻¹ SFA. Similarly, CAT activity increased with increasing SFA by 9.33–17.80%, 7.15–33.77% and 25.41–45.29% at 111.0, 166.5 and 333.0 mg L⁻¹ SFA, respectively. In avermectin treatment, the POD and CAT activity decreased significantly compared to the SFA treatments.

3.3. Effect of SFA on non-enzymatic antioxidants

The effects of SFA on the flavonoid, total phenol and proline content of the leaves of treated tomato plants are illustrated

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