Toxicological Responses of Confused Flour Beetle, *Tribolium confusum* du Val (Coleoptera: Tenebrionoidea) to various Isothiocyanate Compounds


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The confused flour beetle, *Tribolium confusum* du Val (Coleoptera: Tenebrionidae), is one of the most serious pests of stored cereals worldwide. It is about 0.25 inch long, reddish-brown in colour and its antennae end club-like, the ‘club’ consisting of four segments. Even under unsuitable conditions, they can easily survive, develop, reproduce and build up high population densities. Therefore, the harvested crops including seeds, grains and cereals suffer a loss of at least 10% from insect pests during storage. Losses of 30% are common throughout large areas of the world.

INTRODUCTION

The confused flour beetle, *Tribolium confusum* du Val (Coleoptera: Tenebrionidae), is one of the most serious pests of stored cereals worldwide. It is about 0.25 inch long, reddish-brown in colour and its antennae end club-like, the ‘club’ consisting of four segments. Even under unsuitable conditions, they can easily survive, develop, reproduce and build up high population densities. Therefore, the harvested crops including seeds, grains and cereals suffer a loss of at least 10% from insect pests during storage. Losses of 30% are common throughout large areas of the world.
The control of these pests mostly relies on use of chemical insecticides and fumigants. However, the continuous use of chemical insecticides for control of pests has resulted in serious problems such as resistance to the insecticides, pest resurgence, elimination of economically beneficial insects, toxicity to humans and wildlife. In addition, many chemical fumigants such as ethylene dibromide and methyl bromide that have been used to control these stored grain insects are being banned or facing phase-out. These problems and demand for pesticide-free foods have increased efforts to find alternative management or fumigants options.

Cruciferae plants produce glucosinolates which are secondary metabolites that can help plants defend themselves against phytophagous insects, fungi and other pests. Synthetic allyl isothiocyanate are commonly used as an insecticides, fungicides, bacterialocides and nematocides in certain cases for crop protection. Methyl isothiocyanate is one of several soil fumigants commonly used to control soil pests and plant pathogenic microbes. Therefore, glucosinolate breakdown products have potential as biodegradable, act on the insect respiratory system, so safe using insect fumigants. The purpose of this study is to investigate toxicological effects of four isothiocyanate components on the confused flour beetle.

**EXPERIMENTAL**

For both bioassays, adults of confused flour beetle were reared at laboratory cultures on wheat grains at 26 °C, 65 % relative humidity with a photoperiod of 12 h light-dark.

Allyl isothiocyanate (AITC, > 98 % GC purity) was purchased from Fluka. Chem. Co. (Germany). Methyl isothiocyanate, phenyl isothiocyanate and ethyl isothiocyanate were purchased from Merck Schuchardt OHG (Germany). In addition, methanol was purchased from Sigma-Aldrich Laborchemikalien GmbH (Germany).

**Laboratory bioassay:** Two bioassays were conducted at laboratory of the University of Mustafa Kemal, Hatay, Turkey. The first bioassay was set up on 11 September and the following on 19 September in 2008. Four pure isothiocyanate (allyl (2-propenyl)isothiocyanate, ethyl isothiocyanate, methyl isothiocyanate and phenyl isothiocyanate) compounds were used as treatment. Methanol and sterilized distilled water were used as control treatment. Four isothiocyanate compounds were dissolved in methanol and stock solutions were prepared. Each of isothiocyanate compounds with 5 different concentrations (0.005, 0.01, 0.05, 0.1, 0.2 µL) were tested on a 90 mm diameter glass petri dishes containing 10 adult confused flour beetle in vitro. Each of glass petri dishes were immediatly sealed by parafilm. Both bioassays were evaluated at 24 h later and number of dead confused flour beetle were counted. Both bioassays were conducted completely randomized design with five replications. Data were analyzed by analysis of variance (ANOVA) with using the SAS software and the Probit analysis (p < 0.05). Means were separated by using the student-newman-keuls (SNK) multiple comparison tests (p < 0.05).
RESULTS AND DISCUSSION

Phenyl isothiocyanate resulted in significant mortality on the confused flour beetle with 0.1 and 0.2 µL concentrations in both bioassays (F: 34.47, df: 4.21, P: 0.0001; F: 27.38, df: 4.21, P: 0.0001, respectively) (Tables 1 and 2). The highest concentration (0.2 µL) of allyl (2-propenyl)isothiocyanate and methyl isothiocyanate caused significant mortality in the first bioassay (F: 34.47, df: 4.21, P: 0.0001) (Table-1). Ethyl isothiocyanate did not cause significant mortality with all concentrations. In the following bioassay, methyl isothiocyanate resulted in significant mortality with 0.1 and 0.2 µL concentrations (F: 27.38, df: 4.21, P: 0.0001) (Table-2). In addition, the highest concentration (0.2 µL) of allyl (2-propenyl)isothiocyanate and ethyl isothiocyanate had significant mortality.

Phenyl isothiocyanate had the lowest LC50 (0.02781, 0.02627) and highest toxicity on the confused flour beetle in both bioassay (Tables 1 and 2). In the first bioassay, phenyl isothiocyanate were followed by allyl (2-propenyl)isothiocyanate (LC50: 0.05262) and methyl isothiocyanate (LC50: 0.07178) toxicities (Table-1). Ethyl isothiocyanate had the highest LC50 (0.12408) and lowest toxicity. In the following bioassay, phenyl isothiocyanate were followed by methyl isothiocyanate (LC50: 0.04494) and allyl (2-propenyl)isothiocyanate (LC50: 0.06342) toxicities (Table-2). Ethyl isothiocyanate had the highest LC50 (0.09989) and lowest toxicity on the confused flour beetle.

Many chemical fumigants such as ethylene dibromide and methyl bromide that have been used to control these stored grain insects are being banned or facing phase-out. Thus, the controlling stored pests need alternative fumigants. Glucosinolate breakdown products are volatile, the effect of these chemicals on the respiratory function of insects, so good candidates for insect fumigants. The family Cruciferae plants, such as cabbage (Brassica oleracea), mustard (Sinapis alba) and crambe (Crambe abyssinica) produce glucosinolates which are secondary metabolites that can help plants defend themselves against phytophagous insects, fungi and other pests. The toxicity of Brassica tissues is derived from glucosinolates that are converted to isothiocyanates, organic cyanides, oxazolidinethiones and ionic thiocyanates by the enzyme myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) in solution. The glucosinolate breakdown products have potential as biodegradable.

The isothiocyanates are the primary products formed from the degradation of glucosinolates. Methyl isothiocyanate is one of several soil fumigants commonly used to control soil pests and plant pathogenic microbes. Allyl isothiocyanate (AITC) is the most toxic compound formed from allyl glucosinolate hydrolysis in B. juncea L. and possibly the most important for biofumigation. Allyl glucosinolate (sinigrin) breaks down in soils to allyl isothiocyanate and allyl cyanide have been suggested for use in controlling soil-borne plant pests. Previous studies were conducted by Borek et al. and Elberson et al. in laboratory experiments, Brassica napus L. resulted in decreasing populations of black vine weevil larvae and wireworms due to their insecticidal effects. Using the pesticidal properties of compounds
### TABLE 1
EFFECT OF VARIOUS ISOTHIOCYANATE COMPOUNDS ON THE CONFUSED FLOUR BEETLE, *Tribolium confusum* UNDER *in vitro* CONDITIONS

<table>
<thead>
<tr>
<th>Isothiocyanate compounds</th>
<th>Per cent of <em>T. confusum</em> mortality / dose (µg mL⁻¹)</th>
<th>n</th>
<th>Intercept (±SE)</th>
<th>Slope (±SE)</th>
<th>X²</th>
<th>LC₅₀</th>
<th>p &lt; 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.005  0.010  0.050  0.100  0.200</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allyl isothiocyanate</td>
<td>2.00d  2.00d  16.00d  74.00a-c  100.00a</td>
<td>250</td>
<td>7.79±1.14  6.81±1.00</td>
<td>45.48</td>
<td>0.07178</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Ethyl isothiocyanate</td>
<td>2.00d  6.00d  0.00d  66.00bc  60.00c</td>
<td>250</td>
<td>2.96±0.55  3.27±0.50</td>
<td>41.98</td>
<td>0.12408</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Methyl isothiocyanate</td>
<td>12.00d 2.00d  18.00d  90.00ab  100.00a</td>
<td>250</td>
<td>5.39±0.66  4.21±0.52</td>
<td>63.91</td>
<td>0.05262</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Phenyl isothiocyanate</td>
<td>4.00d  4.00d  78.00a-c  100.00a 100.00a</td>
<td>250</td>
<td>9.45±1.09  6.07±0.72</td>
<td>70.60</td>
<td>0.02781</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

*Numbers within a row not followed by the same letter are significantly different (p < 0.05) by SNK and Probit analysis. Numbers of *T. confusum* tested. LC₅₀ Lethal concentrations of essential oils (in µg mL⁻¹) at the 50 % (LC₅₀) levels of probit mortality.

### TABLE 2
EFFECT OF VARIOUS ISOTHIOCYANATE COMPOUNDS ON THE CONFUSED FLOUR BEETLE, *Tribolium confusum* UNDER *in vitro* CONDITIONS

<table>
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<tr>
<th>Isothiocyanate compounds</th>
<th>Per cent of <em>T. confusum</em> mortality / dose (µg mL⁻¹)</th>
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<th>Intercept (±SE)</th>
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<th>X²</th>
<th>LC₅₀</th>
<th>p &lt; 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.005  0.010  0.050  0.100  0.200</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Allyl isothiocyanate</td>
<td>0.00c  0.00c  20.00c  76.00ab  100.00a</td>
<td>250</td>
<td>10.72±1.59  9.39±1.39</td>
<td>45.11</td>
<td>0.06342</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Ethyl isothiocyanate</td>
<td>4.00c  4.00c  4.00c  56.00b  84.00a</td>
<td>250</td>
<td>4.21±0.66  4.21±0.62</td>
<td>45.98</td>
<td>0.09989</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Methyl isothiocyanate</td>
<td>14.00c 4.00c  24.00c  94.00a  100.00a</td>
<td>250</td>
<td>5.18±0.60  3.84±0.45</td>
<td>72.53</td>
<td>0.04494</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Phenyl isothiocyanate</td>
<td>4.00c  10.00c  74.00ab  100.00a 100.00a</td>
<td>250</td>
<td>8.33±0.91  5.27±0.58</td>
<td>81.54</td>
<td>0.02627</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

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released by macerated *Brassica* tissues could be alternative for control of soil-inhabiting pests. In their studies, larvae of masked chafer beetles (*Cyclocephala* spp.) were placed in soil amended with *Brassica juncea* L. (PI 458934) tissue. Allyl isothiocyanate levels were positively correlated to larval mortality, with the 8 % *B. juncea* treatment resulting in 100 % larval mortality with an average allyl isothiocyanate concentration of 11.4 mg per liter of soil atmosphere. Tsao et al. reported that allyl thiocyanate, allyl isothiocyanate and allyl isocyanate which are the glucosinolate breakdown from crambe used as fumigant for the house fly (*Musca domestica*) due to their lethal concentrations. They found LC$_{50}$ of allyl thiocyanate, allyl isothiocyanate and allyl isocyanate were 0.1, 0.13, 0.63 µg cm$^{-3}$. Allyl thiocyanate, the most potent fumigant against the house fly was the most toxic against the lesser grain borer as well. The high fumigant toxicity of the glucosinolate breakdown products against the lesser grain borer may have important economic impact on stored grain insects management. These naturally occurring chemicals were also insecticidal as fumigant on other stored grain insects such as the sawtoothed grain beetle (*Oryzaephilus surinamensis* L.) and the red flour beetle (*Tribolium castaneum* Herbst). In addition, Tsao et al. reported that the fumigation 24 h LC$_{50}$ against the house fly (*Musca domestica* L.) of allyl thiocyanate, allyl isothiocyanate, allyl cyanide and l-cyano-2-hydroxy-3-butene was 0.1, 0.13, 3.66 and 6.2 µg cm$^{-3}$, respectively. The fumigation 24 h LC$_{50}$ against the lesser grain borer (*Rhyzopertha dominica* Fabricius) of allyl thiocyanate, allyl isothiocyanate, allyl cyanide and l-cyano-2-hydroxy-3-butene were 0.55, 1.57, 2.8 and > 19.60 µg cm$^{-3}$, respectively. The fumigation toxicity of some of the glucosinolate products was close to or better than that of the commercial insect fumigants such as chloropicrin (LC$_{50}$: 0.08 and 1.3 µg cm$^{-3}$ against *M. domestica* and *R. dominica*, respectively) and dichlorovos (LC$_{50}$: < 0.02 and 0.29 µg cm$^{-3}$ against *M. domestica* and *R. dominica*, respectively) in their laboratory tests. Significantly increased CO$_2$ expiration was found in insects exposed to the vapour of allyl isothiocyanate, allyl thiocyanate and allyl isocyanate. Allyl isothiocyanate was also found to increase the CO$_2$ expiration of the American cockroach (*Periplaneta americana* L.).

**Conclusion**

The current studies showed that phenyl isothiocyanate resulted in significant mortality on the confused flour beetle with 0.1 and 0.2 µL concentrations in both bioassays. In the first bioassay, the highest concentration (0.2 µL) of allyl (2-propenyl)isothiocyanate and methyl isothiocyanate caused significant mortality. In the following bioassay, methyl isothiocyanate resulted in significant mortality with 0.1 and 0.2 µL concentrations. In addition, the highest concentration (0.2 µL) of allyl (2-propenyl)isothiocyanate and ethyl isothiocyanate had significant mortality.

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phenyl isothiocyanate were followed by methyl isothiocyanate (LC$_{50}$: 0.04494) and allyl (2-propenyl)isothiocyanate (LC$_{50}$: 0.06342) toxicities. Ethyl isothiocyanate had the highest LC$_{50}$ (0.12408) LC$_{50}$ (0.09989) and lowest toxicity on confused flour beetle. Therefore, phenyl isothiocyanate, allyl (2-propenyl)isothiocyanate and methyl isothiocyanate can be alternative fumigant for controlling of the stored pests.

REFERENCES


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