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Original Research Article

Wood Vinegar as an Alternative Insecticide in Controlling Rice Weevil, *Sitophylus oryzae* (Coleoptera: Curculionidae)

Najihah Othman, Nor Hidawati Elias, Nurhadijah Zainalabidin*

Faculty of Chemical Engineering Technology, Universiti Malaysia Perlis, UniMAP, Kompleks Pusat Pengajian Jejawi 3, 02600 Arau, Perlis.

*Corresponding Author: Nurhadijah Zainalabidin, Faculty of Chemical Engineering & Technology, Universiti Malaysia Perlis, UniMAP, Kompleks Pusat Pengajian Jejawi 3, 02600 Arau, Perlis; nurhadijah@unimap.edu.my

Abstract: Rice production faces many challenges including damages due to pest infestation in storage after harvest. Stored rice grains are often under threat and damage from pests such as the rice weevil which resulted in a reduction of the quality and quantity. The application of chemical insecticides helps to control this pest; however, it gives negative impacts on the environment and human health. In contrast, the application of plant-based insecticides including the use of wood vinegar can become an alternative to the conventional method. This work describes the effectiveness of wood vinegar as a bioinsecticide to inhibit the rice weevil's activity by assessing the repellency, mortality and antifeedant activity at different concentrations of 50, 100, 150 and 200 ppm. Wood vinegar showed high repellency of 98.3% towards rice weevils at 200 ppm after 5 hours of exposure times. An increase in concentration affects the mortality rate, which the highest mortality of 97% recorded at 200 ppm wood vinegar concentration. The lethal concentration (LC_{50}) of wood vinegar was calculated to be 77.62 ppm. The antifeedant activity resulted in the reduction of weight loss in stored rice with the lowest reduction of 2% at 200 ppm. This promising result reflects the ability of wood vinegar as an insecticidal agent in the management and control of stored-grain pests.

Keywords: Insecticidal activity; wood vinegar; rice weevil

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1. Introduction

About one and half of the world's population, including nearly all of East and Southeast Asia, is completely dependent on rice as a staple food. Rice is economically important and it is the most widely cultivated and consumed cereal in Malaysia. With the local population increasing in numbers, crop yield development needs to be raised adequately and sustainably to satisfy the growing demand (Herman *et al.*, 2015). However, it was reported that about 30 % to 40 % of pre-harvest and post-harvest crop losses worldwide are due to arthropod pest infestation (Mitchell *et al.*, 2016). Rice weevil or scientifically known as *Sitophilus oryzae* is one of the most important worldwide distributed insect pests that attacks stored cereals including rice, maize, wheat and peas (Nguyen *et al.*, 2016). Many of the latter grains are preferred and cause huge economic losses, where both adults and larvae feed on carbohydrates in rice grains causing weight loss and contamination (Saad & Abdelgaleil, 2018) with up to 100% of stored rice grain may be destroyed by the rice weevil (Naseri & Borzoni, 2016).

Several management strategies have been recorded for the handling of insect pests in storage facilities (Syed Ahmad *et al*., 2018) with fumigation using phosphine and methyl bromide said to play important functions in the prevention of insect pests in stored products. However, Sorensen *et al.* (2016) and Romano *et al*. (2016) stated that the insect pest is resistant to some insecticides such as malathion and phosphine which made it more difficult to handle. Synthetic pesticides and their metabolites often have high persistence in soil, water and crops themselves and thus impact the atmosphere and human health during the preparation, application and consumption of crops (Mpumi *et al.*, 2016).

Due to these challenges, alternatives to the existing synthetic insecticides must be pest-specific, non-harmful to other organisms, biodegradable and less costly. One of the alternatives to control this insect is by using essential oils from plant parts which show interaction, fumigant, repellent and anti-feeding behaviour to some coleopteran insect pests infesting stored products. Among them, the essential oils of tea tree, *Eucalyptus*, cinnamon, Rosemary, Thyme, and lemongrass are variously studied (Abd El-Salam, 2010; Lee *et al*., 2001). However, the fumigant activity in volatile oils is due to the presence of monoterpenes (Koul *et al.*, 2008; Mossa, 2016) takes the longest time and needs the highest concentration to be a good repellent on rice weevil.

Wood vinegar which is liquid smoke produced by the wood pyrolysis process of charcoal (Dewi *et al.*, 2020; Komarayati & Santoso, 2011) is a natural and organic material that can be used as a plant-based insecticide. The chemical components contained in wood vinegar is effective in inducing growth and preventing insect pest attack and plant diseases, for example, it increased pest resistance to whitefly in mulberry plants. Besides that, the uses of wood vinegar can reduce the overuse of chemical insecticides and to provide environmentally friendly and effective pest control (Jothtyangkoon *et al.*, 2017; Kim *et al.*, 2008). Thus, this study aims to assess the insecticidal activity of wood vinegar for the control of insect pests, especially the rice weevil.

2. Materials and Methods

2.1 Materials Preparation

At least 900 rice weevils obtained from infested rice were bred in-house in a transparent 3L plastic container placed in a kitchen cabinet to avoid sunlight, at a temperature range between 23°C to 28°C and the relative humidity ranging from 40% to 80%. The rice weevils were fed with rice and allowed to breed and grow until adulthood. Wood vinegar produced by a local manufacturer made from 100% mangrove trees was purchased from local stores.

2.2 Repellency Test

The test was conducted using the method described by Gomathi and Rathinam (2017) and Khani *et al.* (2011) with modifications. The filter papers were divided uniformly into two parts as test areas which were treated and control part. Half of the filter papers were applied with wood vinegar solution at different concentrations of 50, 100, 150 and 200 ppm and labelled as a treated part. Meanwhile, the other half part area of the filter papers were immersed in distilled water and labelled as control. Both parts were air-dried until the solutions were completely evaporated. Each filter paper that has been applied with different concentrations of wood vinegar was attached with the control part and placed in a petri dish. Ten starved adult rice weevils which were starved 24 h prior to the experiment were put inside the centre of each petri dish and properly covered with a lid. The number of rice weevils that were present at both the treated and control parts were observed, and data were recorded every 30 mins up to 5 h. The percentage of repellency values for this test have been calculated following Equation 1 where PR is the value of percentage repellency, NC is the number of rice weevil present in the control part and NT is the number of rice weevil present in the treated part (McDonald *et al.*, 1970).

$$\mathrm{PR} = \left(\frac{\mathrm{NC} - \mathrm{NT}}{\mathrm{NC} + \mathrm{NT}}\right) \times 100 \tag{1}$$

2.3 Contact Activity

The test was conducted using the method described by Abdelgaleil *et al.* (2016), Kerdchoechuen *et al*. (2010) and Khani *et al*. (2011) with some modifications. Ten adult rice weevils were placed inside the petri dish. About 1 mL of wood vinegar from different concentrations (0, 50, 100, 150, and 200 ppm) was directly sprayed onto the rice weevil and placed in the dark place. About 5 g of rice was placed in the petri dish as a source of food. The mortality rates have been recorded after 24 h and LC_{50} values were calculated. Abbott's correction formula has been used to calculate the percentage of rice weevil mortality following Equation 2 where P is the percentage of corrected mortality, T is the percentage of mortality in the treated sample and C is the percentage of mortality in the control sample (Abbott, 1925; Pavela *et al.*, 2020).

$$\mathrm{P} = \left(\frac{\mathrm{T} - \mathrm{C}}{100\ \mathrm{C}}\right) \times 100 \tag{2}$$

2.4 Antifeedant Test

Antifeedant activity was performed according to Gomathi and Rathinam (2017) and Khani *et al.* (2011) with some modifications. The wood vinegar was prepared into 0, 50, 100, 150, and 200 ppm concentrations. These solutions have been applied by mixing it with 5 g of rice grains with each treatment acting as a treated part. The other rice grains that were not mixed with anything acted as a control part. The treated parts of the rice grains have been air-dried. Then, ten starved adult rice weevils were released into each of the plastic containers and left to feed the treated and controlled rice grains. Each plastic container was closed and left for 7 days to allow the rice weevil to feed on the rice grains. After 7 days of the feeding period, the grains' weight losses were weighed. The percentage weight loss of the rice grains was calculated following Equation 3 where WL is the percentage of weight loss, IW is the initial weight of rice and FW is the final weight of rice (Khani *et al.*, 2011).

$$\mathrm{WL} = (\mathrm{IW} - \mathrm{FW}) \times 100/\mathrm{IW} \tag{3}$$

2.5 Statistical Analysis

Statistical analysis was performed using one-way analysis of variance ANOVA to understand the interaction of independent variables with dependent variables. Tukey test was done to analyse the differences between treatments. Probit analysis was used to observe the binomial response variables by evaluating the reaction of rice weevils at different concentrations of each chemical and the concentrations were compared in which the response exists. The probit analysis output, which is LC_{50}, a lethal concentration to kill 50% of the population, was used to analyse the differences in chemical amounts required to obtain the same result in each various chemical used (Finney & Stevens, 1948; Parsaeyan *et al.*, 2020).

3. Results

3.1 Repellent Activity

Table 1 presents the repellent activity of wood vinegar at different concentrations for a 5-h period of exposure. The increase in concentration resulted in increased percentage repellency (PR) of 70%, 71.7%, 78.3%, and 98.3% for 50, 100, 150 and 200 mg/L of wood vinegar, respectively, after an exposure of 5 h. The increase in PR is also dependent on the period of exposure where when the exposure time increases from 1, 2, 3, 4 to 5 h, the repellency increased from 55.0, 63.3, 66.7, and 71.7 to 98.3%, respectively. Overall, this situation might be due to the existence of biochemical constituents that emit or possess strong repellency activity. The result indicated that the effect of different concentrations of wood vinegar to repel the rice weevils in the control part was statistically significant with $p \leq 0.05$. However, the Tukey test further confirmed that there was no significant difference between the treatments.

Table 1. Repellent activity of wood vinegar against rice weevil at different exposure times

	Time (h)	**Concentration (mg/L)**			
		50	100	150	200
Percentage repellency, PR (%)	1	45.0±0.40	50.0±0.63	53.3±0.33	55.0±0.17
	2	56.7±0.77	60.0±0.37	55.0±0.50	63.3±0.50
	3	60.0±0.43	61.7±0.43	60.0±0.37	66.7±0.40
	4	60.0±0.30	66.7±0.70	63.3±0.50	71.7±0.27
	5	70.0±0.30	71.7±0.50	78.3±0.27	98.3±0.13
Mean Repellency (%)		58.34±0.44	62.02±0.53	61.98±0.39	71±0.29

*Percentage of repellency, PR (%) = [(NC-NT)/(NC+NT) × 100. The values are expressed as Mean ± Standard error for three sample replications of 10 adult rice weevils during 5 hours of treatment time.

3.2 Contact Activity

Contact activity was performed to determine the mortality rate of adult rice weevils that had been exposed with several concentrations of wood vinegar and the bio insecticidal activity of wood vinegar against adult rice weevils was evaluated based on the mortality rate of the weevil after direct exposure for 24 h. The results of mortality rate against concentrations are presented in Figure 1.

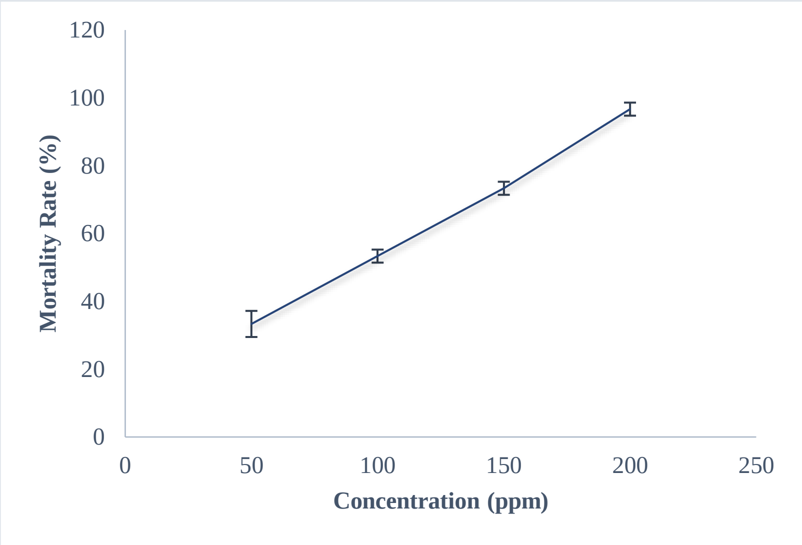

Figure 1. Mortality rate of adult rice weevils against the wood vinegar concentration

The mortality rate of rice weevils increases with the increment of wood vinegar concentration within 24 h exposure. The lowest concentration of 50 rpm showed lowest mortality of 33% while highest concentration of 200 rpm resulted in highest mortality recorded to be 97%. The results of different wood vinegar concentrations against the percentage mortality of rice weevils are statically significant with $p \leq 0.05$, which suggests that the concentrations considerably affect pest mortality. Tukey test with critical values of 19.96 confirmed the significant difference ($p < 0.05$) between treatments.

Table 2. LC_{50} value of wood vinegar applied by contact towards the rice weevil

Concentration (ppm)	% mortality	Empirical probit	Regression equation	LC_{50} value
50	33	4.56	y = 3.475x + (–1.574)	77.62 ppm
100	53	5.08		
150	73	5.61		
200	97	6.88		

Probit analysis was performed to determine the lethal concentration of wood vinegar as presented in Table 2. LC_{50} values were estimated based on the average percentage of mortality against log concentration. As shown in Table 2, the lethal concentration of wood vinegar that was capable of killing as much as 50% of rice weevils populations was at a concentration of 77.62 ppm. This shows the efficacy of the wood vinegar in killing the rice weevil even at low concentrations.

3.3 Antifeedant Activity

The aim of antifeedant test is to study the antifeedant activity of wood vinegar on the weight loss of rice infested by adult rice weevils. Figure 2 below presented a result of weight loss of rice against different concentrations of wood vinegar. The decrease in weight loss of rice is inversely proportional to the increased concentration of wood vinegar after 7 days.

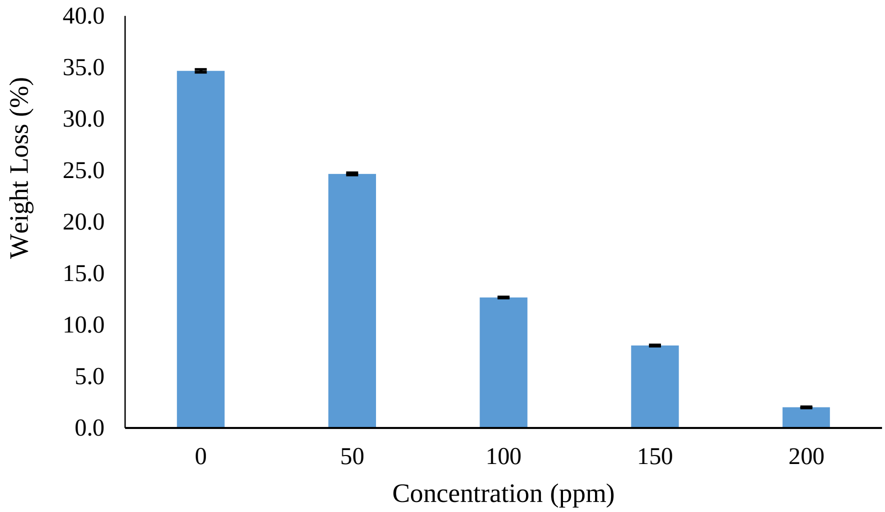

Figure 2. Rice weight loss against the wood vinegar concentrations

Figure 2 above shows a decreasing pattern in weight loss percentage as the concentration of wood vinegar increases. An increase in concentration affect the percentage of weight loss in rice due to rice weevils' loss in appetite after being exposed to the wood vinegar for a period of time. Normally, a decrease in feeding rate shows a greater antifeedant activity. Wood vinegar was able to inhibit rice weevils from eating the rice grains, therefore the feeding activity decreased, as proven by a drop in rice weight loss percentage. As seen from Figure 2 above, the weight loss of rice grains for the control part (0 ppm) is higher than treated part while after 7 days of exposure, the weight loss of rice grains decreased from 34.7% to 2.0% as the concentration of wood vinegar increased from 0 ppm to 200 ppm. The effect of different concentrations of wood vinegar on feeding activity was statically significant with $p \leq 0.05$. Tukey test confirmed the significant difference ($p < 0.05$) between the control and treatment with concentrations ranging from 100, 150, and 200 ppm.

4. Discussion

4.1 Repellent Activity

The result of repellent activity as discussed above is in agreement with Khani *et al.* (2011), who studied the effect of tropical medical plant extract against rice weevils, where it was demonstrated that the plant extract of *Piper nigrum* and *Jatropha curcas* were influenced by the extract concentrations. Based on 1%, 2%, 3%, 4%, and 5% concentrations of both extracts over time durations (1, 2, 3, 4, and 24 h), the highest concentrations (5%) of both extracts have highest repellency activity against the rice weevils which were 80% and 100% as time passed. These findings are also in agreement with those of a previous study obtained by Ishii *et al.* (2010), who studied the repellent test against adult rice weevils by using common spices including fresh chilli, black paper, dried bark of cinnamon, lemongrass and dry chilli powder. It was found that the cinnamon methanol extract showed the highest repellent effect against rice weevils during each exposure times interval (6 h) at a concentration of 10 mg/mL. In another study, when tested with the extract of *Amomum cardamomum* under various concentrations (25%, 50%, 75%, and 100%), the highest repellence activity on adult rice weevils was observed at the highest concentration (100%) after 1 h treatment time (Widiyaningrum *et al.*, 2019). A comparison of this study proves that increments in time and concentration positively affect the repellency of wood vinegar and other plant extracts which scientifically represents the actions of chemical constituents' present. Generally, in wood vinegar, this condition may be related to the presence and impact of active chemical substances such as phenol, acetic acid, ester and more which release or contain significant repellency activity. Some studies reported that these three compounds are toxic to insects (Daniels & Miller, 2015; Michałowicz and Duda, 2007; Puterka *et al.*, 2003). As in this study, mangrove wood vinegar was used. It was known that this type of wood vinegar contains pyroligneous acid, used for ages as a sterilising agent, deodoriser, and wider applications in agriculture as an insecticide, fertiliser, antimicrobial and growth-promoting agent (Grewal *et al.*, 2018; Loo *et al.*, 2001). Application of pyroligneous acid from plant biomass as an insecticide was found efficient in controlling insect pests such as *Anticarsia gemmatalis* and *Spodoptera frugiperda* (Ferreira *et al*., 2013; Petter *et al*., 2013).

4.2 Contact Activity

Research on the toxic and repellent effects of natural biopesticides has been studied where differences in concentrations affect mortality rates of rice weevils caused by bioactive compounds contained in both Neem and Mahogoni (Das *et al.*, 2015). This study reported as the concentration of the extract increases, so does the rate of mortality. This finding is also consistent with Younoussa *et al.* (2020), which showed that mortality increased with increasing volume of concentration. Mya *et al.* (2015) described that the highest quantity of kaffir lime extract may result in the greatest percentage of rice weevil mortality. Therefore, it can be concluded that the solution treatment affected rice weevil mortality rate depending on the concentration and period of exposure. In addition, the other factor that influenced mortality rates due to the presence of chemical compounds in wood vinegar such as phenol, acetic acid, ester and others. These compounds act as insecticide and are toxic to the pests that cause the death of the rice weevils. With the increase in concentration, the number of bioactive compounds that react as the insecticide will also increase. Ferreira *et al*. (2013) reported an increase in the concentration of hexane fraction of pyroligneous acid cause the increase in the mortality of *S. frugiperda* with 60 ppm showing the lowest mortality of 10% while 36,000 ppm showed the highest mortality of 100% within 24 h

4.3 Antifeedant Activity

Khani *et al.* (2011) studied on exposure of tropical plant extracts against adult rice weevils and revealed that the highest concentration of *J. curcas* extract and *P. nigrum* (10 μL/g) have the lowest percentage of weight loss. This showed that plant extracts are efficient as an antifeedant agent who can subsequently help to protect the stored rice grains and other post-harvest products. This study was also supported by Wahba (2020) who described that the increase in concentrations can increase the antifeedant activity where the highest concentration (2000 mg/g) is the best concentration to reduce the appetite of rice weevils to consume the grains. A study reported by Omar *et al*. (2007) on antifeedant activities of terpenoids on rice weevils also suggest that pests treated with the highest concentration resulted in the lowest weight loss of the stored grain. This finding supports the application of wood vinegar as a possible approach that could reduce the weight loss of the grains by manipulating the weevil's appetite from consuming and damaging the stored products.

5. Conclusions

Based on this research, it can be concluded that wood vinegar possesses bio-insecticidal activity in killing the rice weevil as well as repellence which is concentration and time-dependent. The results obtained from the repellency test shows that wood vinegar has the highest number of repellency activity. Overall, this repellency test result indicates that the repellent activity of wood vinegar is strong and able to repel rice weevils. The contact activity of wood vinegar showed its bio-insecticidal activity against rice weevils with LC_{50} of wood vinegar applied towards rice weevils calculated to be 77.62 ppm. Based on the antifeedant test, wood vinegar has strong antifeedant activity against the adult rice weevils by reducing the feeding rates, thus, subsequently, reducing the percentage weight loss of rice grains.

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Conflict of Interest: The authors declare there is no conflict of interest.

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