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Effects of Wood Vinegar Mixted 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,

Planthoppers, such as the brown planthopper *Nilaparvata lugens*, the small brown planthopper *Laodelphax striatellus* and the whiteback planthopper *Sogatella furcifera,* are the major pest of rice plants in many Asian countries (Kiritani, 1979; Kenmore et al., 1984; Hare, 1994). Planthoppers directly damage the rice plants by sucking assimilates from stems and leaves (Sogawa, 1973). For example, the damage of *N. lugens* is so severe as to cause whole plants to become yellowish and rapidly dry, a pattern known as 'hopper-burn' phenomenon (Wang et al., 2005). Planthoppers also indirectly damage the rice plants by transmitting various plant viruses that induce diseases. The *L. striatellus* is by far the most important vector of the rice stripe virus transmission that severely damages the growing rice plants (Nault, 1994; Nemoto et al., 1994; Lijun et al., 2003).

For the control of insect pests in environment-friendly ways, natural products that have activities to kill or interrupt growth, metabolism or reproduction of insects are potentially useful substitutes for synthetic chemical insecticides (Bingaman and Christians, 1995). Wood vinegar, technically known as pyroligneous acid, is a condensed liquid of the smoke generated by charcoal production (Yoshimoto, 1994; Kishimoto, 1997). Wood vinegar contains 80-90% water and 10-20% organic compounds including more than 200 chemical components with mainly acetic acid. It also contains various kinds of phenol, carbonyl and alcohol compounds. Wood vinegar is widely used in agricultural crop production toward plant growth stimulantion, germination, soil disinfection and the control of weed, disease and pest (Kim et al., 2000; Kim et al., 2001; Lee and Huh, 2002; Mu et al., 2003; Yatagai and Unrinin, 1989; Rico et al., 2007). Recently various use of wood vinegar is growing and its production has been increased to about 14,000 ton annually (Jang, 2004). In the aspect of plant protection, the wood vinegar has been used for the control of microbes such as bacterial and fungal diseases of various crop plants (Seo et al., 2000; Radhakrishnan et al., 2002). It is also used for the control of termites (Yatagai et al., 2002). Although the wood vinegar has a great potential for pest control, its use has not been developed for practical application.

Here we examined the wood vinegar's effect on the efficiency of various insecticides. When mixed with insecticides the wood vinegar may influence the insecticidal activities in various ways. Our study suggests that wood vinegar enhances the activity of insecticides when applied together.

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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 ($14\times10\times5$ cm^3) 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 (1.36×10^4 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 of effect wood vinegar on planthopper, mortalits the wood vinegar stock solution was diluted at 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 effects of wood vinegar on the activities of representative insecticides, we treated the planthoppers with mixtures of wood vinegar and various insecticides

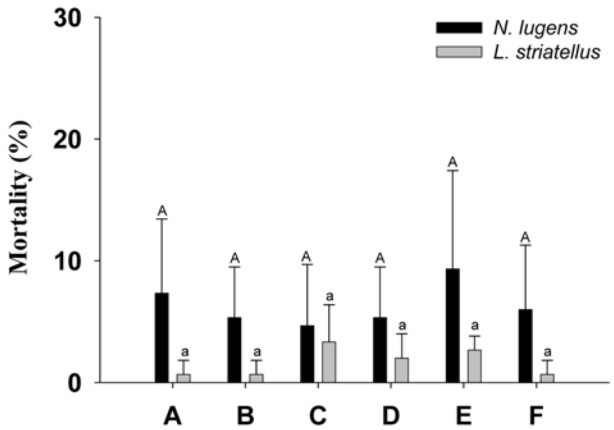

Fig. 1. Effects of wood vinegar on the mortality of *N. lugens* and *L. striatellus*. Wood vinegar was diluted at 10, 100, 300, 500 and 1000 times. Planthoppers were treated as follows: water-treated (A), 10, 100, 300, 500 and 1000-diluted wood vinegar (B, C, D, E and F, respectively). Mortality was determined at 3 h after the treatments.

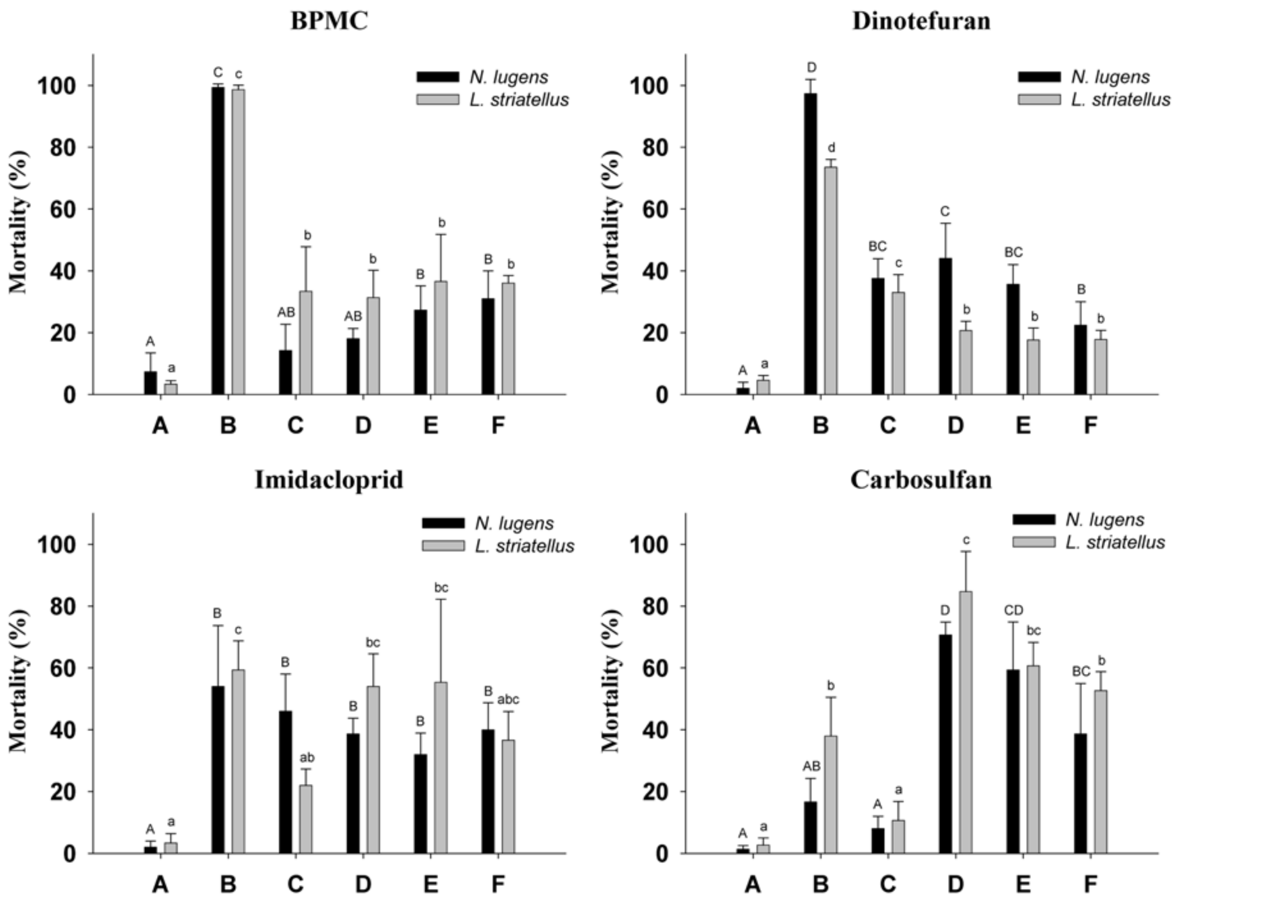

Fig. 2. Effects of insecticides and their mixtures with various dilutions of wood vinegar on the mortality of planthoppers. BPMC, dinotefuran, imidacloprid and carbosulfan were used at dilutions of 20, 30, 50 and 50%, respectively. Planthoppers were treated as follows: water-treated control (A), 100% insecticide only (B), diluted insecticide only (C), mixtures of the insecticide with 300, 500 and 1,000-diluted wood vinegar (D, E and F, respectively). Mortality was determined at 3 h after the treatments.

(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

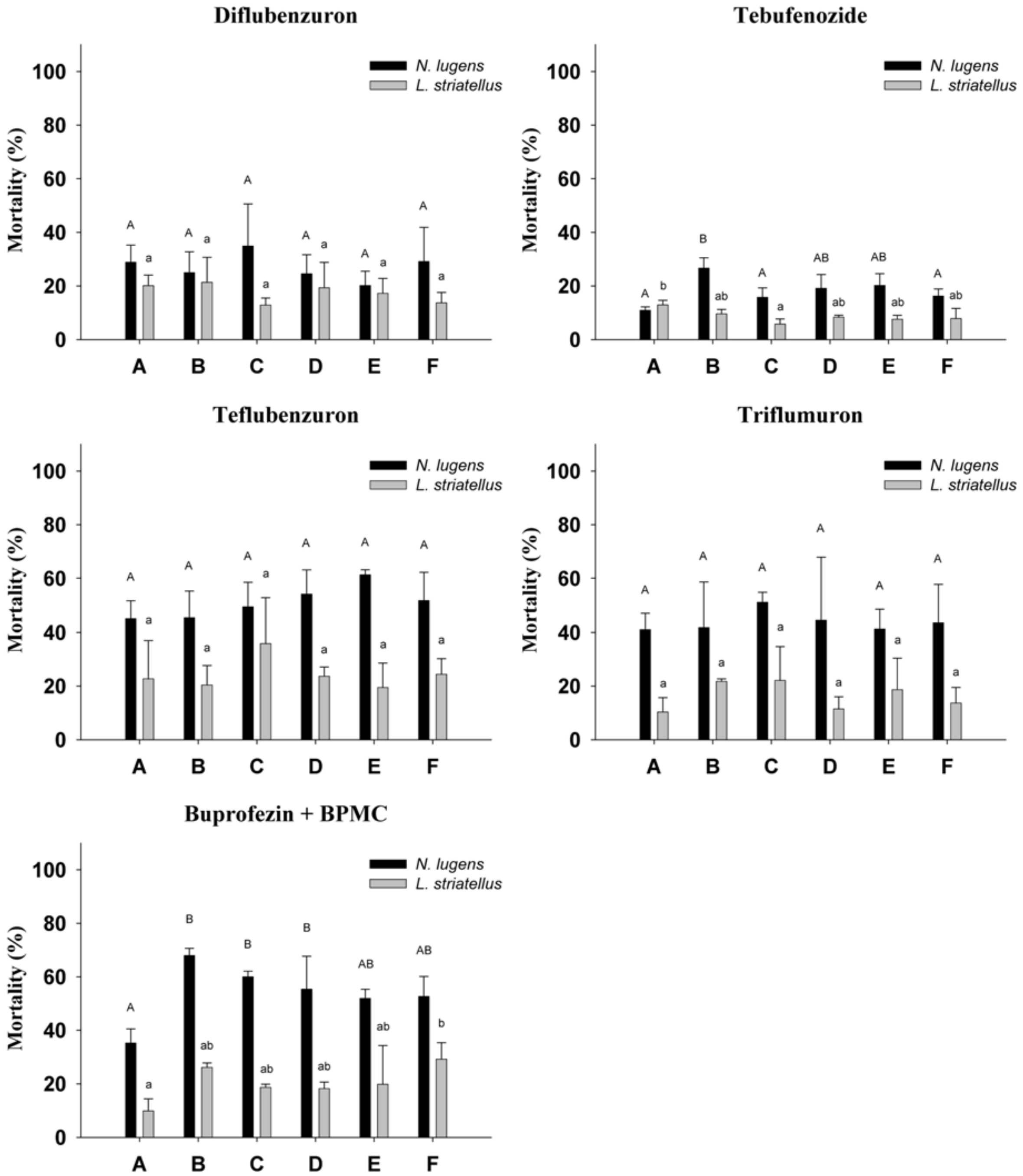

Fig. 3. Effects of IGRs and their mixtures with various doses of wood vinegar on the mortality of planthoppers. 50%-diluted IGRs were used to make mixture solutions with wood vinegar. Planthoppers were treated as follows: water-treated control (A), 100% IGR only (B), diluted IGR only (C), mixtures of 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.

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.

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

Mixtures	Dilutions	ΔA/min[a]	AChE concentrations (mU/ml)	Protein contents (mg/ml extract)	Specific activities (mU/mg protein)
Water-treated		0.31±0.040	160.7	10	16.1
Wood vinegar	300	0.29±0.032	148.6	10	14.9
	500	0.37±0.121	189.7	10	19.0
	1,000	0.33±0.169	170.1	10	17.0
Carbosulfan	50%	0.25±0.066	129.6	10	13.0
Carbosulfan + Wood vinegar	50% + 300	0.10±0.039	50.7	10	5.1
	50% + 500	0.24±0.016	121.4	10	12.1
	50% + 1,000	0.19±0.068	98.9	10	9.9

[a]the absorbance ratio values (ΔA/min) represent the mean±SD of three samples determined at different times.

Table 2. AChE activities of body extracts prepared from *L. striatellus*.

Mixtures	Dilutions	ΔA/min[a]	AChE concentrations (mU/ml)	Protein contents (mg/ml extract)	Specific activities (mU/mg protein)
Water-treated		0.31±0.009	159.7	10	16.0
Wood vinegar	300	0.30±0.015	155.9	10	15.6
	500	0.30±0.017	156.3	10	15.6
	1000	0.38±0.114	197.2	10	19.7
Carbosulfan	50%	0.15±0.011	77.5	10	7.8
Carbosulfan + Wood vinegar	50% + 300	0.08±0.001	42.8	10	4.3
	50% + 500	0.10±0.023	53.3	10	5.3
	50% + 1000	0.11±0.014	58.2	10	5.8

[a]the absorbance ratio values (ΔA/min) represent the mean±SD of three samples determined at different times.

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 characteristics 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 carbosulfan. For example, wood vinegar might enhance the penetration of carbosulfan 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.

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