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*Full Length Research Paper*

# Insecticidal activities of ethanol extracts from thirty Chinese medicinal plants against *Spodoptera exigua* (Lepidoptera: Noctuidae)

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**Chinese medicinal plants extracts of a group of 30 species from 20 different botanical families, were screened for contact toxicity and antifeedant activities against *Spodoptera exigua*. The extracts from eight of the Chinese medicinal plants showed strong activity against the insect. The most effective plant extracts were from *Litsea cubeba* (Lour.) Pers., *Brucea javanica* (L.) Merr. and *Artemisia argyi* Lévl. et Vant. Three of the extracts exerted strong antifeedant activity to the insect.**

**Key words:** Plant extract, contact toxicity, antifeedant activity, *Spodoptera exigua* Hübner.

## INTRODUCTION

The *Spodoptera exigua* Hübner is a polyphagous insect pest, and is considered a major pest in many agricultural areas of the world in vegetable, field, and flower crops (Taylor et al., 2008). At present, controlling of *S. exigua* is primarily dependent on chemical insecticides. As the farmers use chemical pesticides repeatedly to control the pest, the *S. exigua* has developed resistance to various insecticides, making its control increasingly difficult (Brewer and Trumble, 1989; Van Laecke and Degheele, 1991). The success of modern agricultural practices is in part due to chemicals for pest control (Dayan et al., 2009), but it has different drawbacks, such as the development of resistance (Castillo et al., 2009) and impacts on non-target organisms (Akhtar and Isman, 2004). With the discovery and utilization of neem-derived insecticides, interest has grown in botanical compounds with insecticidal or antifeedant activity (Isman, 1994; Isman et al., 1997).

More recently, non-neem extracts derived from different plant species have been proved to have insecticidal activity against different pest species. Aqueous extracts of nine medicinal plants belonging to families Apiaceae, Asteraceae, Lamiaceae, etc showed significant toxicity to the sweet potato white fly, *Bemisia tabaci* Genn (Ateyyat et al., 2009).

Erler et al. (2009) tested eight botanical materials to control mushroom phorid fly and suggested that hot-water extracts of *Origanum onites* and *Pimpinella anisum* may be potential alternatives to conventional pesticides. Elango et al. (2010) found that the hexane extracts of *Aegle marmelos* and *Andrographis paniculata* had potential ovicidal and oviposition deterrent activities against *Culex tritaeniorhynchus* Giles. The botanical extracts have been used as conventional insecticides for centuries, and the active compounds can reduce the potential chances of insect resistance to the natural complex (Feng and Isman, 1995). In the past, active phytochemicals from botanicals have been successfully used as a lead to develop new synthetic pesticides, which are in worldwide use today (Klocke, 1987). Therefore, the study of the effects of plant materials extracts upon pests can lead to the discovery of alternative insecticides. However, systematic studies of Chinese medicinal plants against insects were very scarcity (Chiu, 1985). In this paper, we report our findings on contact and antifeedant bioassay activity of Chinese medicinal plants on *S. exigua*. Isolation of active compounds in one of the most active species will be undertaken in our future work.

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**Table 1.** Chinese medicinal plants studied for insecticidal activities.

| Botanical name | Common name | Family name | Parts used |
|---|---|---|---|
| *Catharanthus roseus* (L.) G. Don | Madagascar periwinkle herb | Apocynaceae | Whole grass |
| *Aristolochia mollissima* Hance | Wooly datchmanspipe herb | Aristolochiaceae | Overground part |
| *Lonicera japonica* Thunb. | Flos lonicerae | Caprifoliaceae | Flower |
| *Quisqualis indica* L. | Fructus quisqualis | Combretaceae | Fruit |
| *Artemisia argyi* Lévl. et Vant. | Argy wormwood | Compositae | Whole grass |
| *Bidens pilosa* L. | Railway beggarticks | Compositae | Whole grass |
| *Carpesium abrotanoides* L. | Fructus carpesii | Compositae | Fruit |
| *Eclipta prostrasta* L. | Yetbadetajo hert | Compositae | Overground part |
| *Rhaponticum uniflorum* (L.) DC. | Radix rhapontici | Compositae | Root |
| *Erysimum bungei* (Kitag.) Kitag. | Wormseed mustard | Cruciferae | Whole grass seed |
| *Perilla frutescens* (L.) Britt. | Fructus perillae | Labiatae | Fruit |
| *Buddleia lindleyana* Fort. | butterfly bush | Loganiaceae | Whole grass |
| *Litsea cubeba* (Lour.) Pers. | Fructus litseae | Lauraceae | Fruit |
| *Astragalus membranaceus* (Fisch.) Bunge | Milkvetch root | Leguminosae | Root |
| *Erythrina variegata* L. | Bark of oriental variegated coralbean | Leguminosae | Root/bark |
| *Fritillatia thunbergii* Miq. | Zhejiang fritillary | Liliaceae | Bulb |
| *Huperzia serrata* (Thunb.) Trev. | Snakefoot clubmoss | Lycopodiaceae | Whole grass |
| *Lycopodium japonicum* Thunb. | Staghorn clubmoss | Lycopodiaceae | Whole grass |
| *Stephania tetrandra* S. Moore | Radix Stephaniae Tetrandrae | Menispermaceae | Root |
| *Menispermum dauricum* DC. | Asiatic moonseed rhizome | Menispermaceae | Rhizome |
| *Ligustrum lucidum* Ait. | Fructus ligustri lucidi | Oleaceae | Fruit |
| *Polygala tenuifolia* Willd. | Thinleaf milkwort root | Polygalaceae | Root |
| *Aconitum ochranthum* C. A. Mey. | Herb of puberulent monkshood | Ranunculaceae | Root |
| *Evodia rutaecarpa* (Juss.) Benth. | Medicinal evodia fruit | Rutaceae | Fruit |
| *Dictamnus dasycarpus* Turcz. | Densefruit pittany root-bark | Rutaceae | Root/bark |
| *Citrus grandis* (L.) Osbeck var. *tomentosa* Hort. | Pummelo peel | Rutaceae | Pericarp |
| *Brucea javanica* (L.) Merr. | Java brucea fruit | Simaroubaceae | Fruit |
| *Saposhnikovia divaricata* (Turcz.) Schischk. | Rdix saposhnikoviae | Umbelliferae | Root |
| *Bupleurum chinense* DC. | Chinese thorowax root | Umbelliferae | Root |
| *Urtica laetevirens* Maxim. | Nettle | Urticaceae | Whole grass |

## MATERIALS AND METHODS

### Plant materials and preparation of crude extracts

The Chinese medicinal plants (Table 1) were purchased in July, 2008 from local traditional medicine markets in Anguo city, Hebei province and cleaned with tap-water for deleted dust and dried in shade under laboratory conditions for more. The plant materials were chopped into small pieces (<2 cm) and dried at room temperature before being ground with a blender. Plant powder (100 g) was extracted three times each with 500 ml ethanol at 28°C for 24 h, and dealt with microwave-oven for 30 min in an 800 W. The three extracts were obtained by filtering with filter paper and then combined. Then, each extraction of plants was evaporated to dryness at 40°C under vacuum conditions and kept at 4°C for bioassay.

### Tested insects

Larvae of *S. exigua* were reared with artificial diet (corn flour and soybean flour mixed with yeast, 20 : 10 : 9, w/w/w) and maintained at 26 ± 1°C, 70 ± 2% RH and a photoperiod of 16 : 8 (L : D) in the laboratory. Third instar larvae for used.

### Bioassays

### Contact toxicity (CT)

Topical application was used to test contact activity of ethanol extracts against *S. exigua*. Dilution of the crude extract was prepared at 10 mg per ml of solvent (10 mg: ml) in acetone. Each extract was applied topically at doses of 0.5 µl per insect (5 µg/insect) dorsally to the thorax of larvae of *S. exigua*, using a Burkard Arnold microapplicator. Controls were determined using acetone. Ten insects were used for each concentration and control, and the experiment was replicated for three times. Both treated and control insects were then transferred to culture boxes (10 insects/ culture boxes) with culture media and kept in incubators at 26 ± 1°C, 70 ± 2% relative humidity. Mortality was observed daily for three days after treatment.

### Antifeedant activity

A leaf-dipping method was used to determine larvicidal activities of test samples against *S. exigua*. Leaf disks (1.5 cm diameter) of Chinese cabbages were prepared. Crude extracts were dissolved in acetone to make the dilutions at 10 mg: ml. Leaf bits of equal size from the cabbage were dipped in test solution for 3 s and then the solvent was evaporated. Such treated bits were transferred to a petri dish (9 cm diameter) with moist filter paper. The third instar larvae of *S. exigua* were starved for 3 h and then released in each replication individually. Ten insects were used for each concentration and control, and the experiment was replicated for three times. Observation on after 24 h were recorded and subjected to analysis of feeding deterrence. The feeding-deterrence index was calculated (Isman et al., 1990) as:

$$(FDI)(\%) = 100 \times (C-T) / C$$

Where *C* is the consumption of control disks, and T is the consumption of treated disks.

### Statistics

Data were analyzed by means of ANOVA procedures, and means were compared by the Tukey's test (SPSS 13.0), at a significance level of $\alpha = 0.05$.

## RESULTS

### Contact toxicity

The ethanol extracts from 30 medicinal plants were tested for their contact efficacy to *S. exigua* by the topical application method at the dose of 5 µg / insect. Table 2 has given contact toxicity of crude extracts on third instar larvae of *S. exigua*. All the crude extracts had weak toxicity to the test insect. Extracts of *Fritillatia thunbergii* gave 33.33% of mortality after 72 h, and *Brucea javanica* gave 30% mortality, no significant differences were detected among the extracts.

Some extracts of Chinese medicinal plants such as *Aconitum ochranthum, Aristolochia mollissima* etc., did not show any contact toxicity.

### Antifeedant activity

Extracts of 7 Chinese medicinal plants including *Artemisia argyi, Evodia Rutaecarpa, Stephania tetrandra, A. ochranthum, B. javanica, Polygala tenuifolia* and *Litsea cubeba* showed a significant antifeedant activity, because 60 to 96.64% FDI was produced when larvae fed the tested leave that treated with extracts in 10 mg/ml. *L. cubeba* and *B. javanica* extracts gave a stronger direct feeding deterrent, the FDI were 96.64 and 86.86% independently.

Other significant values of FDI (30 to 60%) were induced by extracts of *Bidens pilosa*, *Carpesium abrotanoides*, *Eclipta prostrasta, F. thunbergii*, *Lonicera japonica, Saposhnikovia divaricata*, *Quisqualis indica*. Otherwise, 12 Chinese medicinal plants did not have any antifeedancy to the larvae of *S. exigua*; among them, *A. mollissima, B. lindleyana, D. dasycarpus, E. variegata, L. lucidum, M. dauricum* and *P. frutescens* did not show any contact toxicity too.

## DISCUSSION

In recent years, interest in screening plants for novel insecticides has increased significantly (Weinzierl, 1998). Searching for plant-derived chemicals that have potential use as crop protectants (insecticides, antifeedants, growth inhibitors) often begins with the screening of plant extracts (Akhtar and Isman, 2004). Chariandy et al. (1999) screened 29 species used in traditional medicine in Trinidad for insecticidal properties using *Aedes aegypti*. Liu et al. (2007) screened 40 species of Chinese medicinal herb from 32 different botanical families for contact, fumigant and feeding deterrent activities against two stored-grain insects *Sitophilus zeamais* and *Tribolium castaneum*. Huang et al. (2007) screened of ethanol extracts from 20 medicinal plants for contact and repellency activities against *Rhizopertha dominica* (Fab.). Huang et al. (1994) isolated nine limonoids active as antifeedants against *S. exigua* from the root bark of Chinese *Melia azedarach* L. Over 2000 species of plants are known to have some insecticidal activity (Klocke, 1989).

Several investigators have reported activity in extracts of species related to our study. The results from our study revealed that some of the plants extracts tested had insecticidal activity against *S. exigua* in varying degrees. *L. cubeba* and *B. javanica* extracts had significant toxicity against the *S. exigua* larvae.

In this study, we also observed that many of the treated larvae were smaller and weaker compared to those in the acetone-treated control. Jiang et al. (2009) assessed the toxicity of essential oil of *L. cubeba* against third-instar *Trichoplusia ni* larvae, and suggested that $\gamma$-terpinene accounted for much of the toxicity of the oils to the insects. It was reported that *B. javanica* and compounds isolated from it had antifeedant and insect growth activity against *Myzus persicae*, *Heliotbis virescens*, *Agrotis ipsilom* and *Spodoptera frugiperda* (Plonshy et al., 1989; Lidert et al., 1987; Kloche et al., 1985). These findings indicate that *L. cubeba* and *B. javanica* extracts may be good alternatives to the chemical insecticides used for *S. exigua* control.

Yang and Tang (1988) reviewed a table of 267 pesticidal plants that comes from experience of Chinese people. Most of Chinese traditional medicinal herbs were studied for pharmaceutical uses, and while many compounds have been isolated, but there is a lack of scientific laboratory data. Though insecticidal plants had been studied severally, most of research work was based on the stage of direct utilization, so the research of active compounds and action mechanism must be enhanced (Lowery and Isman, 1995).

This study indicates that isolation of active compounds from the most active species based on these extracts

**Table 2.** Contact and antifeedant efficacy from 30 medicinal plants extracts against *Spodoptera exigua.*

| Plant species | Contact toxicity (%) | | | Feeding-deterrency Index (FDI %) |
|---|---|---|---|---|
| | 24 h | 48 h | 72 h | |
| *Aconitum ochranthum* | — | — | — | 75.32 ± 3.46 bc |
| *Aristolochia mollissima* | — | — | — | — |
| *Artemisia argyi* | 13.33 | 20 | 26.67 | 78.85 ± 4.31 bc |
| *Astragalus membranaceus* | 3.33 | 3.33 | 3.33 | — |
| *Bidens pilosa* | — | — | — | 57.72 ± 3.76 def |
| *Brucea javanica* | 20 | 26.67 | 30 | 86.86 ± 3.78 ab |
| *Buddleia lindleyana* | — | — | — | — |
| *Bupleurum chinense* | 13.33 | 20 | 30 | 8.09 ± 2.29 h |
| *Carpesium abrotanoides* | — | — | — | 48.32 ± 5.72 f |
| *Catharanthus roseus* | — | — | — | 10.08 ± 2.16 h |
| *Citrus grandis* | 0 | 3.33 | 3.33 | — |
| *Dictamnus dasycarpus* | — | — | — | — |
| *Eclipta prostrasta* | — | — | — | 54.20 ± 5.71 ef |
| *Erysimum bungei* | 3.33 | 10 | 10 | 48.86 ± 5.80 f |
| *Erythrina variegata* | — | — | — | — |
| *Evodia rutaecarpa* | 10 | 20 | 20 | 70.09 ± 5.55 cd |
| *Fritillatia thunbergii* | 26.67 | 30 | 33.33 | 45.04 ± 6.41 fg |
| *Huperzia serrata* | 6.67 | 6.67 | 10 | — |
| *Ligustrum lucidum* | — | — | — | — |
| *Litsea cubeba* | 6.67 | 10 | 10 | 96.64 ± 1.94 a |
| *Lonicera japonica* | — | — | — | 31.59 ± 4.35 g |
| *Lycopodium japonicum* | 0 | 3.33 | 3.33 | — |
| *Menispermum dauricum* | — | — | — | — |
| *Perilla frutescens* | — | — | — | — |
| *Polygala tenuifolia* | 6.67 | 6.67 | 6.67 | 73.20 ± 4.80 bc |
| *Quisqualis indica* | 10 | 16.67 | 16.67 | 32.36 ± 6.34 g |
| *Rhaponticum uniflorum* | 13.33 | 20 | 20 | — |
| *Saposhnikovia divaricata* | 6.67 | 10 | 10 | 33.50 ± 5.45 g |
| *Stephania tetrandra* | 13.33 | 16.67 | 20 | 66.85 ± 6.06 cde |
| *Urtica laetevirens* | 3.33 | 10 | 16.67 | 15.01 ± 3.57 h |

—, inactive, Feeding-deterrence index (FDI) (%) =100×(C–T)/C, C is the consumption of control, and T the consumption of treated insects.

may be feasible. The next work is to select the most insecticidal active plants from which to isolate the active compound(s).

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