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## Research Article

# Field Efficacy of *Nicotiana tabacum* L. var Virginia Extract against Coffee Borer Beetle (*Hypothenemus hampei*) Attacking Coffee Berries in Plantation Area

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The extract of *Nicotiana tabacum* L. var Virginia origin of Ponorogo (EHRE-Nt) was applied under field conditions against the coffee borer beetle *Hypothenemus hampei* (CBB). Three extract formulations containing 150, 300, and 450 ml of EHRE-Nt in 100 liters of water were prepared and sprayed on 0.5 hectares each. A nontreatment was applied as a control on 0.5 hectares of plantation. The intensity of the CBB attacks was observed for six weeks on a Robusta coffee plantation in the Kalibening area, South Semarang, Central Java, Indonesia. An extract formulation containing 450 ml of EHRE-Nt resulted in the lowest intensity of CBB attacks from weeks one to six (1.6% to 2.2%). The attacks of the control field were 11.3% to 13.5%. The highest efficacy level of 85.4% was obtained with the 450 ml EHRE-Nt formulation. These results demonstrate that EHRE-Nt has potential as a bio-insecticide for coffee berries from CBB attacks.

## 1. Introduction

The coffee borer beetle *Hypothenemus hampei* Ferrari (abbreviated as CBB) is the major coffee pest that is harmful for coffee berries on the coffee plantation. Globally, it has been causing 25% annual losses in the production of harvest coffee over US\$500 million in the world [1]. CBBs attacks also induced substantial losses on coffee plantations in Indonesia. For an average yield of total Indonesian coffee about 1.25 million hectares, it is more than US\$6.7 million

per year of losses due to CBBs attack. So, the yield losses in a hectare are about 50 kg per year [2].

Adult females of CBBs make holes in the endosperm of coffee beans to put their eggs into the holes. The eggs are transformed into larvae in only around four days. The larvae are then proliferated as the pupae after 15 days and then come out from the coffee berries as mature beetles after seven days [3, 4]. So, between eggs and adult phases, they got the feed and nutrients from the coffee berries where they live [5, 6].

The proliferation of CBBs is also influenced by the temperature and availability of coffee berries on the coffee plantation. The CBBs eggs are optimally proliferated at 30–32°C, while the larvae, pupae, and adult beetles are at 27–30°C. The holes in coffee berries are made by adult female beetles at the temperature range of 20–33°C, whereas at a temperature below 15°C or above 35°C, the beetles fail to make holes in coffee berries, or the holes could be made by them without any eggs inside. The peak of the intensity of CBBs attack on coffee berries on the plantation is around May to July, where the coffee plants mostly produce coffee berries at these periods [7, 8].

The CBBs can attack both immature and mature coffee berries. The damaged immature coffee berries due to CBBs attack are then rotten and fall. Differently, the attack of CBBs on the mature coffee berries caused flawed coffee beans [9]. In both cases, the coffee bean quality and yield productivity are significantly jeopardized if the insects are not eradicated.

The use of synthetic insecticide is widely known to eradicate CBBs attack on a coffee plantation. A carbamate group from widely used commercial pesticides with the active compound of 85% carbaryl is an example of a white crystalline synthetic insecticide. This insecticide effectively kills CBBs beetle pest by contact and stomach poisoning as the effect from its active compound. However, indiscriminate use of carbaryl and also carbofuran insecticide leaves the residue and leads to the emergence of resistant to many insects such as the peach potato aphid *Myzus persicae* [10], the German cockroach *Blattella germanica* [11], the striped stem borer *Chilo suppressalis* [12], and also *Hypothenemus hampei* [13, 14]. The hazardous residue can be absorbed into the coffee beans and can also contaminate the environment for a long-term period [13]. Therefore, the use of synthetic insecticides should be substituted by natural insecticides (bioinsecticides) due to the low toxicity of bioinsecticides and its safe application for the environment.

Some methods for controlling CBBs were achieved by integrated pest management (IPM). They are, firstly, the application of the biological control agents as natural enemies of CBBs; secondly, sanitation harvesting of coffee berries as a CBBs food source which is left on the trees and the soil surface after harvesting; and thirdly, the application of the bioinsecticides to eradicate or prevent the CBBs attack [15, 16].

The use of soil fungus *Beauveria bassiana* against CBBs was reported in Columbia [17] and also in India [18]. The results showed a significant decrease in the CBB population due to the presence of infected CBB by a mixture of *B. bassiana* strains. The infected CBBs failed to proliferate in coffee berries. The other biocontrol agents, i.e., *Cephalonomia stephanotis* Betrem (*Hymenoptera: Bethyridae*) and *Phymastichus coffea* LaSalle (*Hymenoptera: Eulophidae*), were also reported as two types the ants as parasitoids of CBBs [19, 20].

The soil fungus *B. bassiana* is widely known as an entomopathogenic fungus against a large number of insect species worldwide. However, the activity of *B. bassiana* is not entirely satisfactory due to its use in large quantities under field conditions. This fungus also needs to mix with other strains or natural compounds to be effectively used as a biological control agent against CBBs [21, 22]. The uses of

two types of ants as parasitoids are not always available against the larvae and pupae of the CBBs. Their availability is highly dependent on their season to breed naturally, or they can only be produced on a limited scale for the proliferation of these two parasitoids to control CBBs population [19, 20]. Thus, the uses of biological control agents require to be combined or mixed with natural insecticides (bioinsecticides) as an IPM to control more effectively and efficiently the CBBs [15, 16].

*Nicotiana tabacum* is well known as a natural insecticide to eradicate the insects. Previous studies reported that *N. tabacum* contains at least 200 hazardous chemicals, including nicotine, phenolic compounds, and diterpene [23–25]. They are the compounds that are mostly found in tobacco leaves. Nevertheless, the composition of *N. tabacum* extract can potentially change depending on the species, place of origin, type of extraction methods, and solvents used [25]. Nicotine, phenolic compounds, and diterpene are toxic to insects, especially for nicotine that acts as a neurotoxin for most pest insects, mammals, and birds [26, 27]. On the other hand, the extract of *Nicotiana tabacum* could reduce the protein content of both coffee bean and skin which is used by CBBs for their growth and development, thus lowering the risk of insect pests [28].

The previous studies reported that *N. tabacum* extract had been used to eradicate and prevent most insects and other pests. The combination of 320 g *Beauveria bassiana*/8 L of water and 30 ml *Nicotiana tabacum* extract/10 L of water was found to be able to reduce the percentage and intensity of coffee fruit attacked by CBB to 1.54% and 0.33%, respectively [29]. The attack of cabbage looper (*Trichoplusia binotalis*), as the significant insects under field conditions in cabbage plantation, was also significantly lower with an application of 3% aqueous extract of *N. tabacum*. The cabbage looper attack was only 9.32% that occurred on the field, while the attack to the control was 34.23% [30]. Other previous studies also reported that *Nicotiana tabacum* L. leaf extract obtained from Ethanolic Heat Reflux Extraction (EHRE) had shown insecticidal activity against some agricultural pests such as *Gryllus bimaculatus* (cricket), *Galleria mellonella* (greater wax moth) larvae, *Tenebrio molitor* (mealworm beetle) larvae, and *Zophobas morio* (darkling beetle) larvae at  $LC_{50}$  values of 38.5 mg/ml, 36.6 mg/ml, 11.1 mg/ml, and 71.1 mg/ml, respectively [31, 32]. The top fraction bio-oil of *N. tabacum* was also reported as a biorepellent to protect human skin from mosquito bites. The result showed that the average percentage of human skin protection against mosquito bites was 57.07% for 6 hours, using 3% bio-oil concentration [24].

This study focuses on the investigation of the ethanolic heat reflux extract of the *Nicotiana tabacum* (EHRE-Nt) to control the intensity of CBBs attack. The main novelty of this study is the application of the EHRE-Nt as a bioinsecticide against a major pest insect, *Hypothenemus hampei* Ferrari (*Coleoptera: Scolytidae*) on Robusta coffee plantation. This study examined the intensity of CBBs attack expressed in percentages of damaged coffee berries. The efficacy level of EHRE-Nt formulation on CBBs attack was also calculated.

## 2. Materials and Methods

**2.1. Materials.** The ethanolic heat reflux extract of *N. tabacum* L. var Virginia (EHRE-Nt), *Lypothanemus hampei* Ferrari (Coleoptera: Scolytidae), and the coffee berries on Robusta coffee plantation were used in this study. Dichlorodiphenyltrichloroethane, organophosphorus pesticides mix, and carbamate standards used for thin-layer chromatography (TLC) analysis were purchased from Sigma-Aldrich, Germany. Silica gel 60G F<sub>254</sub> TLC plates were also purchased from Merck Co., Germany.

**2.2. Biopesticide Preparation.** Tobacco leaves were taken from Ponorogo District (East Java, Indonesia). The procedure for obtaining a high yield of EHRE-Nt was explained in the previous study [23]. Ethanolic heat reflux extraction was used for our previous study to produce EHRE-Nt. The extraction was achieved at 6 hours to obtain a high yield of EHRE-Nt with the optimum temperature at 70°C, 150 rpm, and a fixed solid-to-solvent ratio at 1:5.

An amount of 150 ml, 300 ml, and 450 ml of the concentrated EHRE-Nt was added to three stainless steel containers containing each 100 l of water. They were named as E1, E2, and E3, respectively. These formulations were sprayed on the coffee plantation. A nontreatment of coffee beans was applied as a control, named as C.

**2.3. Location of Efficacy Assay.** The coffee plantation is located in the Kalibening area, Kebondalem Village, in Jambu district (South Semarang, Central Java, Indonesia). The coordinate of the area is 7°16'44" S and 110°20'11" E, with an altitude of 650–710 m above sea level (m.a.s.l.), an average temperature of 18.6°C, and an average annual rainfall of 2801 mm [33]. Kalibening is surrounded by four mountains, i.e., Ungaran, Merbabu, Sumbing, and Sindoro (see Figure 1). Thus, the climate and soil conditions in this area are suitable for Robusta coffee production. There were a total of 2 hectares that are used for this experiment, i.e., 1.5 hectares for the field assays and 0.5 hectares for the control. The experiments were carried out for six weeks.

**2.4. Characterization of the Extract.** Characterization of chemical compounds in the EHRE-Nt was conducted by a GC-MS from Agilent Technologies 7890 Series with auto-sampler, 5975 Mass Selective Detector, and Chemstation Data System. An electron impact using ionization mode with 70 eV of electron energy was set for this instrument. The samples of EHRE-Nt were injected into a capillary column HP Ultra 2L with 30 × 0.25 mm ID and 0.25 μm film thicknesses. The GC-MS analysis was conducted at the Regional Health Laboratory (Labkesda), Jakarta.

The presence of any residual of organochlorine, organophosphate, and carbamate was characterized using thin-layer chromatography (TLC) with methyl alcohol p.a as a mobile phase. These TLC assays were conducted at the Bureau of Testing and Certifications for Quality of Commodities, Agency for the Industrial and Commercial Affairs,

Central Java Provincial Government, Republic of Indonesia. Silica gel 60G F<sub>254</sub> TLC plates (Merck Co., Germany) were used as the stationary phase. The residues of As, Pb, Cd, and Hg were also analyzed by the atomic absorption spectroscopy (AAS) method. These assays were conducted at Laboratory for Testing Quality of Medicinal, Food and Cosmetics, Faculty of Pharmacy, Universitas Indonesia. A hydride vapor generation (HVG) was used as a flame selection of AAS for Arsenic (As) detection, while for Pb and Cd, O<sub>2</sub>-C<sub>2</sub>H<sub>2</sub> was used as a flame of AAS. A flame of Mercury vapor unit (MVU) was then used for Hg detection by AAS.

**2.5. Randomized Block Design (RBD).** RBD by triplicate was used as a model for this experiment. Three blocks of coffee plants were used for the assays (Block I–III). A block was used for control (Block IV). Each block consists of three replications of each extract solution in randomized three sample coffee plants on 0.5 hectares. The model of RBD is presented in Table 1. They were observed for six weeks on the coffee plantation.

**2.6. The Intensity of CBBs Attack and Efficacy EHRE-Nt as Bioinsecticide.** The intensity of CBBs attacks (*I*) was calculated using the formula shown in equation (1), where *x* and *y* are the numbers of damaged coffee berries due to CBBs attack and amount of nondamaged coffee berries, respectively:

$$I = \frac{x}{(x + y)} \cdot 100\%. \quad (1)$$

The efficacy criteria of bioinsecticide using EHRE-Nt can be calculated based on the number of coffee berries attacked on the coffee plantation. Observation of coffee berries damage due to CBBs before application of EHRE-Nt showed no significant difference between treatment blocks. Therefore, the efficacy level (*E*) of the extract formulation can be calculated using the Abbot formula shown in equation (2) below with *Ca* and *Ta* as the intensity of CBBs attack to the control block after application of the EHRE-Nt (%) and intensity of CBBs attack to treatment block after application of the EHRE-Nt (%) respectively:

$$E = \frac{Ca - Ta}{Ca} \cdot 100\%. \quad (2)$$

The EHRE-Nt formulation was effectively used as bioinsecticide to prevent CBBs attack on coffee plants if the efficacy level of the extract formulation is greater than 50% [14, 34].

**2.7. Morphology of Coffee Bean by Scanning Electron Microscopy (SEM).** SEM (JSM-6510 LA, JEOL Ltd., Japan) was used to compare the morphological differences between coffee bean samples, which were infected and uninfected by CBBs. This morphological comparison is intended to see the impact of damage on coffee beans due to CBB attacks on a microscale. The coffee bean samples (infected and

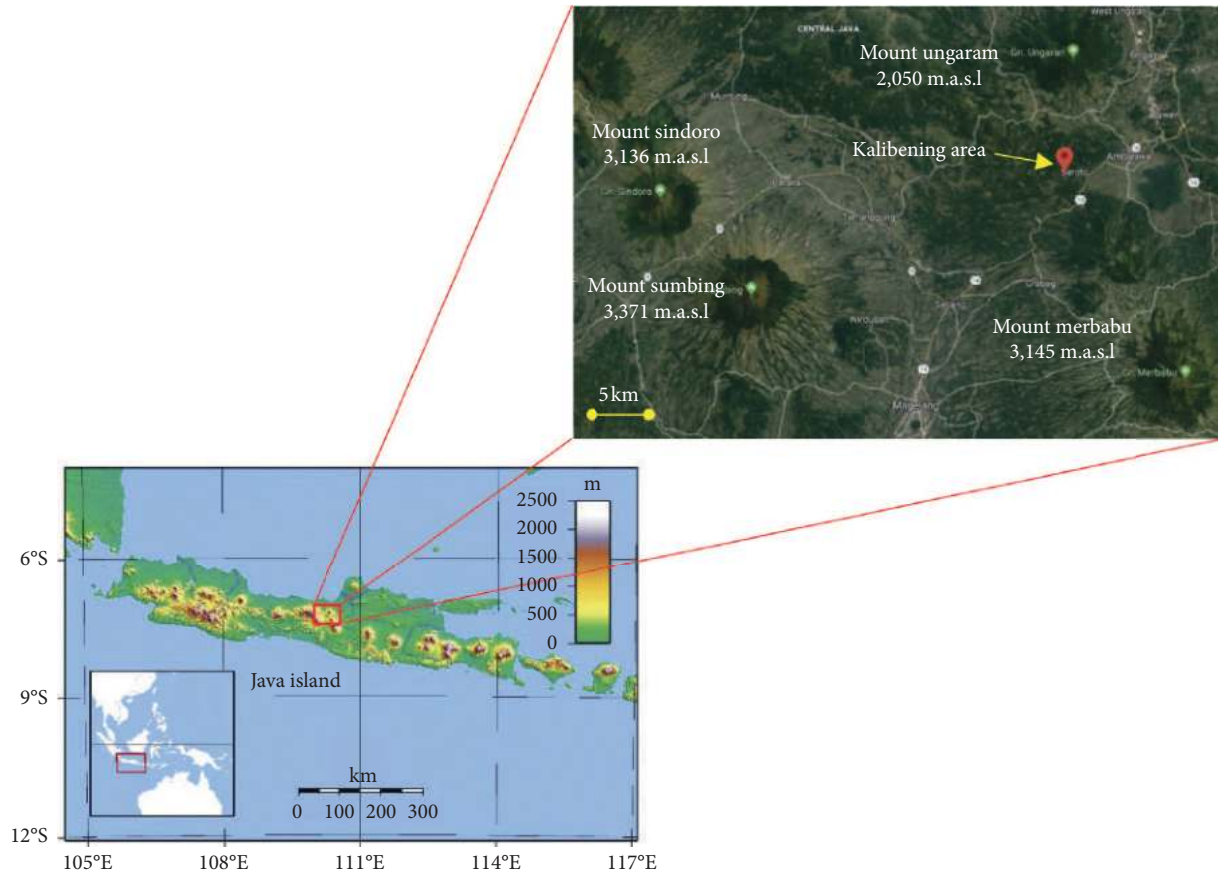


FIGURE 1: Geographic location of the Kalibening area (a robusta coffee plantation). This location is surrounded by four mountains, i.e., Ungaran, Merbabu, Sumbing, and Sindoro. Kalibening is located at Kebondalem village, Jambu district, South Semarang, Central Java, Indonesia (source: modified from Google Maps).

TABLE 1: The triplicated randomized block design (RBD) in this experimental design.

Block (@area = 0.5 hectare)	Extract <sup>†</sup> (ml)	Sample name <sup>‡</sup>
I	150	E1-T01
	150	E1-T02
	150	E1-T03
II	300	E2-T04
	300	E2-T05
	300	E2-T06
III	450	E3-T07
	450	E3-T08
	450	E3-T09
Control	0	C-T10
	0	C-T11
	0	C-T12

<sup>†</sup>Each extract was diluted into 100 l of water, except the control. <sup>‡</sup>E1, E2, and E3 are the extract solutions that consist of 150, 300, and 450 ml of the concentrated extract, respectively. They are then sprayed into the randomized plants on a total of 2 hectares. T01 until T12 are the randomized coffee plants used in this study. C is the control without treatment.

uninfected) were coated with gold under vacuum conditions. The accelerated voltages and samples diameter observed were 15 kV and ten  $\mu\text{m}$ , with 1000X magnification. The observation was carried out at around the cross section

of both samples of coffee beans. The SEM analysis was performed at Sentra Teknologi Polimer, Agency for the Assessment and Application of Technology (BPPT).

### 3. Results and Discussion

**3.1. Characteristics of the Extract.** The characterization of chemical compounds in the EHRE-Nt by GC-MS analysis was carried out using Chemstation Databases System. The results are shown in Table 2.

The GC-MS result showed the presence of 16 different chemical compounds (see Table 2). There are two major chemical compounds in the extract, i.e., nicotine and linoleic acid. The contents of nicotine and linoleic acid obtained by GC-MS analysis were 6.30% and 3.72%, in which these two contents larger than the other compounds. These results are similar to those found in other studies [35–38]. The nicotine content of *Nicotiana tabacum* L. leaves extract obtained from Heat Reflux Extraction (HRE) technique at 6 hours was found to be 6.3% by HPLC [35]. Huang et al. [38] found that another compound with a percent of relative content more than 20%, i.e., androsta-3,5-dien-7-one (21.06%), which are not found in our result [38]. However, GC-MS results of the nicotine compound obtained in Shen and Shao [37]; Hossain and Salehuddin [36]; and also Huang et al. [38] were

TABLE 2: The GC-MS<sup>†</sup> spectral analysis of EHRE-Nt<sup>‡</sup>.

No.	RT <sup>§</sup> (min)	Name of the compound	Molecular formula	Molecular weight	Peak area (%)
1	12.76	Nicotine	C <sub>10</sub> H <sub>14</sub> N <sub>2</sub>	162	49.18
2	28.21	Hexadecanoic acid, methyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270	1.80
3	28.66	Hexadecanoic acid (Palmitic acid)	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	2.01
4	28.73	Hexadecanoic acid, ethyl ester	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284	2.06
5	29.14	Methyl 10-trans,12-cis-octadecadienoate	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294	0.62
6	29.19	11-Octadecanoic acid, methyl ester	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296	2.43
7	29.38	Methyl 9-cis,11-trans-octadecadienoate	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294	0.72
8	29.41	Methyl 9-Octadecenoate	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296	2.64
9	29.56	Methyl stearate	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298	0.47
10	29.76	9,12-Octadecadienoic acid (Linoleic acid)	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	280	29.01
11	31.46	Methyl 20-methyl-heneicosanoate	C <sub>23</sub> H <sub>46</sub> O <sub>2</sub>	354	0.91
12	31.63	13-Docosenoic acid	C <sub>22</sub> H <sub>42</sub> O <sub>2</sub>	338	1.86
13	31.77	Ethyl pentadecanoate	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270	0.53
14	32.73	15-Tetracosenoic acid, methyl ester	C <sub>25</sub> H <sub>48</sub> O <sub>2</sub>	380	1.89
15	34.14	22-Tricosenoic acid	C <sub>23</sub> H <sub>44</sub> O <sub>2</sub>	352	1.91
16	35.72	Stigmastan-3,5-diene	C <sub>29</sub> H <sub>48</sub>	396	1.96

<sup>†</sup>Gas chromatography-mass spectrometry analysis was conducted at the regional health laboratory (Labkesda), special capital region (DKI) of Jakarta. No.: 2.3/1582. <sup>‡</sup>The ethanolic heat reflux extract of *N. tabacum* L. var Virginia origin of Ponorogo. <sup>§</sup>Retention time for each detected compound expressed in minutes.

detected less than our GC-MS result, i.e., 2.90%, 3.60%, and 4.25%, respectively [36–38]. On the contrary, we found the substance of linoleic acid, which is not found in Huang et al. [38]. In most cases, the nicotine is the predominant compound in *Nicotiana tabacum* and *Nicotiana rustica*, with the range 0.5–8% [25, 39].

The mass-spectral chromatograms and chemical structures of nicotine and linoleic acid in the EHRE-Nt are shown in Figures 2 and 3. The presence of nicotine and linoleic acid compounds was proven by the pattern of fragmentations that occurred in each mass-spectral chromatograms (see Figures 3 and 4). The patterns of fragmentation of these two compounds were similar to the standard of nicotine and linoleic acid obtained from the Chemstation Databases System. A molecular ion peak ( $M^+$ ) of nicotine was achieved at  $m/z$  161.1, while linoleic acid has  $M^+$  at  $m/z$  280.3. These molecular ion peaks were 97% and 99% similar to the molecular weight of nicotine and linoleic acid standards, i.e., 162 and 280 g/mol.

TLC qualitatively characterized any residuals of organochlorine, organophosphate, and carbamate as hazardous chemicals that could be found in the EHRE-Nt. AAS methods also examined any residual substances of As, Pb, Cd, and Hg. Seven types of hazardous components were examined to the EHRE-Nt (see Table 3). The results showed that there were no hazardous residues in the extract except a tiny amount of Arsenic (As) substance. It was  $<1 \mu\text{g/g}$  of extract (under the limit of  $1 \mu\text{g/g}$  as an allowable limit). Thus, the EHRE-Nt would not be harmful if it is exposed to the plants as a natural insecticide nor contacted with human skin.

**3.2. The Intensity of CBBs Attack.** The intensity of CBBs attack on coffee berries is shown in Figures 4–6. Figure 4 shows the coffee berry with infection by CBB and without. Figure 5 shows the CBBs and the impact of their attacks in a coffee berry. The morphological differences of damaged

coffee bean samples due to CBBs attack and undamaged coffee bean as control were examined using scanning electron microscope (SEM) on a microscale. The results are shown in Figure 6.

The damaged coffee bean in a microscale due to the *Hypothenemus hampei* (CBBs) attack was shown in Figure 6(b). The coffee bean appears rough and visibly damaged morphological structures. This condition occurs due to the coffee bean that is consumed by the larvae and pupae of CBBs. They can consume coffee beans starting from the hole where they live in and spread until the whole of the coffee bean. So, if compared to the undamaged coffee bean as a control, then undamaged coffee bean tends to be more subtle than the damaged coffee bean (see Figures 6(a) and 6(b)). For immature coffee berries, this condition can damage endosperm cells. If the endosperm cells are broken, then coffee berries with the seeds inside become rotten and fall. For mature coffee berries, the damaged condition causes perforated coffee beans. The defective coffee beans also significantly affect the composition of their chemical compounds inside, especially at caffeine and reducing sugars. So, this condition affects the taste of the coffee and will degrade the quality of the coffee [4, 40].

The percentages of the intensity of CBBs attack to coffee berries on a coffee plantation in the block I–IV for six weeks of observation are shown in Figure 7. They were obtained based on the calculation result using (1).

Figure 7 shows that the lowest intensity of CBBs attack was achieved by formula E3 (450 ml EHRE-Nt in 100 l water), i.e.,  $1.5 \pm 0.10\%$ . This value was achieved at the 3<sup>rd</sup>-week observation. The highest intensity of CBBs attack was achieved by formula E1 (150 ml EHRE-Nt in 100 l water), i.e.,  $6.0 \pm 0.25\%$ . It was reached at the 4<sup>th</sup>-week observation, while the range of C or control (without treatment) to the intensity of CBBs attack that occurs was between  $11.3 \pm 0.28\%$  and  $13.5 \pm 0.50\%$ . They were achieved at the 1<sup>st</sup> to 6<sup>th</sup> week's observation. Thus, the average intensity of CBBs attack of C, E1, E2, and E3 formulas was 12.6%, 4.3%,

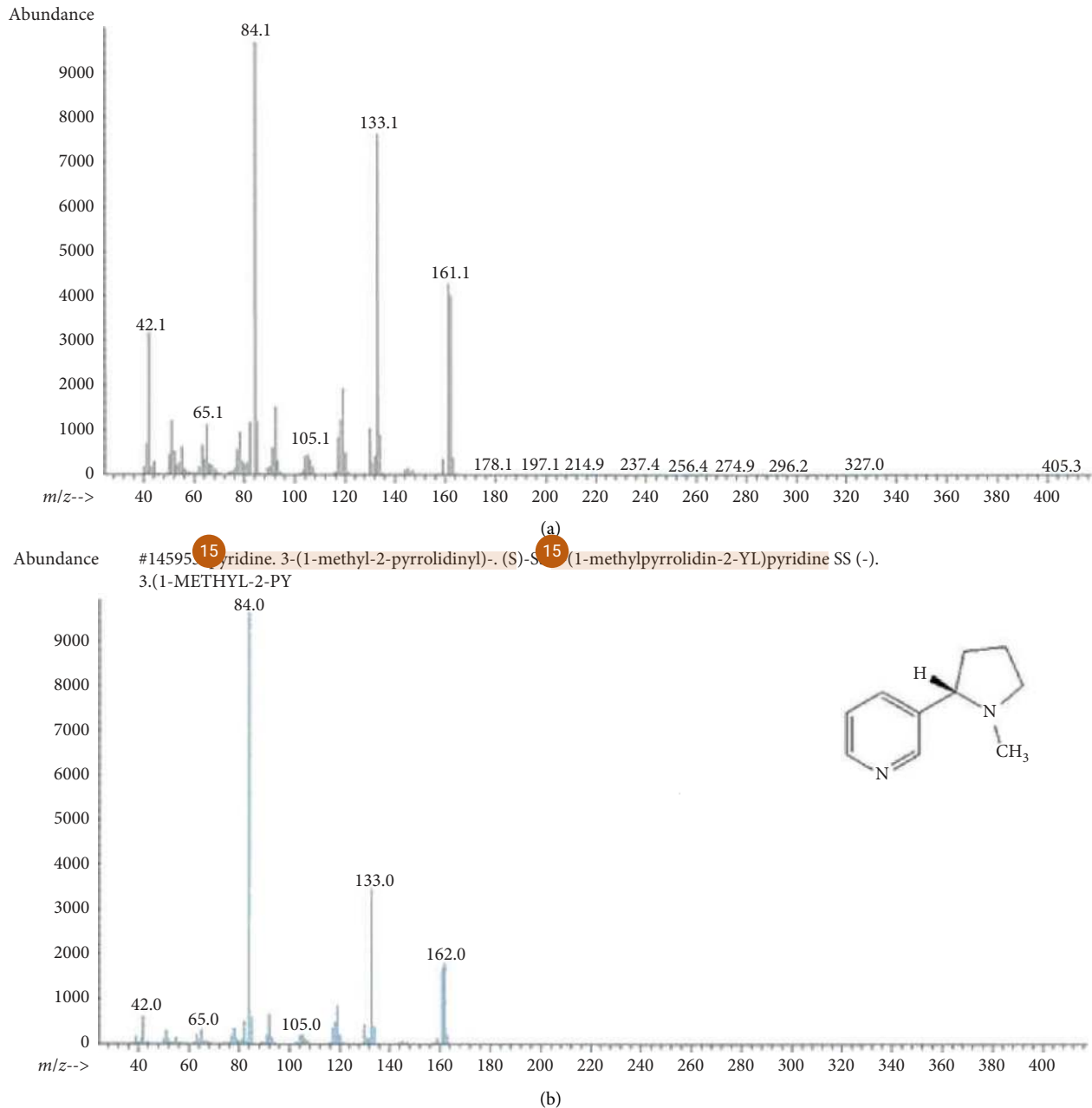


FIGURE 2: The mass-spectral chromatogram of nicotine in the ethanolic heat reflux extract of *N. tabacum* L. var Virginia origin of Ponorogo (a) and the nicotine standard (b).

3.1%, and 1.8%. These results expressed that all applications of three extract formulations of E1, E2, and E3 showed a significantly lower intensity of CBBs attack to coffee berries on the coffee plantation than the control.

The efficacy level of EHRE-Nt as a bioinsecticide against CBBs attack was then calculated using (2). They were obtained based on the average percentages of the intensity of CBBs attack. The results are shown in Table 4 above.

The highest efficacy level of EHRE-Nt formulation as bioinsecticide against the *Hypothenemus hampei* (CBBs)

attack was achieved by E3 formulation; it was 85.4%. The lowest efficacy level of the extract formulation as a bioinsecticide was achieved by E1 formulation; it was 66.1%. However, the overall percent values of the efficacy level of the extract formulations as a bioinsecticide were higher than 50% as a minimum value for bioinsecticide effectiveness (see Table 4). Thus, the EHRE-Nt has a high efficacy as a bioinsecticide to protect coffee berries on a coffee plantation against CBBs attack. Furthermore, EHRE-Nt had the lowest intensity of CBB attacks ( $1.5 \pm 0.1\%$ ) and even the lowest extract concentration (4.5 ml/l water) if

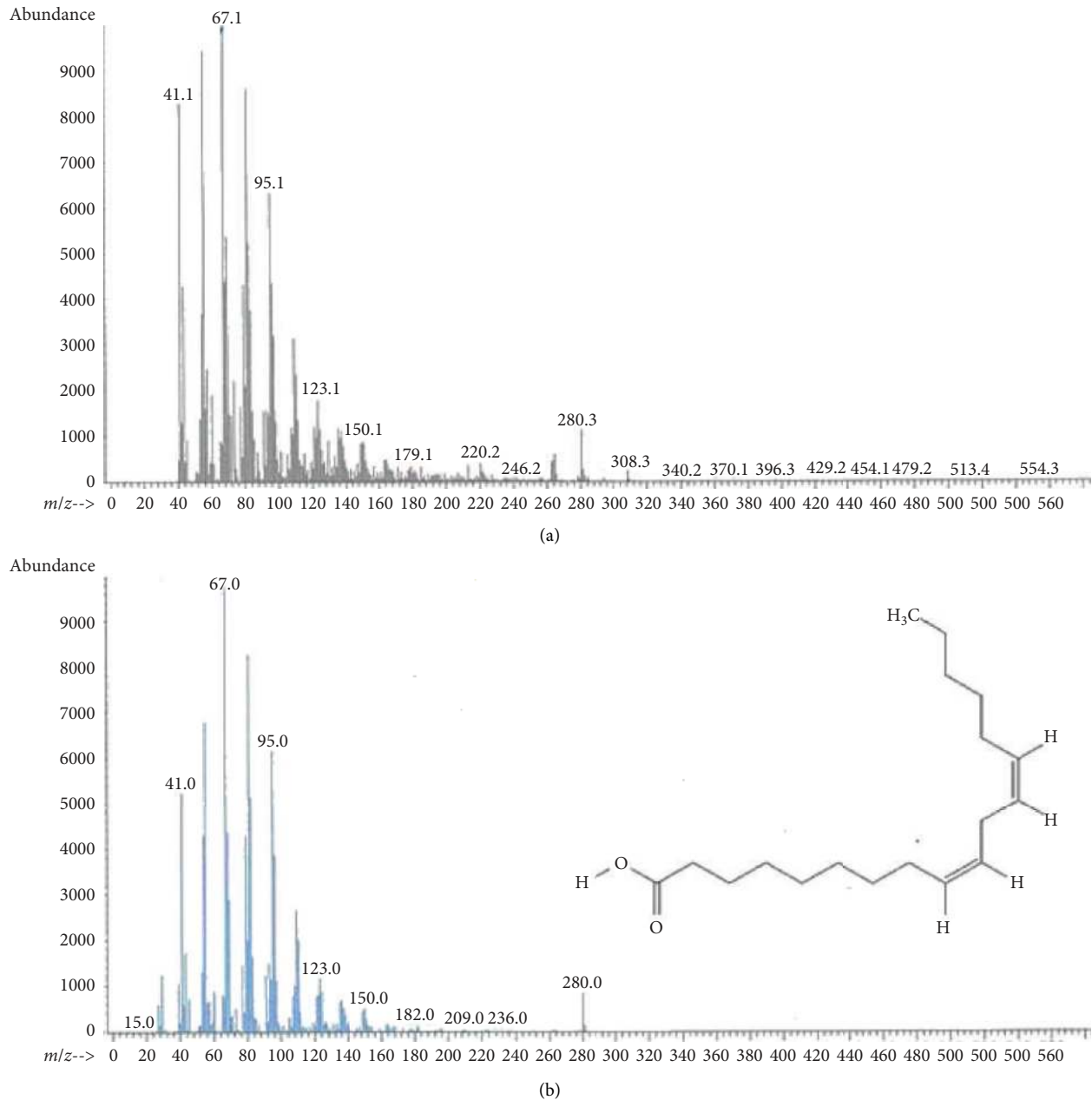


FIGURE 3: The mass-spectral chromatogram of linoleic acid in the ethanolic heat reflux extract of *N. tabacum* L. var Virginia origin of Ponorogo (a) and the linoleic acid standard (b).

compared with the result of other previous studies as can be seen in Table 5. This showed that EHRE-Nt is effective to be used as a prospective bioinsecticide against CBB at low extract concentration.

This study shows that the highest efficacy level of EHRE-Nt was achieved by E3 formulation of 450 mL EHRE-Nt in 100 L water or equals to 0.45% in terms of concentration. The result of GC-MS spectral analysis also indicated that the nicotine content of EHRE-Nt was not more than 50%, which further means that the actual concentration of nicotine in E3 formulation was only about 0.23%. This value is far below the lower limit of ingested nicotine, causing a fatal outcome of 0.5–1 g

corresponding to an oral  $LD_{50}$  of 6.5–13 mg/kg (0.65–1.3%) [43, 44]. Furthermore, nicotine was assigned as a botanical insecticide with dissipation half-life of 2.51 days, thus making it readily dissipated from treated plants [45]. In addition, acute oral toxicity test showed that 5 g *Nicotiana Tabacum* L. bio-oil, with nicotine as the most dominant compound, per kg body weight of female Wistar rats was not toxic due to the absence of mortality and no significant change of the body weight and behavior of the rats [46]. It could be concluded that besides its far below toxicity level to humans and animals, the EHRE-Nt shows highly potential bioinsecticidal activity.



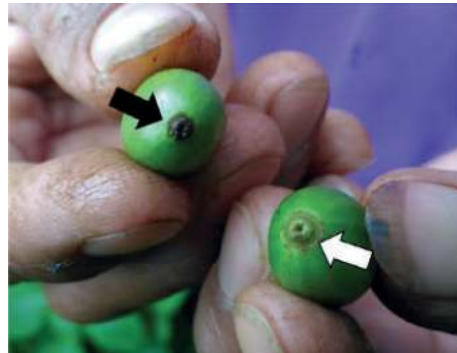
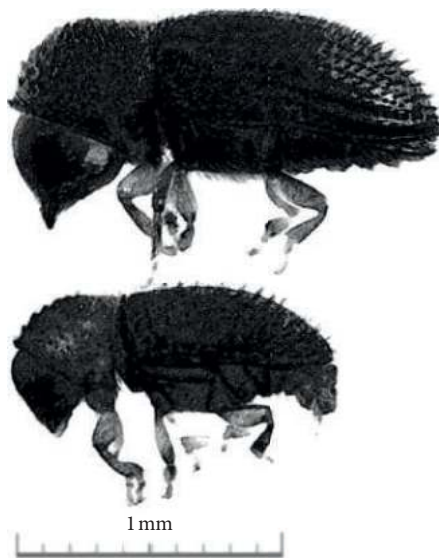


FIGURE 4: Field sampling to coffee berries show a half-mature coffee berry that is infected by the CBB (black arrow) and uninfected by the CBB (white arrow). An infected coffee berry indicated by the presence of a black-hole on the surface of a coffee berry.

TABLE 3: Residual assay of EHRE-Nt<sup>†</sup>.

Hazardous chemicals	Results	Methods
(1) Organochlorine	Negative	TLC <sup>‡</sup>
(2) Organophosphate	Negative	TLC
(3) Carbamate	Negative	TLC
(4) Arsenic (As)	<1 µg/g	AAS-HVG <sup>§</sup>
(5) Pb	Negative	AAS-O <sub>2</sub> -C <sub>2</sub> H <sub>2</sub>
(6) Cadmium (Cd)	Negative	AAS-O <sub>2</sub> -C <sub>2</sub> H <sub>2</sub>
(7) Hg	Negative	AAS-MVU <sup>⊥</sup>

<sup>†</sup>The ethanolic heat reflux extract of *N. tabacum* L. var Virginia origin of Ponorogo. <sup>‡</sup>Thin-layer chromatography. <sup>§</sup>Atomic absorption spectroscopy–hydride vapour generation. <sup>⊥</sup>Atomic absorption spectroscopy–mercury vapour unit.



(a)



(b)

FIGURE 5: Continued.

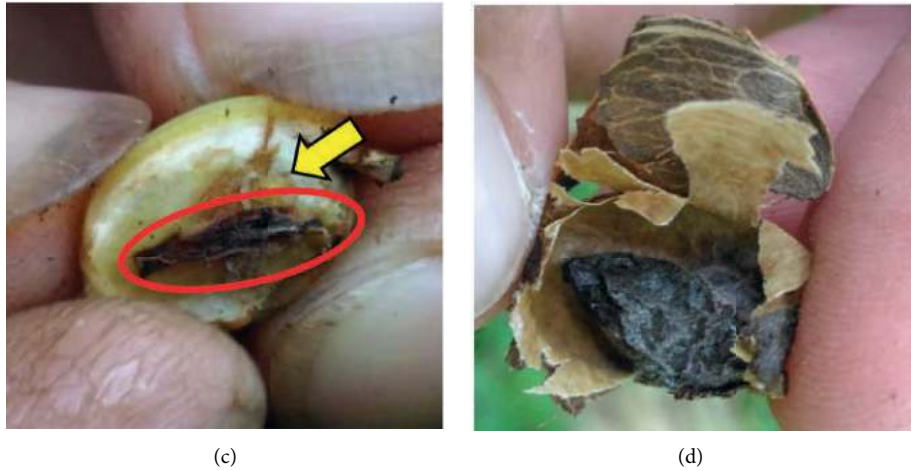


FIGURE 5: (a) Lateral view of female (top) and male (down) of CBBs (*H. hampei*). Field samplings show (b) dorsal view of female CBB (upper arrow), a hole (lower arrow) appeared in a coffee berry. (c) A half-mature coffee berry with cross section of the hole (circled) with larvae of CBB (arrow) appears in the picture. (d) A rotten coffee berry infected by CBB.

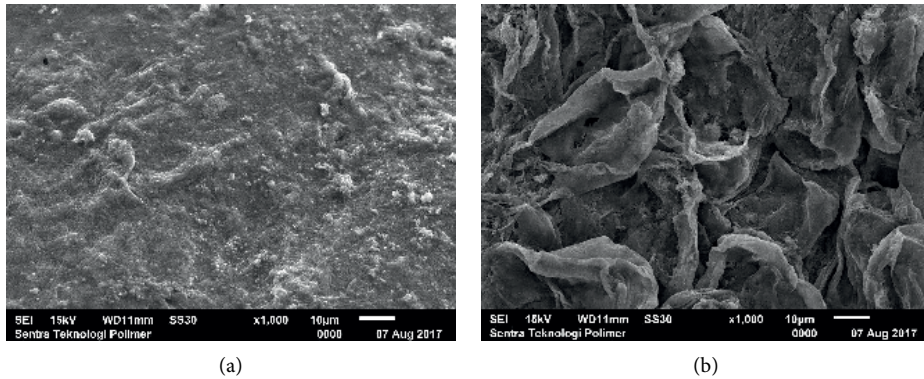


FIGURE 6: Scanning electron micrograph of a cross section coffee bean both in microscales with 1000X magnification. (a) An uninfected coffee bean as a control. (b) An infected coffee bean by CBB.

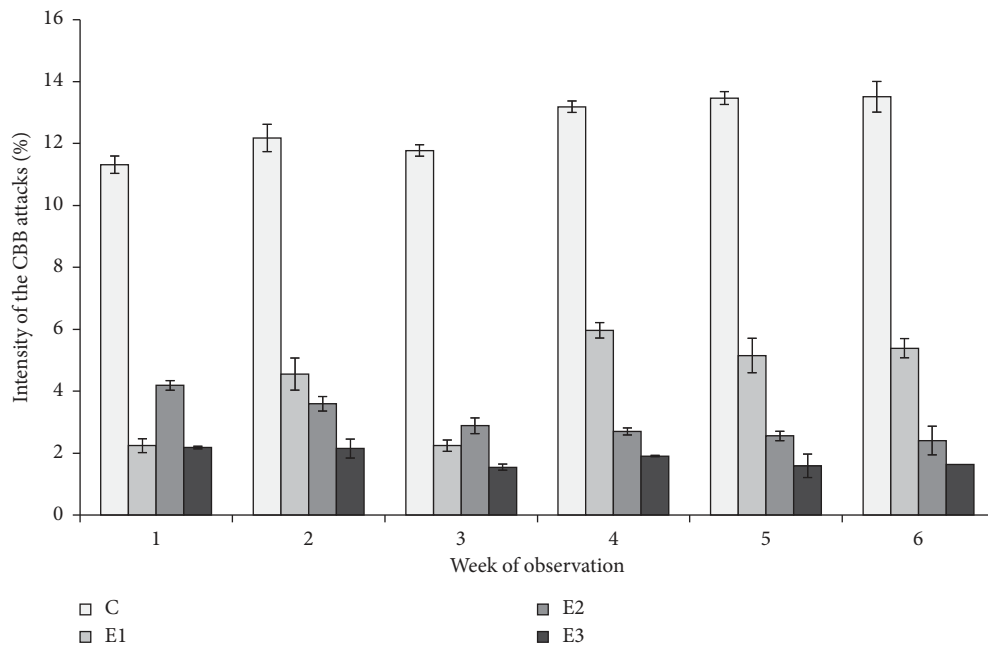


FIGURE 7: The percentage of intensity of the CBB attacks during six weeks of observation (mean  $\pm$  SD;  $n = 3$ ). C is the control. E1, E2, and E3 are 150 ml, 300 ml, and 450 ml of the ethanolic heat reflux extract of *N. tabacum* L. var Virginia origin of Ponorogo (EHRE-Nt), each diluted with 100l of water.

TABLE 4: The efficacy level of EHRE-Nt<sup>†</sup> against coffee borer beetle attacks.

EHRE-Nt <sup>†</sup> formulation	Efficacy level as a bioinsecticide (%)
E1 <sup>‡</sup>	66.14
E2 <sup>§</sup>	75.71
E3 <sup>‡</sup>	85.42

<sup>†</sup>The ethanolic heat reflux extract of *N. tabacum* L. var Virginia origin of Ponorogo. <sup>‡</sup>150 ml EHRE-Nt in 100 l of water. <sup>§</sup>300 ml EHRE-Nt in 100 l of water. <sup>‡</sup>450 ml EHRE-Nt in 100 l of water.

TABLE 5: Comparison of *N. tabacum* as a bioinsecticide against CBB<sup>†</sup> attacks on coffee plants.

Bioinsecticide	Concentration <sup>‡</sup> (ml/l water)	The intensity of the CBB <sup>†</sup> attacks (%)	Reference
Volatile compounds of <i>Nicotiana tabacum</i> (companion plant)	In the form of gas	10.6 ± 1.8	Castro et al. [41]
<i>Cassia spectabilis</i> and <i>Nicotiana tabacum</i> (1 : 1)	15.0	4.4 ± 0.2	Wiriyadiputra, [42]
<i>Nicotiana tabacum</i> (EHRE-Nt <sup>§</sup> )	4.5	1.5 ± 0.1	This study

<sup>†</sup>The coffee borer beetle, *Hypothenemus hampei* (Ferrari). <sup>‡</sup>The concentration of each formulation was obtained based on the volume of the concentrated extract that diluted in water, except in Castro et al., [42], due to the form of gas. There is no concentration data from this reference. <sup>§</sup>The ethanolic heat reflux extract of *N. tabacum* L. var Virginia origin of Ponorogo.

## 4. Conclusions

The ethanolic heat reflux extract of *N. tabacum* L. var Virginia origin of Ponorogo (EHRE-Nt) has strong potential and is effectively used as a bioinsecticide against coffee borer beetle (*Hypothenemus hampei*) on the coffee plantation; it is concluded based on the results of this study that the EHRE-Nt can be quickly produced in a larger scale to supply the availability of the insecticide. A mixture of EHRE-Nt with other plant extracts, e.g., neem (*Azadirachta indica*) extract, as well as a combination of the EHRE-Nt with soil fungus *B. bassiana* or other natural predators against *H. hampei* on a coffee plantation, would very potentially be developed for further investigation. The results are expected to increase the efficacy level of bioinsecticide against coffee borer beetles. The use of EHRE-Nt also proves that the extract neither leaves any hazardous residue nor chemicals. So, it is safely used for the environment and is harmless if exposed to human skin.

## Nomenclature

- I: Intensity of CBBs attack (%)  
 x: Amount of damaged coffee berries due to CBBs attack  
 y: Amount of nondamaged coffee berries  
 E: Efficacy level of the EHRE-Nt as a bioinsecticide (%)  
 Ca: Intensity of CBBs attack to the control block after application of the EHRE-Nt (%)  
 Ta: Intensity of CBBs attack to treatment block after application of the EHRE-Nt (%)  
 E1: 150 ml EHRE-Nt in 100 l water  
 E2: 300 ml EHRE-Nt in 100 l water  
 E3: 450 ml EHRE-Nt in 100 l water  
 C: Control without treatment.

## Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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