Bioactivities of jimsonweed extract, *Datura stramonium* L. (Solanaceae), against *Tribolium castaneum* (Coleoptera: Tenebrionidae)

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Received: 06.04.2010

**Abstract:** In this research, the efficacy of plant extract from *Datura stramonium* L. (Solanaceae) was tested against the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), for its toxic and antifeedant activity. The dried and powdered plant was extracted with 150 mL of absolute ethanol (99%, Merck) using a rotary evaporator extractor at 45 °C. Experiments were designed to measure the nutritional indices, such as relative growth rate (RGR), relative consumption rate (RCR), efficiency of conversion of ingested food (ECI), and feeding deterrence index (FDI). Treatments were evaluated by flour disk bioassay in the dark at 27 ± 1 °C and 60 ± 5% RH. Aliquots of 10 μL of acetone (control) or acetone extract (947-3007 mg L$^{-1}$) were spread evenly on the flour disks. The solvent was allowed to evaporate, and then 10 adult insects were introduced into each treatment. After 72 h, nutritional indices were calculated. On the basis of the designed experiments, the calculated lethal concentrations of LC$_{50}$ and LC$_{90}$ of *D. stramonium* were 3936 and 15373 mg L$^{-1}$, respectively. Results indicated that nutritional indices varied significantly as plant extract concentrations increased. As the concentration of *D. stramonium* increased, RGR, RCR, and ECI indices were reduced, and the most significant effect was observed at 3007 mg L$^{-1}$. On the other hand, the FDI index increased with increasing extract doses and, as a result, insect food consumption was reduced. The results of data analysis showed that there is a significant difference between the plant and different concentrations in the feeding deterrence index of *T. castaneum*.

**Key words:** Antifeedant indices, *Datura stramonium*, plant extract, *Tribolium castaneum*

**Introduction**

Harvest grains are basic human food products (Padín et al. 2002). The presence of pests constitutes a serious ongoing problem in grain stocking and its derived industry (Pérez Mendoza et al. 2004). Worldwide, between 5% and 10% of the total weight of cereals, oil plants, and legumes are lost due to the presence of pests (Hill 1990).

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is one of the primary pests infesting stored grains. It is widely spread worldwide and very destructive. Due to this high rate of infestation, synthetic insecticides have been used to control it.

Resistance and toxicity problems derived from synthetic insecticides have made it necessary to find
more effective and healthier alternatives. Presently, plant extracts are the most commonly tested alternative products (Papachristos and Stamopoulus 2002; Umoetok and Gerard 2003; Zhang et al. 2004; Tapondjou et al. 2005; Ferrero et al. 2006; Sánchez Chopa et al. 2006; Stefanazzi et al. 2006; Wang et al. 2006). Different biological activities of plant derivatives have been demonstrated for the control of stored-grain pests (Golob et al. 1999; Rajendran and Sriranjini 2008). A wide variety of higher plants may provide new sources of natural pesticides, antifungals, antifeedants, and repellence (Grainge and Ahmed 1988; Arnason et al. 1989; Ananthakrishnan 1992; Çetinsoy et al. 1998; Turkusay and Onogur 1998; Tunaz et al. 2009). Plant extracts are among the numerous plant-derived substances studied and have various effects on insect pests, including stored-product insects (Grainge and Ahmed 1988; Jacobson 1989; Shaaya et al. 1991). Some researchers reported that the acetone extracts of *Piper nigrum* L. and the hexane extract of the dried fruit of *Piper guineense* Schumach. and Thonn. were toxic to 4 species of stored-product insects and were repellent to *Tribolium castaneum* (Herbst) (Su 1983; Sighamony et al. 1984). Georges et al. (2008) reported that *Datura innoxia* Mill. (Solanaceae) compounds possess insecticidal activity [e.g. mosquitocidal against *Ochoteratus triseriatus* Say (Diptera: Culicidae); larvicidal against *Helicoverpa zea* Boddie, *Heliothis virescens* Fabricius (Lepidoptera: Noctuidae), and the whitefly *Bemisia tabaci* Genn. (Homoptera: Aleyrodidae)]. In addition, whole-plant extracts of the perennial common herb *Datura stramonium* L. were defined as toxic for their insecticidal and antifeedant properties against *Dysdercus cingulatus* Fabricius (Hemiptera: Pyrrhocoridae), *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae), and *Pericallia ricini* Fabricius (Lepidoptera: Noctuidae) (Prakash and Rao 1997). Furthermore, the effectiveness of the compounds extracted from different parts of *Datura* spp. on spider mites were investigated under laboratory conditions (Mateeva et al. 2003; Zhang et al. 2006). In addition, Kumral et al. (2009) evaluated the acaricidal, repellent, and oviposition deterrent activity of the seed and leaf extracts of *D. stramonium* on *Tetranychus urticae* Koch (Acari: Tetranychidae).

*Datura stramonium* is a plant distributed throughout most of the temperate regions of the world. In ancient herbal medicine, it was used internally to treat madness, epilepsy, and melancholy. Externally, it formed the basis of ointments for burns and rheumatism. More recently, preparations from the plant have been used as ingredients in some asthma medicines. With this exception, however, the plant is generally considered too toxic for medical applications.

In this study, we assessed the potential of *D. stramonium* plant extract for bioactivities against adults of *T. castaneum*.

Materials and methods

**Insect rearing**

*Tribolium castaneum* adults used as test insects were obtained from laboratory cultures and maintained in the dark in incubators at 27 ± 1 °C and 60 ± 5% RH. *Tribolium castaneum* was cultured on wheat flour ground from whole wheat grain mixed with yeast (10:1, w:w). For the flour disk bioassay, insects were starved for 24 h before use.

**Extraction of plant material**

The plant samples (*D. stramonium* seeds and leaves) used in the experiment were collected from flowering plants between the end of August and the beginning of October from the medicinal plants research station at Shahed University, Tehran, Iran. The plant samples were identified by a member of the Faculty of Agricultural Sciences, Department of Botany, Shahed University, according to the Index of Botanical Plants of Iran. Fresh seeds and leaves were dried under indoor conditions for 5 days. The dried seeds and leaves were powdered with a grinder.

Plant materials (50 g each) were extracted with 200 mL of absolute ethanol (99%, Merck) using a Soxhlet extractor at room temperature for 2 days. The extraction was completed in 3 cycles. The solvent was removed with a rotary evaporator at 55 °C and 200 mm Hg. After filtering through a Buchner funnel, the filtrates were concentrated to dryness by rotary evaporator under low pressure. The extract obtained was a solid composition of *Datura* seed and leaf extract, and it was dissolved in 99% ethanol. The basic concentration was 5000 mg L⁻¹, and the next concentrations (974, 1609, 2254, and 3007) were determined using the N1V1 = N2V2 method from stock concentration.
Contact toxicity

The extract of *D. stramonium* was tested for contact insecticidal activity by impregnating filter papers with 99% ethanol. Petri dishes, 8 cm in diameter, were used to confine the insects. Filter paper disks (Whatman No. 1) cut to be 8 cm in diameter were attached to the bottom of the Petri dishes. A series of concentrations of *D. stramonium* extract (1000, 2000, 3000, 3500, 4000, 4500, and 5000 mg L$^{-1}$) were prepared in acetone, while acetone alone was used for control. The filter papers were exposed to the abovementioned concentrations. Then, 10 adults (1-7 days old) were placed in each Petri dish, and each concentration was replicated 5 times. Petri dishes were kept in incubators until end-point mortality of the adults was reached. The numbers of dead and living insects were counted 3, 6, 9, 12, 24, and 48 h from the beginning of exposure. The mortality data used to estimate LC$_{50}$ and LC$_{90}$ values were analyzed by probit analysis using PROC PROBIT SAS software (SAS Institute 1997).

Flour disk bioassay

Flour disks were prepared according to the method used by Xie et al. (1996) with modifications from Huang et al. (2002). Aliquots of 200 μL of a suspension of wheat flour in water (10 g in 50 mL) were dropped into a Petri dish (8 cm in diameter) to form the disks. The disks were left in the fume hood overnight to dry, and then they were equilibrated at 27 ± 1 °C and 70%-80% RH for 24 h. The weights of the flour disks ranged from 35 to 39 mg, and their average moisture content was 13.5 ± 0.1%. Flour disks were treated with acetone solution with drops from a sampler (10 μL) containing 4 sublethal concentrations, including 947, 1609, 2254, and 3007 mg L$^{-1}$, of *D. stramonium* extract, and with acetone alone for the control. After solvent evaporation, the disks were placed in glass vials (diameter 2.5 cm, height 5.5 cm). A total of 10 group-weighed, unsexed adults, 1-3 days old, were added to each pre-weighed vial, which contained 2 disks. Adult insects were starved for 48 h before treatment. For each concentration, 4 replicates were prepared. After a 72 h exposure, the glass vials with flour disks and live insects were weighed again, and insect mortality, if any, was recorded. Nutritional indices were calculated, as previously described by Huang et al. (2000) with some modifications, as relative growth rate (RGR) = $\frac{(A-B)}{B} \times$ day, where $A =$ weight of live insects on the third day (mg)/no. of live insects on the third day, $B =$ original weight of insects (mg)/original no. of insects; relative consumption rate (RCR) = $\frac{D}{B} \times$ day where $D =$ food biomass ingested (mg)/no. of live insects on the third day; and efficiency of conversion of ingested food.

\[ \text{(ECI%) } = \frac{\text{RGR}}{\text{RCR}} \times 100. \]

For antifeedant activity, the formula described by Isman et al. (1990) was modified in calculating the feeding deterrence index, the consumption in control disks and $T =$ food consumption in treated disks, as the control and treated disks were placed in separate vials in no-choice tests.

Data analysis

Data from the flour disk bioassay were subjected to one-way ANOVA (P < 0.05) after checking for normality with, and means were separated by Tukey's Studentized Range Test at the 5% significance level using SPSS ver. 16 software.

Results

Contact toxicity

Probit analysis of the concentration-mortality response of *T. castaneum* indicated that the adults were susceptible to *D. stramonium* extract. The highest mortality trend in all concentrations was achieved at exposure times ranging from 12 to 24 h after the beginning of the experiment (Figure 1). There were significant differences between tested concentrations after 24 and 48 h. As the concentration increased, mortality of adults also increased, and the highest mortality was seen at a concentration of 5000 mg L$^{-1}$. LC$_{50}$ and LC$_{90}$ values for *T. castaneum* adults after 24 h of exposure to *D. stramonium* extract were 3936 and 15373 mg L$^{-1}$, respectively (Table 1).
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Flour disk bioassay

The RGR, RCR, ECI, and FDI values of *T. castaneum* adults fed on different concentrations of *D. stramonium* extract-treated flour disks for 72 h are given in Table 2. With increasing concentrations of *D. stramonium* extract, the RGR and RCR values of *T. castaneum* adults were significantly reduced (RGR: df = 4, 15, F = 22.51, P = 0; RCR: df = 4, 15, F = 12.46, P = 0). At a concentration of 2254 mg L\(^{-1}\), RGR was reduced from 0.143 to 0.071 mg for each milligram of insect weight (mg mg\(^{-1}\), daily) in comparison to the control, a nearly 50% reduction. There was a nearly 70% reduction in both RGR and RCR at a concentration of 3007 mg L\(^{-1}\). Similarly, ECI in adults was significantly reduced with increasing concentrations. ECI was reduced from 84.90% in the control to 43.86% at a concentration 3007 mg L\(^{-1}\). The FDI of adult insects increased significantly (ECI: df = 4, 15, F = 5.29, P = 0.007; FDI: df = 4, 15, F = 22.13, P = 0) with increasing concentrations. With a concentration increase from 947 to 3007 mg L\(^{-1}\), FDI increased from 34.93% to 97.21%. For *T. castaneum* adults, extract of *D. stramonium* significantly (P < 0.05) reduced RGR, RCR, and ECI values as extract concentrations increased. Feeding deterrence indices showed that the extract had antifeedant action against *T. castaneum* adults at higher concentrations, with a 97% reduction in feeding at a concentration of 3007 mg L\(^{-1}\) (Figures 2 and 3).

Table 1. Probit analysis data of *Datura stramonium* extract against adults of *Tribolium castaneum* resulting from 24- and 48-h contact toxicity tests.

<table>
<thead>
<tr>
<th>Exposure time (h)</th>
<th>n(^a)</th>
<th>(\text{LC}_{50}) (Fiducial limit)(^b) (mg L(^{-1}))</th>
<th>(\text{LC}_{90}) (Fiducial limit)(^b) (mg L(^{-1}))</th>
<th>Slope ± SE</th>
<th>d.f.(^c)</th>
<th>(\chi^{2d})</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td>350</td>
<td>3936 (3381-4330)</td>
<td>15,373 (1007-36,057)</td>
<td>2.165 ± 0.385</td>
<td>5</td>
<td>6.57</td>
</tr>
<tr>
<td>48 h</td>
<td>350</td>
<td>1954 (1579-2274)</td>
<td>6585 (5298-9356)</td>
<td>2.428 ± 0.337</td>
<td>5</td>
<td>6.03</td>
</tr>
</tbody>
</table>

\(^{a}\)number of insects tested, excluding controls; \(^{b}\)95% lower and upper fiducially limits are shown in parenthesis; \(^{c}\)degree of freedom; \(^{d}\)chi-square (chi-square is significant, P < 0.05).

Discussion

The present study demonstrates that the leaf and seed extracts of jimsonweed, *D. stramonium*, has contact toxicity against *T. castaneum* adults.
The results can be compared with other studies on *D. stramonium*. Larvicidal effects of *D. stramonium* against *T. castaneum* were also observed (Pascual-Villalobos and Robledo 1997). In addition, the acaricidal activity of this plant against the 2-spotted mite, *T. urticae*, was demonstrated (Kumral et al. 2009). Toxic effects of the seed and leaf extracts of the plant may be related to the presence of certain alkaloids. Moreover, some studies showed that certain alkaloids are present in different amounts in different parts of *Datura* spp., which explains why leaf extracts were more effective than seed extracts (Philipov and Berkov 2002; Berkov et al. 2006). Additionally, the whole-plant extracts of *D. stramonium* were found to have repellent and antifeedant properties against some insect pests, such as the rice moth, *C. cephalonica* St. (Lepidoptera: Pyralidae), *D. cingulatus* (Fabricius), *S. litura*, and *P. ricipit (Devaraj and Srilatha 1993; Pascual-Villalobos and Robledo 1997; Prakash and Rao 1997).

In this study, *D. stramonium* extract had significant effects on the nutritional indices and mortality of *T. castaneum* adults at different concentrations. It significantly reduced the growth rate and food consumption and utilization of *T. castaneum* adults at higher concentrations.

The effect of other plant extracts on nutritional indices was studied by different researchers. Huang and Ho (1998) demonstrated the toxicity and

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**Table 2.** Mean relative growth rate (RGR), relative consumption rate (RCR), efficiency of conversion of ingested food (ECI), and feeding deterrence index (FDI) of *Tribolium castaneum* adults fed different concentrations of *Datura stramonium* extract-treated flour disks for 72 h.

<table>
<thead>
<tr>
<th>Extract concentration (mg L⁻¹)</th>
<th>RGR ± SE (mg mg⁻¹, daily)</th>
<th>RCR ± SE (mg mg⁻¹, daily)</th>
<th>ECI ± SE (%)</th>
<th>FDI ± SE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.143 ± 0.02 a</td>
<td>0.255 ± 0.02 a</td>
<td>84.90 ± 0.08 a</td>
<td>-</td>
</tr>
<tr>
<td>947</td>
<td>0.116 ± 0.01 a</td>
<td>0.227 ± 0.03 ab</td>
<td>79.81 ± 0.03 a</td>
<td>34.93 ± 0.11 bc</td>
</tr>
<tr>
<td>1609</td>
<td>0.102 ± 0.01 ab</td>
<td>0.213 ± 0.02 ab</td>
<td>76.44 ± 0.02 a</td>
<td>34.93 ± 0.11 bc</td>
</tr>
<tr>
<td>2254</td>
<td>0.071 ± 0.01 b</td>
<td>0.166 ± 0.04 b</td>
<td>71.30 ± 0.02 ab</td>
<td>63.88 ± 0.16 ab</td>
</tr>
<tr>
<td>3007</td>
<td>0.022 ± 0.02 c</td>
<td>0.081 ± 0.05 c</td>
<td>43.86 ± 0.29 b</td>
<td>97.21 ± 0.24 a</td>
</tr>
</tbody>
</table>

Means within a column with different letters are significantly different (Tukey’s test, 5%); one-way ANOVA was applied for data.

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*Figure 2.* Comparing RGR and RCR indices of *Tribolium castaneum* after feeding on various concentrations of *Datura stramonium* extract.

*Figure 3.* Comparing ECI and FDI indices of *Tribolium castaneum* after feeding on various concentrations of extracts of *Datura stramonium*. 
antifeedant activity of the methylene chloride extract of cinnamon, *Cinnamomum aromaticum* Nees, on *T. castaneum* adults and larvae. Our results showed that *D. stramonium* extract had a higher level of toxicity and antifeedant activity than *Cinnamomum aromaticum* Nees extract. Moharrammipour et al. (2002) and Shahkarami et al. (2004) also demonstrated that *Ferula asaefida* L. extract and the essential oil of *Artemisia aucheri* Boiss. had antifeedant property on *T. castaneum* adults. Other studies showed that some secondary plant compounds, such as eugenol and its constituents, had contact toxicity for *T. castaneum*, but its sublethal doses had no considerable antifeedant property (Huang et al. 2002). However, the extract of *D. stramonium* is composed of certain alkaloids (such as scopoline, hyoscyamine, meteloidine, and apoatropine), terpenoids, and flavonoids that are believed to be responsible for many of this plant’s insecticidal properties (Pavela 2004; Berkov et al. 2006).

This study suggests that *D. stramonium* extract may be a potential grain protectant due to its combined contact and antifeedant activity against *T. castaneum* adults. The results of this study highlight the potential benefit of further research into suitable formulations, as well as cheaper and more potent synthetic analogs.

**Acknowledgments**

We would like to thank the Agricultural section of Medicinal Plants Research Center of Shahed University, for providing financial support for this project. We also appreciate the cooperation and support we received from the Department of Plant Protection, Shahed University, Tehran, Iran.

**References**


