Effects of Wood Vinegar Mixed with Insecticides on the Mortalities of *Nilaparvata lugens* and *Laodelphax striatellus* (Homoptera: Delphacidae)

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Abstract: Effects of wood vinegar on the activity of various insecticides were determined by measuring the mortality of two species of rice planthoppers, *Nilaparvata lugens* and *Laodelphax striatellus*. Wood vinegar itself did not show insecticidal activity on planthoppers. When the planthoppers were treated with wood vinegar mixed with one of insecticides such as BPMC, dinotefuran, imidacloprid, carbosulfan or insect growth regulators, the planthopper mortality induced by carbosulfan was greatly increased by the wood vinegar in comparison with a single carbosulfan treatment. Wood vinegar showed no effect on other insecticides. In addition, the wood vinegar-carbosulfan mixture significantly reduced AChE activity of planthoppers, which is a target molecule of carbosulfan. This result suggests that wood vinegar has a synergistic effect on the insecticidal activity of carbosulfan. Our study provides information on a potential role of wood vinegar in facilitation of activity of specific insecticides.

Key words: Carbosulfan, *Laodelphax striatellus*, *Nilaparvata lugens*, Wood vinegar,
MATERIALS AND METHODS

Wood vinegar
The wood vinegar was obtained from the Gangwon Mokcho Industry Incorporation, Yeongwol, Korea. Its quality is determined to be specific gravity (Be-4.1), acid amount (4.6-5.5%), pH (below 2.5), tar (below 4.0) and refraction ratio (above 8.0, % Brix).

Insects
The colonies of *N. lugens* and *L. striatellus* were supplied from Yeongnam Agricultural Research Institute, Milyang, Korea. Both species were reared on young rice plants (*Oryza sativa*) in a insect rearing room at the condition of 28°C, 70% r. h. and a 16 h light/8 h dark (16L/8D) photoperiodic cycle. For experiments, rice plants in a Petri dish (5 cm in diameter) were placed in the center of a plastic box (14 × 10 × 5 cm³) and planthoppers were released into the box.

Treatments with wood vinegar and insecticides
Four representative insecticides to be used were selected: O-sec-butylphenyl N-methylcarbamate (BPMC), carbosulfan, dinotefuran and imidacloprid. In addition, five kinds of insect growth regulators (IGR) were tested: diflubenzuron, tebufenozide, teflubenzuron, triflumuron and buprofezin+BPMC. The wood vinegar and insecticides were prepared at various dilutions with water. The mixture solutions of each insecticide and wood vinegar were prepared as follows. Half-recommended or lower dilutions of insecticides were mixed with 300, 500 or 1,000-fold dilutions of the wood vinegar. The solutions of the wood vinegar, insecticides or the mixtures were sprayed onto planthoppers infesting rice plants in the laboratory. Mortalities of the planthoppers were determined 3 h after the treatments with representative insecticides or 5 days after treatments with IGRs.

Acetylcholine esterase assay
Planthoppers were homogenized with 0.3 ml of 0.1 M Tris-HCl buffer (pH 7.8) containing 0.5% Triton X-100 in a 1.5 ml microcentrifuge tube on ice. The homogenate was centrifuged at 10,000 rpm for 20 min at 4°C, and the supernatant was transferred to a new tube. Protein concentration was determined by the Bradford assay (Bradford, 1976).

Acetylcholine esterase (AChE) activity was measured according to the method of Ellman et al. (1961) with some modifications. The reaction mixture contained 100 mM Tris-HCl (pH 7.8), 0.4 mM 5,5-dithio-bis 2-nitrobenzoic acid (DTNB), 5 mM acetylcholine iodide, and 10 µl enzyme solution. The reaction rate was monitored for 10 min at room temperature and the AChE activity was determined by measuring OD values at 405 nm at 30 sec intervals using a Tecan Sunrise microplate reader (Tecan, San Jose, CA). We used the ratio of changed absorbance (ΔA/min) as the enzyme reaction speed. The AChE concentration of extracts was determined using extinction coefficient (1.36×10⁴ cm/M) of 5-thio-2-nitrobenzoic acid (Lee et al., 1991). One unit (U) of AChE activity was defined as the hydrolysis of 1 µmol of substrate per min under the assay conditions. AChE activity is presented as the slope of the reaction rate created by the increase in absorbance over time.

Statistic analysis
Statistical analysis of data was conducted with SPSS 12.0 program (SPSS Inc., 2004) for Windows. The data was analyzed by one-way analysis of variance (ANOVA) or Student’s *t*-test. Data which were not normally distributed were analyzed by the Tukey method (*P*<0.05).

RESULTS

Effect of wood vinegar on planthopper mortality
To determine the effect of wood vinegar on planthopper, mortalities were recorded for 10, 100, 300, 500 and 1000 fold ratio and sprayed onto either *N. lugens* or *L. striatellus*. Mortalities of water-treated planthoppers of both species were less than 10% (Fig. 1). In addition, its rate was not significantly changed by the treatments with various dilutions of the wood vinegar solution.

Effect of wood vinegar mixed with representative insecticides on the planthopper mortality
To determine the effect of wood vinegar on the activities of representative insecticides, we treated the planthoppers with mixtures of wood vinegar and various insecticides.
Effects of Wood Vinegar Mixtures with Insecticides in the Mortalities of Nilaparvata lugens and Laodelphax striatellus (Homoptera: Delphacidae)

(Fig. 2). Treatments with recommended doses (100%) of BPMC, dinotefuran, imidacloprid and carbosulfan showed planthopper mortalities of 99.3, 97.3, 54.0 and 16.7% for N. lugens, and 98.6, 73.5, 59.3 and 38.0% for L. striatellus (B). We determined the effective dose of each insecticide that elicited less than 50% mortalities in both species. In the case of BPMC and dinotefuran, treatments with half of recommended doses elicited more than 60% mortalities in both species of planthoppers. Therefore, we used 20 and 30% dilutions of recommended doses of BPMC and dinotefuran, respectively, which elicited less than 60% mortality of both species. In imidacloprid and carbosulfan treatments less than 60% mortalities were elicited by 50%-diluted insecticides (C). When the planthoppers were treated with mixtures of either 20% BPMC, 30% dinotefuran or 50% imidacloprid with various dilutions of wood vinegar, the mortalities of both species did not significantly change from the single insecticide treatments (D, E and F). In addition, the mortalities were not influenced by various dilutions of wood vinegar. However, when the planthoppers were treated with 50% carbosulfan mixed with wood vinegar, the mortalities of both species were greatly increased. Its effects were higher when carbosulfan was mixed with a large amount of wood vinegar.

Effect of wood vinegar mixed with IGRs on planthopper mortality

To determine the effect of wood vinegar on the activities of IGRs, we treated the planthoppers with wood vinegar mixed with various IGRs (Fig. 3). The mortality was not increased by the treatments with recommended doses (100%) of all IGRs except for buprofezin+BPMC. In addition, the effects of treatment with 50% dilutions of recommended doses of all IGRs were similar with those of recommended doses. Furthermore, IGRs mixed with various dilutions of wood vinegar did not significantly change the mortalities of planthoppers.

Effects of the wood vinegar mixture with carbosulfan on AChE activity

To determine the mechanism by which wood vinegar enhances the insecticidal activity of carbosulfan, we
determined whether the activity of AChE, which is a target molecule of carbosulfan, of planthoppers is changed by the mixture treatment. Wood vinegar, 50% dilution of carbosulfan or the mixtures were exposed to both species of planthoppers and the AChE activities were determined (Table 1 and 2). AChE activities of both species were not changed by wood vinegar alone. However, its level was reduced by the 50% dilution of carbosulfan. With the mixtures of carbosulfan and various doses of wood vinegar, the AChE activities were reduced further. Its activity was the lowest when carbosulfan was mixed with 300 dilution of wood vinegar.

Fig. 3. Effects of IGRs and their mixtures with various doses of wood vinegar on the mortality of planthoppers. 50%-diluted IGRs were used to make mixture solutions with wood vinegar. Planthoppers were treated as follows: water-treated control (A), 100% IGR only (B), diluted IGR only (C), mixtures of the IGR with 300, 500 and 1,000-diluted wood vinegar (D, E and F, respectively). Mortality was determined at 5 d after the treatments.
DISCUSSION

Wood vinegar itself did not show any insecticidal effect on planthoppers. Thus we focused on finding alternative role(s) of wood vinegar in the mixture with insecticides. We treated the planthoppers with mixtures of wood vinegar and several representative insecticides that are used for planthopper control and IGRs. Among them, only the mixture with carbosulfan had highly enhanced effect on both species of planthoppers while the mixtures with other insecticides and IGRs did not. This result suggests that wood vinegar has a potential to increase the activity of insecticides, particularly in the case of carbosulfan. In addition, its effect might be specific to a certain chemical characteristic because other of the insecticides tested did not show an increased effect when mixed with wood vinegar.

Insecticides exert their effect only when they act on a target tissue within an organism. There are several steps in the mode of action of insecticides (Ishaaya et al., 2007). Firstly, insecticides must enter the insect body, for example, by penetrating the body surface or through the mouth, spiracle or anus. The body surface of most insects are covered with lipophilic cuticle layers. The penetration rate through the cuticle layer is different for each insecticide.

Secondly, the insecticides must reach target tissues within the body to elicit their actions. At the target sites, differences in affinity between the insecticides and target molecule exist. Thirdly, the insecticide molecules may change their structure, size or activity within the insect body by interaction with biomolecules within hemolymph or tissue of insects. For example, mixed function oxidase (MFO) of insects can metabolize various kinds of insecticides and reduce their toxicity (Brattsten, 1979; Ahmad, 1983).

Carbosulfan is one of carbamate insecticides that act on AChE in the nervous tissue of insects. This compound binds to AChE and inhibit its activity that result in abundance of acetylcholine at the synapse, which eventually induce paralysis of the body (Oakeshott et al., 2005). When the mixture of carbosulfan and wood vinegar was exposed to planthoppers, the AChE activity was significantly reduced. This result indicates that wood vinegar enhances the activity of carbosulfan at the target site. AChE is a target molecule of many other carbamate insecticides. However, the activity of BPMC, which is of the same carbamate class, did not increase when mixed with wood vinegar. Thus, this suggests that the effect of wood vinegar may be specific to a certain chemical characteristic that may not be common to carbamate insecticides. In addition, wood vinegar may influence another step in the action of

Table 1. AChE activities of body extracts prepared from N. lugens.

<table>
<thead>
<tr>
<th>Mixtures</th>
<th>Dilutions</th>
<th>∆A/min* a</th>
<th>AChE concentrations (mU/ml)</th>
<th>Protein contents (mg/ml extract)</th>
<th>Specific activities (mU/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water-treated</td>
<td>0.31±0.040</td>
<td>160.7</td>
<td>10</td>
<td>16.1</td>
<td></td>
</tr>
<tr>
<td>Wood vinegar</td>
<td>300</td>
<td>0.29±0.032</td>
<td>146.6</td>
<td>10</td>
<td>14.9</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.37±0.121</td>
<td>189.7</td>
<td>10</td>
<td>19.0</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>0.33±0.169</td>
<td>170.1</td>
<td>10</td>
<td>17.0</td>
</tr>
<tr>
<td>Carbosulfan</td>
<td>50%</td>
<td>0.25±0.066</td>
<td>129.6</td>
<td>10</td>
<td>13.0</td>
</tr>
<tr>
<td>Carbosulfan + Wood vinegar</td>
<td>50% + 300</td>
<td>0.10±0.039</td>
<td>50.7</td>
<td>10</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>50% + 500</td>
<td>0.24±0.016</td>
<td>121.4</td>
<td>10</td>
<td>12.1</td>
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<tr>
<td></td>
<td>50% + 1,000</td>
<td>0.19±0.068</td>
<td>98.9</td>
<td>10</td>
<td>9.9</td>
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Table 2. AChE activities of body extracts prepared from L. striatellus.

<table>
<thead>
<tr>
<th>Mixtures</th>
<th>Dilutions</th>
<th>∆A/min* a</th>
<th>AChE concentrations (mU/ml)</th>
<th>Protein contents (mg/ml extract)</th>
<th>Specific activities (mU/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water-treated</td>
<td>0.31±0.009</td>
<td>159.7</td>
<td>10</td>
<td>15.6</td>
<td></td>
</tr>
<tr>
<td>Wood vinegar</td>
<td>300</td>
<td>0.30±0.015</td>
<td>155.9</td>
<td>10</td>
<td>15.6</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.30±0.017</td>
<td>156.3</td>
<td>10</td>
<td>15.6</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>0.38±0.114</td>
<td>197.2</td>
<td>10</td>
<td>19.7</td>
</tr>
<tr>
<td>Carbosulfan</td>
<td>50%</td>
<td>0.15±0.011</td>
<td>77.5</td>
<td>10</td>
<td>7.8</td>
</tr>
<tr>
<td>Carbosulfan + Wood vinegar</td>
<td>50% + 300</td>
<td>0.08±0.001</td>
<td>42.8</td>
<td>10</td>
<td>4.3</td>
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<tr>
<td></td>
<td>50% + 500</td>
<td>0.10±0.023</td>
<td>53.3</td>
<td>10</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>50% + 1,000</td>
<td>0.11±0.014</td>
<td>58.2</td>
<td>10</td>
<td>5.8</td>
</tr>
</tbody>
</table>

*a the absorbance ratio values (∆A/min) represent the mean ±SD of three samples determined at different times.
carbosulfan. For example, wood vinegar might enhance the penetration of carbofuran into the planthoppers because it contains a large amount of acetic acid that may influence the permeability of cuticle layer. Further study is required to determine the mechanism of synergistic effect of wood vinegar on the carbosulfan action. In addition, since wood vinegar is a mixture of numerous organic compounds, key molecule(s) that elicit synergistic effect with insecticides should be identified.

Our results indicate that natural products such as wood vinegar could be beneficial for the control of insect pests. This study is potentially important toward reduction of chemical insecticide overuse and provides information on pest control in an environment-friendly way.

ACKNOWLEDGMENT

We are grateful to Dr. Do-Yeon Kaw at Yeongnam Agricultural Research Institute, Milyang, Korea for providing planthopper colonies. This work was supported by a research grant of Agricultural Research and Development Promotion Center, Korea.

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