Effects of Three Plant Extracts on Growth and Development of Dodder and Soybean and on Protective Enzymes of Host

Jing Wan1*, Jun Xu1*, Mingyan Yang1, Zhende Yang*, Qinghe Huang*, Shufang Zhao

1. Forestry College of Guangxi University, Nanning, 530004, P.R. China
2. School of Chemistry and Chemical Engineering of Guangxi University, Nanning, 530004, P.R. China

* The authors who contributed equally to this work

Corresponding authors email: dzyang68@126.com. Authors

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Abstract The ethanol extracts of Melia azedarach bark and leaves, Eucalyptus robusta, leaves, Sapium sebiferum leaves were studied which treated to dodder and its host soybean seedlings in different concentrations, the impact of three plants extracts on dodder and soybean seedlings were evaluated at the 15 days after spraying. The results showed that three extracts in low concentrations had no significant influence on the growth and development of dodder and soybean seedling. In high concentrations (0.25 g/mL), Eucalyptus robusta leaves extract caused damage to soybean seedling and dodder reached at 64% and 70% respectively, while the damage of Melia azedarach bark extract was 78% to dodder and only 7% to soybean seedlings. On the other hand, the treatment of Eucalyptus robusta leaves and Melia azedarach bark extract led to superoxide dismutase (SOD) activity of soybean's leaves rising, the highest values were 2.37 times and 2.0 times respectively as much as the control groups. But the effects in activities of peroxidase (POD) and catalase (CAT) were different, which the highest value of POD activity caused by Eucalyptus robusta leaves extract was 2.28 times compared to the control group. Whereas CAT activity rose highest under Melia azedarach bark extract treatment, the maximum was 1.58 times than the control group, which suggested the damage to soybean caused by Eucalyptus robusta leaves extract was associated with its lower activity in CAT.

Keywords Plant extract; Dodder (Cuscuta chinensis Lam.); Melia azedarach; Eucalyptus robusta; Sapium sebiferum; Soybean (Glycine max); Host plant; Protective enzymes

Introduction

Plant is the natural treasury of active compounds, and the secondary metabolites of plant such as flavonoid, alkaloid, nitriles, terpenes, phytoalexin etc. played roles in insecticidal, antibiotic and weedling (Guo, 2003). Since the 1980s, people had been aware of the evil consequences to human being, animals and our environment by the long-term use of chemical pesticide, people have started to exploit biological pesticides with the characteristic of various effects, easily degradable, not easy for pests to produce drug-resistance, pollution-free as well as not easy for humans and animals to get poisoned. Biological pesticides include pesticide, biocide, and herbicide. Some researches showed that biological herbicide has great application value (Habib and Abdul Rahman, 1988; Lee et al., 1997; Duke et al., 2000; Li and He, 2004; Liu et al., 2005; Gao, 2008).

Dodder, Cuscuta chinensis Lam., belonging to Convolvulaceae, Cuscutoideae, Cuscuta, is a kind of annual parasitic herb, whose roots and leaves are degraded; stems can climb, and haustoria stretch into host plant’s skin to get its nutrition (Qin, 2009, China’s Forest Pest, 4: 10). Distributed in most part of China, dodder are important plant quarantine objects, and its host plants exist widely, grow rapidly, do serious long-term harm to crops and garden plants. Since soybean is a common host plant to dodder, in this research, we treated the dodder and soybean seedling with the ethanol extracts of Melia azedarach bark and leaves, Eucalyptus robusta leaves, Sapium sebiferum leaves in different concentrations to study their effects on the growth of dodder and the activity of protective enzymes in soybean, in order to provide...
theory foundation for choice of safe, effective biological pesticide preventing dodder.

1 Results and Analysis

1.1 Effects of three plant extracts on growth and development of dodder and soybean

Three plant extracts had certain effects on growth and development of dodder and soybean (Table 1). Three plant extracts in low concentrations had no significant injury symptoms on dodder and soybean seedling. While in high concentrations (0.25 g/mL), the extracts gave a minimum damage rate of 48%, the most injury rate was up to 78%, which was specifically manifested in growth retardation, the reduction of haustorium number and twine circle number, wilting and withering. Under the treatment of plant extracts with high concentrations, the injury rate of all plant extracts was over 54%, except that of *Melia azedarach* bark was only 7%; for instance, *Eucalyptus* leaves extracts had a rate of 64%. *Melia azedarach* bark extract was detrimental to dodder but very little to soybean seedlings, which suggested the inhibitory effect of *Melia azedarach* bark extract on growth and development of dodder.

1.2 Effects of three plant extracts on protective enzymes of soybean

SOD is the first defense line that protected plant cells from free radical (Hernández et al., 2001), for it can clear the $O_2^-$ away by transforming it to $H_2O_2$ and $O_2$. It plays an important role in protecting plant cells from reactive oxygen species (ROS) and very commonly exists in plants. Under the high concentration (0.25 g/mL), the treatment of all extracts except *Melia azedarach* leaves caused the SOD activity increase of the soybean seedlings leaves. The activity value induced by *Eucalyptus* leaves extract was 2.26 times of the control group, whereas the values of SOD activity, which were induced by *Sapium sebiferum* leaves and *Melia azedarach* leaves at a concentration of 0.05 g/mL, respectively, were only 0.81 and 0.88 times of the control group (Figure 1).

POD plays an essential role in enzymatic system of scavenging active oxygen of plants, namely, which further decomposes $H_2O_2$, the dismutation product of SOD, into $H_2O$ and $O_2$. CAT was the other path to the degradation of $H_2O_2$ in plants, that is, CAT and SOD together clear away $H_2O_2$ and $O_2$ *in vivo* to minimize the formation of hydroxyl radical. POD activity in soybean leaves had changed under treatments of extracts of *Eucalyptus* leaves and *Melia azedarach* leaves, which obviously showed in the following two cases. POD activity, treated by *Eucalyptus* leaves at 0.05 g/mL and 0.01 g/mL, were 2.28 times and 2.04 times respectively of the control group. Treated *Melia azedarach* leaves at 0.25 g/mL, 0.05 g/mL and 0.01 g/mL, the POD activity increased to 2.0 times, 1.75 times and 1.48 times respectively of the control group (Figure 2). Extracts of *Sapium sebiferum* leaves and *Melia azedarach* bark had no significant effect on POD activity of soybean seedlings leaves. Comparing with POD, CAT activity was much significantly affected by *Sapium sebiferum* leaves and *Melia azedarach* bark extracts, whereas under *Eucalyptus* leaves extract treatment, the CAT activity was only slightly higher than the control group (Figure 3).
Table 1 The inhibition effects of three plant extracts on dodder’s growth and development and on host’s damage

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>Concentration (g/mL)</th>
<th>Symptom</th>
<th>Damage degree (%)</th>
<th>Fresh weight (g/plant)</th>
<th>Symptom</th>
<th>Damage degree (%)</th>
<th>Fresh weight (g/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eucalyptus leaves</td>
<td>0.00</td>
<td>Normal growth and development</td>
<td>0.0</td>
<td>0.145±0.01a</td>
<td>Normal growth and development</td>
<td>0.0</td>
<td>2.6±0.19b</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>No wilting, slow growing</td>
<td>16.0</td>
<td>0.134±0.03a</td>
<td>Leaves yellowing</td>
<td>8.0</td>
<td>2.1±0.10ab</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>Part wilting, loose twining</td>
<td>44.0</td>
<td>0.104±0.01a</td>
<td>Dead spots partly appearing in leaves</td>
<td>22.0</td>
<td>1.8±0.12a</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>Wilting, dead</td>
<td>70.0</td>
<td>0.115±0.03a</td>
<td>Dead spots fully appearing in leaves</td>
<td>64.0</td>
<td>1.9±0.29a</td>
</tr>
<tr>
<td>Sapium sebiferum leaves</td>
<td>0.00</td>
<td>Normal growth and development</td>
<td>0.0</td>
<td>0.127±0.02a</td>
<td>Normal growth and development</td>
<td>0.0</td>
<td>2.7±0.12a</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>No obvious damage</td>
<td>6.0</td>
<td>0.12±0.02a</td>
<td>Chlorotic leaves appearing</td>
<td>7.0</td>
<td>2.9±0.33a</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>Wilting, few twines</td>
<td>44.0</td>
<td>0.108±0.02a</td>
<td>Leaves partly appearing dead</td>
<td>24.0</td>
<td>2.6±0.37a</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>Same as above</td>
<td>48.0</td>
<td>0.103±0.03a</td>
<td>Lots of leaves dead</td>
<td>54.0</td>
<td>2.9±0.19a</td>
</tr>
<tr>
<td>Melia azedarach leaves</td>
<td>0.00</td>
<td>Normal growth and development</td>
<td>0.0</td>
<td>0.153±0.01a</td>
<td>Normal growth and development</td>
<td>0.0</td>
<td>2.6±0.19a</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>No wilting, slow growing</td>
<td>18.0</td>
<td>0.154±0.05a</td>
<td>Not obvious hurt</td>
<td>4.0</td>
<td>2.7±0.34a</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>Slowly growing, few twine</td>
<td>30.0</td>
<td>0.105±003a</td>
<td>Leaves yellowing</td>
<td>10.0</td>
<td>2.2±0.25a</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>Wilting, Half of leaves death</td>
<td>60.0</td>
<td>0.065±0.01a</td>
<td>Large area dead spots appearing in leaves</td>
<td>58.0</td>
<td>2.0±0.16a</td>
</tr>
<tr>
<td>Melia azedarach bark</td>
<td>0.00</td>
<td>Normal growth and development</td>
<td>0.0</td>
<td>0.121±0.02a</td>
<td>Normal growth and development</td>
<td>0.0</td>
<td>2.6±0.19a</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>Slow growing, few twine</td>
<td>28.0</td>
<td>0.081±0.01a</td>
<td>No obvious hurt</td>
<td>4.0</td>
<td>3.1±0.51a</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>Wilting, no twine</td>
<td>54.0</td>
<td>0.101±0.01a</td>
<td>No obvious hurt</td>
<td>5.0</td>
<td>2.5±0.32a</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>Mostly dead</td>
<td>78.0</td>
<td>0.08±0.01a</td>
<td>Leaves yellowing slightly</td>
<td>7.0</td>
<td>2.5±0.27a</td>
</tr>
</tbody>
</table>

Note: Data of the experiment in the table was the value of the average ± standard error repeated five times; Multiple comparisons were done with Duncan new multiple range method, and there is no significant difference (p<0.05) among the same letters after the same column by Duncan test.

2 Discussion
It is crucial to the chemical control of dodder in protecting the normal growth of the host plant; the use of plant extracts is an attempt for prevention and treatment of dodder. After studying the effects of several weed extracts on the growth of dodder and its host, Hobib and Abdual Rahman (1988) pointed out that the Burmudagrass methanol extract included substances that suppressed dodder growth and affected the security of the host alfalfa, in addition, and there were 10 components identified. Zhou et al (2008) pointed out that the extracts of *Eucalyptus, Sapium, Melia* all had the active substances for deinsectization and weeding (Wei, 2005; lin et al., 2007; Zhou et al., 2008); Jiang (2004) indicated that mersosin was the major active substance in *Melia azedarach* bark. The results of this study showed that *Eucalyptus robusta* leaves extract at the high concentration (0.25 g/mL)
caused strong damage to soybean seedling and dodder, while the *Melia azedarach* bark extract at high concentration only did great harm to dodder but safe for soybean, which suggested *Eucalyptus* extract could not be used for the prevention and treatment of dodder in soybean fields, whereas the *Melia* bark extract would be expected to develop a biological pharmaceutical for the control of dodder in soybean fields.

Plants can produce a certain amount of reactive oxygen free radicals in normal environment or in adversity. In normal physiological condition, production and scavenging of active oxygen in plants is in a state of dynamic equilibrium. CAT, SOD, POD and other enzymes coordinate with each other to keep the reactive oxygen free radicals in the plants at a low level, so the plant cells are preserved from harm; in contrast, the metabolic balance of production and scavenging of active oxygen was damaged under stress conditions, that is, excessive accumulation of reactive oxygen leads to the plant cell membrane lipid peroxidation, which resulted into the destruction of cell membrane structure and function; demonstrating the injury symptoms (Hernandez et al., 2003; Apel and Hirt, 2004). It was indicated that activity changes of these three enzymes in plants could be used to evaluate the extent of plant hurt, which provided a theoretical basis for the selection of the secure extract for the host soybean.

This study demonstrated the extracts of *Eucalyptus robusta* leaves and *Melia azedarach* bark had different effects on the protective enzyme system of the host soybean leaves. Although both treatment groups caused the obvious elevation of SOD activity, producing more poisonous H$_2$O$_2$, they had obviously different effects on POD and CAT, which were eliminating H$_2$O$_2$. When the soybean was dealt with the extract of *Eucalyptus robusta* leaves, activity of POD went up obviously, while the CAT activity merely was a little higher compared to the control group; when the soybean was dealt with the extract of *Melia azedarach* bark, activity of CAT went up obviously, while the POD activity was no significantly affected. Taking the extent of hurt into consideration, we can draw the conclusion that the degradation of poisonous H$_2$O$_2$ in soybean leaves is mainly coupled with CAT, then POD, namely, SOD activity obvious elevation produced much poisonous H$_2$O$_2$; whereas, the enzyme activity of CAT, carried on eliminating H$_2$O$_2$, was relatively weak, caused H$_2$O$_2$ accumulation *in vivo*, which was the physiological mechanism of emerging obvious injury symptoms in host soybean. On *Jerusalem artichoke*, Huang et al. (2011) found that the activity of POD and SOD was obviously higher than that of CAT, therefore they concluded that the superoxide anions in *Jerusalem artichoke* leaves is mainly degraded by SOD-POD, then SOD-CAT. Similar to the above-mentioned study, in this study, we found that the effect of SOD-CAT in degradation of superoxide anions in soybean leaves is apparently superior to SOD-POD. After the treatment by the extracts of *Eucalyptus robusta* leaves, low activity of CAT in soybean seedling leaves can't effectively eliminate the poisonous H$_2$O$_2$, developing obvious injury symptoms; on the contrary, after treated by the extracts of *Melia azedarach* bark, higher activity of CAT in soybean seedling leaves can effectively degrade the poisonous H$_2$O$_2$, developing no significant injury symptoms.

In this study, it was concluded that the ethanol extract of *Melia azedarach* bark can inhibit the growth and development of the dodder but secure to the host soybean; however, what substance on earth that inhibit the growth and development of dodder still remains unknown. In order to investigate the substance that significantly inhibit the growth and development of dodder, further research is needed on isolation and
identification of the ethanol extract of *Melia azedarach* bark. This current work plays a great significance on the development for new bio-pesticide of dodder control.

3 Methods and Materials

3.1 Preparation of plants ethanol extracts

Three plants, *Melia azedarach* bark and leaves, *Eucalyptus robusta* leaves, *Sapium sebiferum* leaves were collected from campus in Guangxi University. The materials were left to dry at room temperature and smashed respectively, and then 500 g from each were taken and macerated in a blender with 95% alcohol in a ratio of 1:10 w/v. The mixture was allowed to stand 24 hours under refrigeration. After filtration, extracts were evaporated to dryness under pressure of 0.5~0.7 at 80°C. The dry extracts were dissolved in water to the desired concentrations: 0.00 g, 0.01 g, 0.05 g, and 0.25 g dry weight material per milliliter of water.

3.2 Controlled experiment of plant extracts to dodder

The host soybean was sowed in the internship base of Forestry College of Guangxi University. 10 cm in length stem of dodder picked from two-year *Duranta repens* cv. golden leaves was inoculated to soybean, when the soybean seedlings reached 40 cm in height. After dodder parasitized successfully, extracts in different concentrations were sprayed on the dodder and soybean. Each concentration repeated five times, while the control group was treated by distilled water. Fifteen days later, treatment groups were evaluated. Dodder control was visually estimated on a scale of 0 (for no effect) to 100 (for 100% effectiveness). The same scale was used to evaluate treatment injury to soybean foliage. Dodder and soybean fresh weights were taken for each treatment. All statistical analyses and tests were conducted according to the methods designated by Habib and Abdul Rahman (1988).

3.3 Protection enzymes activity of soybean seedling assay

Protection enzymes activity was assayed, when the soybean seedlings were treated by extracts after fifteen days. POD activity was determined with the quaiac-based phenol colorimetric method. CAT activity was measured with ultraviolet spectrometry. SOD activity was measured with NBT illumination method (Chen and Wang, 2006; Zheng, 2006).

3.4 Data analysis

Experiment data were analyzed with software of Excel and SPSS17.0; all data were expressed as the average ± standard error. Multiple comparisons were done with Duncan new multiple range method, and there was no significant difference (p<0.05) among the same letters after the same column by Duncan test.

Authors’ Contributions

JW and JX analyzed the data and wrote the manuscript. ZDY conceived and designed the experiment as well as modified the manuscript. JW, JX, MYY, QHQ and SFZ preformed the experiment. All authors have read and approved the final manuscript.

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