The potency of coconut shell wood vinegar and essential oils as botanical insecticides to control *Hypothenemus hampei* (Coleoptera: Curculionidae)

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**Abstract.** *Hypothenemus hampei* is the major pest on a coffee plantation invested coffee berry in the field then unwittingly carried to storage. The aim of this research was to evaluate the potential of coconut shell wood vinegar and essential oils to control *H. hampei*. The research conducted in the Pest and Disease Laboratory of IIBCRI and Pakuwon Experimental Field started from January until December 2018. The test of clove oil and citronella oil residue conducted at 0, 1, 2, 3, 4, and 5 days after treatment (DAT) at a concentration of 2%; 3%; 4%; 5%; control (aquadest) and α-cyhalothrin insecticides (2 ml/l), respectively. Each treatment used 15 adults of *H. hampei* replicated 3 times. Coconut shell wood vinegar formulated into 2 formulas. Each formula was applied with different essential oil concentrations as 6 treatments and replicated 4 times. Result showed that the botanical insecticide formula of coconut shell wood vinegar + 5% clove oil caused mortality of *H. hampei* until 80-95%, and coconut shell wood vinegar + 5% citronella oil caused mortality of *H. hampei* reached 73.34 - 88.33%. Overall, the best botanical insecticide formula is a combination of coconut shell wood vinegar + clove oil.

1. **Introduction**

*Hypothenemus hampei* (Coleoptera: Curculionidae) or coffee berry borer (CBB) is an important pest of coffee plant that caused damage reached 28.97-37.11%. The damage intensity of CBB is different in each type of coffee field. It is affected by vegetation surrounding the coffee field and the cultivation type applied in the area [1]. Females of *H. hampei* causing damage by boring the berries to lay their eggs. The larvae eat the coffee seeds as soon as they hatched from the eggs. It causes berries damaged and reduces the coffee bean quality. This species is known to cause an increased caffeine and water level in coffee beans [2]. The *H. hampei* damage the coffee berry since the berries ripen and still stayed inside the berries until they fall out and even in the storage [3].

The control techniques against *H. hampei* that common use are mechanical, chemical, and organic control using bio-pesticides. Farmers prefer to use chemical control that applying chemical pesticides to reduce the pest population. Inadvisable insecticide application is potential cause resistance and resurgence problems, new pest emergence, and negative affect on living organisms and the environment.

The wood vinegar has been used for several years as fungicides, herbicides, and insecticides because it repellent [4] and antifeedant [5] characters. The wood vinegar of coconut shells was known to cause mortality of adults and larvae and damage on *Rhyzopertha dominica* eggs [6]. Coconut shells wood vinegar at a concentration of 12.5% caused mortality 53% of *Nilaparvata lugens* [7] and at 0.5% effective in protecting corn and soybean seeds from contaminant fungi in storage warehouses [8]. It also reported cause mortality to termites *Odontotermes* sp. at 81.71%, *Ferrisa virgata* at 95.12% [9], *H. hampei* at 48.87% in the laboratory [9], and also reported not phytotoxic to the plant [7].
2. Materials and methods
This study was conducted in the Pest and Diseases Laboratory and experimental field of Indonesian Industrial and Beverages Crops Research Institute, Sukabumi, West Java, started from January until December 2018. The adult of *H. hampei* (CBB) used in the research were collected from several coffee fields in the West Java area that were reared in the Pest and Diseases Laboratory of IIBCRI.

2.1. The rearing of *H. hampei*
Coffee berries that have the boreholes of *H. hampei* were collect from the coffee plantations in West Java. Collected berries were then sterilized according to the berries sterilization method [10] in the laboratory to remove the carried fungi from the field. Coffee berries soaked with liquid soap for 15 minutes, and then washed with flowing water. The clean berries were then soaked again in sodium hypochlorite 2% solution for 5 minutes and rinsed with distilled water. After the sterilization process, the sterilized berries were air-dried for a couple of hours before placed into a plastic container, covered with gauze, and kept in the tray. After two weeks, adults emerged from the berries. The *H. hampei* was reared in the laboratory for two months before used.

2.2. The wood vinegar production
The raw material of wood vinegar was coconut shells collected from coconut sellers in Parungkuda Market, Sukabumi. The coconut shell was crushed into small chips (diameter ± 5 cm) then sun-dried for 5-7 days. The pyrolysis process of dried coconut shell chips was using a tank connected with a pipe to catch the smoke. The smoke was absorbed through the lid of distillers that were connected to the condenser. Smoke condensed in condenser using water as cooler. The condensation process produced a brown to a black liquid known as wood vinegar. The wood vinegar was stored in a container for the next usage.

2.3. The efficacy test of clove oil residue against *H. hampei*
The test of clove oil residue was carried out at 0, 1, 2, 3, 4, and 5 days after treatment (DAT) at concentration of 2% (CO1); 3% (CO2); 4% (CO3); 5% (CO4); control (aquadest) (CO5) and chlorpyrifos insecticides (2 ml/l) (CO6) as a comparison, respectively. The sterile coffee berries were sorted into berries with symptoms *H. hampei* and healthy (without boreholes). The healthy berries were placed in a petri dish then sprayed with clove oil according to concentration treatments. After sprayed, the coffee berries were aerated for 5 minutes until the berries dry then moved into a petri dish covered with gauze. Each Petri has 10 treated coffee berries and infested by 15 adults of *H. hampei*. The infestation treatment was done at 0, 1, 2, 3, 4, 5 DAT and repeated 3 times. The *H. hampei* mortality was observed at 7 DAT by counting the dead adults. Observation of borer holes on coffee berries was carried out by cut open the berries.

2.4. The efficacy test of citronella oil residue against *H. hampei*
The test of citronella oil residue was carried out at 0, 1, 2, 3, 4, and 5 days after treatment (DAT) at concentration of 2% (CtO1); 3% (CtO2); 4% (CtO3); 5% (CtO4); control (aquadest) (CtO5) and chlorpyrifos insecticides (2 ml/l) (CtO6) as a comparison, respectively. The coffee berries from the field were sterilized then sorted to determine the berries without bored symptom. The healthy berries were placed in a petri dish then sprayed according to the treatment. The coffee berries were then air-dried for 5 minutes or until the berries dry then placed in a petri dish covered with a filter paper mat. Each petri filled with 10 treated berries and then infested with *H. hampei* adult for 15 each Petri. *H. hampei* adult infestation was carried out at 0, 1, 2, 3, 4, 5 days after treatment (DAT) [11] repeated three times. Observation of adults’ mortality was carried out 7 DAT. It also observed the number of holes in the coffee berries by splitting the berries attacked by *H. hampei*.
2.5. The efficacy test of Wood Vinegar Insecticides Formula to control H. hampei

2.5.1. Bio-insecticides formulation. Wood vinegar prepared from the coconut shell was formulated with essential oil (Citronella Oil and Clove Oil) purchased from Chemical Shop dissolved with distillate water into the desired concentration. Wood vinegar, essential oil, and Tween 80 emulsifier were mixed using a spatula to obtain the bio-insecticides formula. The essential oil used in this treatment was Clove Oil for formula I and Citronella Oil for Formula II. The composition of each formula was:

Formula I:
- Coconut shell wood vinegar + clove oil (2%) + tween 80 (0.5%) (A);
- Coconut shell wood vinegar + clove oil (3%) + tween 80 (0.5%) (B);
- Coconut shell wood vinegar + clove oil (4%) + tween 80 (0.5%) (C);
- Coconut shell wood vinegar + clove oil (5%) + tween 80 (0.5%) (D);
- Distilled water control (E); chemical insecticides control (F).

Formula II:
- Coconut shell wood vinegar + citronella oil (2%) + tween 80 (0.5%) (G);
- Coconut shell wood vinegar + citronella oil (3%) + tween 80 (0.5%) (H);
- Coconut shell wood vinegar + citronella oil (4%) + tween 80 (0.5%) (I);
- Coconut shell wood vinegar + citronella oil (5%) + tween 80 (0.5%) (J);
- Distilled water control (K); chemical insecticides (L).

Control of each treatment was distilled water and chemical insecticides with active material α-sihalotrin that commonly used to control H. hampei.

2.5.2. Laboratory treatment. The formula of bio-insecticides was applied using a spraying bottle to clean berries that placed in the plastic containers. The treated berries then aerated for a couple minutes before 15 adults of H. hampei placed in the containers. The mortality of insects was observed at 24, 48, and 72 DAT. Each treatment was repeated 4 times.

2.5.3. Field treatment. Research conducted at coffee Arabica plantation in the Pakuwon experimental field of IIBCRI, Sukabumi West Java. The formula of bio-insecticide was applied using a pressure sprayer in each plot according to the treatment and was repeated 3 times according to field condition. The formula applied in field observation was as follow:
- Coconut shell wood vinegar + clove oil (4%) + tween 80 (0.5%) (A);
- Coconut shell wood vinegar + clove oil (5%) + tween 80 (0.5%) (B);
- Coconut shell wood vinegar + citronella oil (4%) + tween 80 (0.5%) (C);
- Coconut shell wood vinegar + citronella oil (5%) + tween 80 (0.5%) (D);
- Distilled water control (E); chemical insecticide control (F)

The concentration was the best concentration obtained from the Laboratory efficacy test.

Observation of H. hampei infestation was done in experimental plots that consisted of 4 x 4 sample trees (16 coffee trees) of each. The observation was performed before the treatment sprayed [12] until 4 weeks after application with interval observation was 2 weeks. All trees in the experimental plots were observed by counting the number of bunch per twig, the number of coffee berries per bunch, and the number of coffee berries with symptoms of H. hampei infestation per bunch. There were 4 twigs observed of each tree as samples. Damage intensity of H. hampei infestation was calculated using formula

\[ IK = \frac{A}{B} \times 100\% \]
where the IK for damage intensity, A for the number of damaged berries, and B for number of observed berries.

2.5.4. Data analysis. The data collected from the Laboratory and Field Treatment was tabulated in Ms. Excel then were analyzed using one-way Analysis of Variance (ANOVA) followed by Least Significant Difference (LSD) test at 5% error level. The data analyzed using R software [13].

3. Results and discussion

3.1. Effect of clove oil residue against H. hampei
Mortality of adult H. hampei in the laboratory after treatment of clove oil at 0, 1, 2, 3, 4, and 5 days after treatment (DAT) present in (figure 1). The highest mortality (more than 50%) occurred in the treatment of 5% clove oil concentration (CO4) which was directly infested at 0 DAT. The average percentage of mortality at other concentrations of clove oil is still below 50%. The effect of clove oil residue on the CBB decreased over time. This result showed that the effect of clove oil against H. hampei was effective causing mortality until 4 DAT.

Clove oil contains eugenol, caryophyllene, humulene, and eugenyl acetate. A study of Jayanudin [14] reported the chemical composition of clove oil using GCMS. Distillation of clove oil fumes contains the biggest component are eugenol 65.03% and transcaryophyllene 20.94%.

![Figure 1. Mortality of H. hampei adult in the laboratory after treatment of clove oil at 0, 1, 2, 3, 4, and 5 days after treatment (CO:n: clove oil concentration n; DAT: Day After Treatment).](image)

3.2. Effect of citronella oil residue against H. hampei
Application of citronella oil with a concentration of 2, 3, 4 and 5% caused more than 50% H. hampei mortalities in the laboratory. Citronella oil residue at all concentrations up to 5 DAT caused H. hampei mortality of more than 80% in average. The highest mortality (93.33%) occurred in the treatment with a 5% concentration (CtO4) and infested 1 DAT (figure 2).

Citronella oil contains chemical material such as citral compound, citronella, geraniol, mirsena, nerol, farsenol, and dipentena that toxic to insects. Besides, citronella oil contains metil heptanon that repellent against insects.
Figure 2. Average of *H. hampei* mortality with the application of citronella oil treatment in the laboratory (CtOn: citronella oil concentration n; DAT: Day After Treatment).

3.3. Effect of Wood Vinegar Insecticides Formula and essential oil against *H. hampei* in laboratory

Coconut shell wood vinegar insecticides formula added with clove oil caused mortality of *H. hampei* of 51.67% - 80% at 1 days after treatment (DAT); 65% - 80% at 2 DAT and 75% - 95% at 3 DAT (table 1). The bore holes in coffee berries that sprayed with wood vinegar + clove oil were 0.05 – 0.275 while at control with distilled water was 1.175 and control of chemical insecticides was 0.88. However, the number of bore holes in coffee berries did not represent the damage intensity of the coffee seeds. The results showed that the combination of WV+CtO is significant towards the water control of all treatment, but insignificant towards the control of synthetic pesticides in treatment of WV+CtO (2%) + Tween 80 (Table 1). The highest average of mortality is 88.33% for 3DAT with the treatment of WV+CtO (2%)+Tween 80 dan WV+CtO (3%)+Tween 80. The lowest average of mortality is 43.34% for the treatment WV+CtO (2%)+Tween 80 on 1DAT. The combination of WV + CtO also affects the amount of borer holes in the coffee berries. The best combination is on WV + CtO (5%) + Tween 80 on 1DAT. The highest number of borer holes was 0.35 on the average of WV + CtO (4%) + Tween 80 treatment in 3 DAT.

Table 1. Percentage of *H. hampei* mortality after treatment of coconut shell wood vinegar + clove oil at 1, 2 and 3 days after treatment (DAT).

| Formulation                  | 1 DAT       | 2 DAT       | 3 DAT       |
|-----------------------------|-------------|-------------|-------------|
| WV+CtO (2%)+Tween 80        | 43.34 ± 3.85c | 50.00 ± 3.85c | 51.66 ± 6.38c |
| WV+CtO (3%)+Tween 80        | 53.33 ± 5.44b | 63.33 ± 15.87b | 65.00 ± 21.34b |
| WV+CtO (4%)+Tween 80        | 68.33 ± 19.15a | 81.67 ± 8.39a | 88.33 ± 6.38a  |
| WV+CtO (5%)+Tween 80        | 73.34 ± 7.70a | 85.00 ± 6.38a | 88.33 ± 8.39a  |
| Control 1 Distilled Water   | 3.34 ± 3.85d  | 3.34 ± 3.85d  | 5.00 ± 3.34d   |
| Control 2 Chemical Pesticides| 36.67 ± 3.85c | 40.00 ± 5.44c | 41.67 ± 6.39c  |

WV=Wood Vinegar, CtO=Citronella Oil, DAT=Days After Treatment; Mean±SD; Averages (±SE) followed by the same letter in the columns are not different by Least Significant Difference 5%.
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Table 2. Averages of bore hole in coffee berries caused by H. hampei treated with coconut shell wood vinegar + citronella oil at 1, 2 and 3 days after treatment (DAT).

| Formulation                  | Averages of borer a hole cause of H. hampei |
|------------------------------|--------------------------------------------|
|                             | 1 DAT* | 2 DAT* | 3 DAT |
| WV+CiO (2%)+Tween 80        | 0.25 ± 0.10c | 0.28 ± 0.13c | 0.33 ± 0.05c |
| WV+CiO (3%)+Tween 80        | 0.1 ± 0.14c | 0.3 ± 0.08c | 0.33 ± 0.10c |
| WV+CiO (4%)+Tween 80        | 0.28 ± 0.10c | 0.33 ± 0.15c | 0.35 ± 0.17c |
| WV+CiO (5%)+Tween 80        | 0.05 ± 0.06c | 0.33 ± 0.05c | 0.33 ± 0.05c |
| Control 1 Distilled Water   | 1.18 ± 0.26a | 1.18 ± 0.26a | 1.55 ± 0.26a |
| Control 2 Chemical Pesticides | 0.88 ± 0.21ab | 0.93 ± 0.13b | 0.93 ± 0.13b |

WV=Wood Vinegar; CiO=Citronella Oil; DAT=Days After Treatment; Mean±SD; * the star behind the DAT showed the treatment was significant; Averages (±SE) followed by the same letter in the columns are not different by Least Significant Difference 5%.

Effectivity test of coconut shell wood vinegar added with citronella oil insecticides formula application in the laboratory caused mortality of H. hampei was 48.33% - 73.33% at the first days after treatment (DAT); 50% - 85% at 2 DAT and 51.67% - 88.33% at 3 DAT (Table 2). This result showed that the formula was still effective causing mortality of H. hampei until 3 DAT. The highest mortality caused by formula wood vinegar with citronella oil 5%.

The result showed that the combination of WV+CO towards H. hampei is significant for the control of water and synthetic pesticides in all treatments. The average mortality of H. hampei is up to 95% for WV+CO (5%) + Tween 80 for 3DAT, and the lowest is on WV+CO (2%) + Tween 80 for 1 DAT with the average percentage of mortality is 51.67% (Table 3). The combination of WV + CO also affects the amount of borer holes in the coffee berries. The best combination is on WV + CO (5%) + Tween 80 on 1 DAT. The highest number of borer holes on average was 14.25 in the WV + CO (2%) + Tween 80 treatment in 3 DAT.

Table 3. Percentage of H. hampei mortality after treatment of coconut shell wood vinegar formula + clove oil in observation at 1, 2, and 3 days after treatment (DAT).

| Formulation                  | Mortality of H. hampei (%) |
|------------------------------|-----------------------------|
|                             | 1 DAT | 2 DAT | 3 DAT |
| WV+CO (2%)+Tween 80         | 51.67 ± 11.39b | 65.00 ± 17.53a | 75.00 ± 10.00a |
| WV+CO (3%)+Tween 80         | 60.00 ± 12.17b | 65.00 ± 23.96a | 78.33 ± 29.00a |
| WV+CO (4%)+Tween 80         | 65.00 ± 22.03ab | 68.33 ± 17.53a | 75.00 ± 21.34a |
| WV+CO (5%)+Tween 80         | 80.00 ± 9.43a | 80.00 ± 10.88a | 95.00 ± 19.90a |
| Control 1 Distilled Water   | 1.67 ± 3.33c | 0 ± 0b | 18.33 ± 3.33b |
| Control 2 Chemical Pesticides | 10.00 ± 12.77c | 10.00 ± 8.61b | 1.75 ± 3.33b |

WV=Wood Vinegar, CO=Clove Oil; DAT=Days After Treatment; Mean±SD; Averages (±SE) followed by the same letter in the columns are not different by Least Significant Difference 5%.

Based on the results of the research, botanical insecticide residues were still effective in killing H. hampei on 3 DAT observations both in the combination of WV + CiO and WV + CO. The highest concentration was found in 5% essential oil treatment with a mortality rate of 95%. The high percentage of deaths is estimated to be due to the effect of giving liquid smoke. Based on [8] report, the application of coconut shell liquid smoke can cause termite mortality to reach 81.71% and 95.12% for mealy bugs. Also, the content of clove oil is potential insecticide because it contains eugenol and eugenol acetate which can be toxic to insects. This is following the opinion which states that clove oil has a contact toxicity effect of Cacopsylla chinensis with the highest LD 50 value, namely 0.730 µg / imago, and
1.795 µg/nymph. Furthermore, it was explained that the field trials showed that clove essential oil caused a significant population decline of 73.01% [15].

Table 4. Averages of bore hole in coffee berries caused by H. hampei treated with coconut shell wood vinegar + clove oil at 1, 2 and 3 days after treatment (DAT).

| Formulation                  | Hole cause H. hampei |
|------------------------------|-----------------------|
|                              | 1 DAT*                | 2 DAT                 | 3 DAT                 |
| WV+CO (2%)+Tween 80          | 0.25 ± 0.10<sup>c</sup> | 12 ± 0.13<sup>c</sup> | 14.25 ± 0.33<sup>c</sup> |
| WV+CO (3%)+Tween 80          | 0.1 ± 0.14<sup>c</sup> | 10.25 ± 0.08<sup>c</sup> | 11.75 ± 0.33<sup>c</sup> |
| WV+CO (4%)+Tween 80          | 0.28 ± 0.10<sup>c</sup> | 9.75 ± 0.15<sup>c</sup> | 11.25 ± 0.35<sup>c</sup> |
| WV+CO (5%)+Tween 80          | 0.05 ± 0.06<sup>c</sup> | 9.75 ± 0.05<sup>c</sup> | 11.25 ± 0.33<sup>c</sup> |
| Control 1 Distilled Water    | 1.18 ± 0.26<sup>a</sup>| 1.5 ± 0.24<sup>a</sup> | 2.75 ± 1.55<sup>a</sup> |
| Control 2 Chemical Pesticides| 0.88 ± 0.21<sup>b</sup>| 0 ± 0.13<sup>b</sup>  | 0.25 ± 0.13<sup>b</sup> |

WV=Wood Vinegar, CO=Clove Oil; DAT=Days After Treatment; Mean±SD; * the star behind the DAT showed the treatment was significant; Averages (±SE) followed by the same letter in the columns are not different by Least Significant Difference 5%.

The use of botanical insecticides can reduce the number of borer holes caused by H. hampei compared to control and synthetic pesticides. It is suspected that there is an antifeedant in the botanical insecticide that is applied. Clove oil is a botanical insecticide that is antifeedant (reduced appetite) [16]. However, the results showed that the antifeedants in botanical insecticide residues did not last long, this was indicated by the increasing number of borer holes in the 3 DAT in each treatment.

3.4. Effect of Wood vinegar Insecticides Formula and essential oil against H. hampei in field

The efficacy test of botanical insecticide formula was conducted in Arabica coffee field in Pakuwon experimental field of IIBCRI, Sukabumi, West Java. Damage intensity of coffee berries was observed to four twigs of each coffee plant. Results showed that the application of the insecticides formula in the coffee field was not significant at the first observation (F5,12=0.777, P=0.585), however, it has shown a significant difference at the second observation (F5,12=9.29, P=0.00***) (figure 3).

The result presented in figure 3 showed that botanical insecticides of coconut shell wood vinegar combined with essential oil application in the field were able to reduce the number of damaged berries at the second observation. This showed that the wood vinegar and essential oil combination was able to suppress the feeding activity of H. hampei. The formula that showed maximum reduction effect was a combination of wood vinegar and clove oil. This result following the statement that clove essential oil are potential compounds that possible to be exploited as strategies to control insect pests [15,16].
Figure 3. Percentage of berries damaged by *H. hampeii* in the field (a: First observation; b: second observation; A: coconut shell wood vinegar + clove oil (4%) + tween 80 (0.5%); B: coconut shell wood vinegar + clove oil (5%) + tween 80 (0.5%); C: coconut shell wood vinegar + citronella oil (4%) + tween 80 (0.5%); D: coconut shell wood vinegar + citronella oil (5%) + tween 80 (0.5%); E: distilled water control; F: chemical insecticide control).

4. Conclusion

Adult of *H. hampeii* that infested on the same day after treatment reached 50% mortality of populations at coffee berry that sprayed with 5% of clove oil. Citronella oil residue at all concentrations up to 5 DAT caused *H. hampeii* mortality of more than 80% on average. The botanical insecticide formula of coconut shell wood vinegar + 5% clove oil caused mortality of *H. hampeii* until 80-95%. The botanical insecticide formula of coconut shell wood vinegar + 5% citronella oil caused mortality of *H. hampeii* was 73.34-88.33%. Overall, the best botanical insecticide formula is a combination of coconut shell wood vinegar + clove oil.

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