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Using Ultrasonic Assisted Extraction to Produce a Bioinsecticide from Cigarette Butt Waste and Green Solvent to Control Armyworm Infestation

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Abstract. Indonesia has one of the highest rates of cigarette consumption in the world, and it has been estimated that up to 75% of cigarette butts end up in nature, where they damage the environment. Since 2019, a new species of armyworm (Spodoptera frugiperda or the fall armyworm) has been attacking maize plants in Indonesia. Therefore, it is proposed to use cigarette butts in the production of a bioinsecticide to control this armyworm. Tobacco, the main ingredient in cigarettes, contains various compounds that can be used as insecticides. These compounds can be extracted using an ultrasonic-assisted extraction method and a variety of solvents, namely, aquadest, 96% ethanol, and NADES as green solvent. The highest extract yield was 27.2±2.0% and the highest mortality rate for the fall armyworm occurred with 96% ethanol extract. The 16-Hentriacontanone compound with the highest peak area of 22.67% was obtained using a gas chromatography mass spectrometry (GC/MS) instrument. All compounds obtained from the GC/MS instrument were simulated with molecular docking to the acetylcholinesterase receptor. The highest docking score was -10.3 kcal/mol for 2,3-Dimethyl-5,6-diphenyl-1,7-dihydrodipyrrolo pyridine and 16-Hentriacontanone, which had a 100% similarity of interactions with the control ligand.

Keywords: Bioinsecticides; Cigarette butt waste; Green solvent; Spodoptera frugiperda; Ultrasonic assisted extraction

1. Introduction

It has been estimated that up to 75% of cigarette butts end up in nature (Cigarette Litter Organization, 2001). One way to reduce the waste from cigarette butts is to convert them into bioinsecticides. The main ingredient of cigarettes, tobacco leaves, contain various compounds that have the potential to be used as insecticides. These compounds include alkaloids, flavonoids, fatty acids, and essential oils (Khalalia, 2016; Kirkova et al, 2016). The advantages of biopesticides are that they are easily degraded, have no long-term residue, are fast acting, and have low mammalian toxicity and low phytotoxicity (Haryuni et al., 2019).

The targeted pest for this bioinsecticide from cigarette butts is the fall armyworm. The loss of harvest from an attack by the *Spodoptera litura* armyworm can reach 80% if the species is not controlled (Marwoto and Suharsono, 2008). Larvae damage crops by biting, chewing, and then eating the lower surface of the leaves. The leaves become transparent

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white, and after the severe damage only the midrib and veins of the leaves remain (Wijanarko et al., 2017). Recently in Indonesia there has emerged a new species of armyworm, *Spodoptera frugiperda,* which infests the maize plants (Ismail, 2019).

Ultrasonic-assisted extraction (UAE) is a fast method for extracting organic compounds (Sholihah et al., 2017). The increased yield from UAE comes from cavitation, which facilitates the disruption of the cell wall by the ultrasound waves (Dianursanti et al., 2020). The solvent used with the extracted tobacco compounds was a green solvent, namely NADES, 96% ethanol and aquadest.

The extracts from the cigarette butts were identified using a gas chromatography mass spectrometry (GC/MS) instrument. Then, those compounds were simulated with molecular docking using the compounds that are abundant in cigarette butt bioinsecticide.

There has not been much research on the production of bioinsecticides using tobacco from cigarette butts. However, previous studies have proven the effectiveness of tobacco extract as an anti-pest bioinsecticide. One study proved that extract from the leaves of Nicotiana tabacum L., var. Virginia was effective in eradicating imago *Gryllus bimaculatus* and larvae *of Galleria mellonella* with LC_{50} values of 38.5 mg/mL and 36.6 mg/mL, respectively (Andjani et al., 2019). However, previous studies on waste from cigarette butts have not varied in the extraction method and type of solvent used to extract the desired compounds in cigarette butts.

This study was conducted to study the effect of solvent types on the yield amount of the crude extract of cigarette butts, to assess the effect of solvent types on the mortality rate of armyworms, to identify the content of compounds contained in the crude extract of cigarette butts by GC/MS analysis, and to identify the docking results of major compounds according to the results of GC/MS analysis with the pest receptor target.

2. Methods

2.1. Materials and Scheme

This research was conducted at the Bioprocess Laboratory and Basic Chemical Process Laboratory, Chemical Engineering Department, Universitas Indonesia, and the Laboratory of Insect Physiology and Toxicology, Department of Plant Protection, Bogor Agricultural University. The cigarette butts were produced by PT HM Sampoerna variant A Mild; they were collected from the smoking area around the Faculty of Engineering, Universitas Indonesia. The *Spodoptera frugiperda* were purchased from Bogor Agricultural University, aquadest from Wiloso, lactic acid from Kimia Ard Yogyakarta, 96% technical ethanol and neem oil from Citra Hidroponik, Indonesia, and choline chloride from HiMedia Laboratories Pvt. Ltd., India. The detail of the research method used in this paper is shown in Figure 1.

2.2. Preparation of Solvents and Extracts of the Cigarette Butts

The NADES solvent was made based on the study by Gibranadhi (2019) by dissolving choline chloride with lactic acid at a mole ratio of 1:1. The solution was stirred with a magnetic stirrer for 45 minutes at 85°C until homogeneous, then the temperature was lowered to a constant 50°C with stirring for 15 minutes. A 96% ethanol solvent and aquadest were prepared.

The material in the form of tobacco powder in cigarette butt filters was collected and dried in the sun for 1 hour, then placed in an oven at 60°C for 2 hours. Then the powder was blended and sieved with Mesh 150 (0.1 mm).

The extraction process was carried out by mixing materials and solvents at a ratio of 1:10 (g material to mL of solvent) and using the ultrasonic-assisted extraction method at a frequency of 53 kHz, a temperature of 40°C, and a sonication time of 20 minutes. Then, the extract was centrifuged at 4500 rpm for 10 minutes. Extracts obtained with 96% ethanol solvent and aquadest were dried using a hot plate at 60°C to evaporate the solvent.

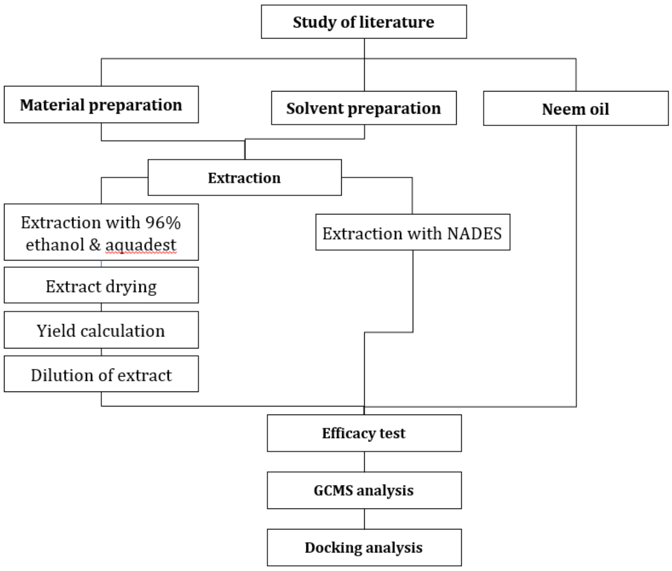

Figure 1 General scheme of research method

2.3. Efficacy Test for Cigarette Butt Bioinsecticides

Bioinsecticides were prepared by dissolving extracts and distilled water to a concentration of 20 mg/mL. Aquadest was previously mixed with an Agristic adhesive at a concentration of 0.1 mL/L distilled water. The diluted extract was applied only to the extract with 96% ethanol solvent and aquadest. Corn leaves with a size of 3 cm × 3 cm were prepared. The bioinsecticide was applied to the leaves using a brush. Five armyworms were used per variation of the solvent type. They were placed in a separate container to avoid the cannibal nature of the fall armyworm. Controls also were tested, namely pure solvents and commercial bioinsecticides of neem oil. The armyworms were monitored for 7 days. The monitoring period was determined based on the duration of larval development (instar larval phase 1 to 5) which was 20 days. That indicated the rapid development, while the phase needed in this research was the 3rd instar phase (Nonci et al., 2019).

2.4. Analysis of the Cigarette Butt Bioinsecticide Content by GC/MS

To analyze the contents of the cigarette butt bioinsecticide, a Perkin Elmer GC/MS instrument was used with these column conditions: oven: initial temp 40°C for 5 min, ramp 20°C/min to 100°C, hold 5 min, ramp 20°C/min to 250°C, hold 13 min, InjAuto = 200°C, volume = 0 μL, split = 80:1, carrier gas = He, solvent delay = 2.00 min, transfer temp = 200°C, source temp = 200°C, scan: 30 to 600Da, column 30.0m × 250μm.

2.5. Analysis of the Interaction between the Bioinsecticide Compounds and the Acetylcholinesterase Receptor

The docking of bioinsecticide compounds and the acetylcholinesterase receptor was done using the AutoDock Vina program. The 9-(3-Iodobenzylamino)-1,2,3,4-Tetrahydroacridine compound was used as control ligand because it is an acetylcholinesterase receptor inhibitor (Harel et al., 2008). The control ligand was docked to obtain a root mean square deviation (RMSD) value ≤ 3.0 Å to determine the active site of the receptor (Jain and Nicholls, 2008). Then, the bioinsecticide compounds were docked to the active side of the receptor and visualized using LigPlot+.

3. Results and Discussion

3.1. The Effects of Solvent Types on the Yield of Cigarette Butt Extract

The average yield was calculated with the result that 96% ethanol was the most effective solvent type for the extraction process of the cigarette butts. It had an average yield of 27.2±2.0%, while aquadest yielded 10.8±0.8%. This was in accordance with the principle of like dissolves like (Zhang et al., 2018). Using solvents that have a polarity similar to the dissolved material increases the amount of extraction (Silva et al., 2007). Cigarettes are processed tobacco products that contain several chemical compounds including alkaloids, flavonoids, fatty acids, and essential oils (Khalalia, 2016; Kirkova et al., 2016). These compounds tend to be more soluble in ethanol than in water (Rustan and Drevon, 2005; Ding et al., 2010; Ferreira and Pinho, 2012; Dhifi et al., 2016).

3.2. The Effect of Solvent Types on the Mortality of Fall Armyworms

The targeted pest was the fall armyworm (*Spodoptera frugiperda*) in instar phase 3. This is the active eating phase of the fall armyworm which causes damage to the leaf growth point (Nonci et al., 2019). The effect of solvent types on the extraction process on the mortality rates of fall armyworms treated for 7 days with bioinsecticides can be seen in Figure 2.

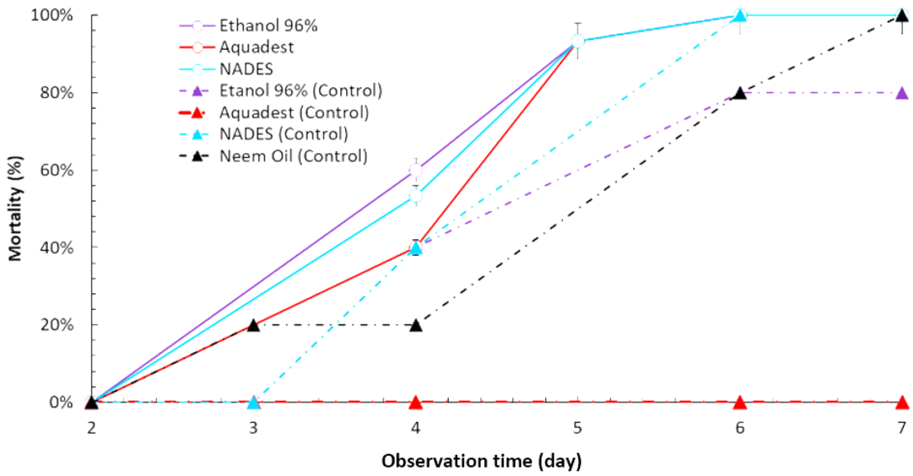

Figure 2 The bioinsecticide effect of cigarette butt waste extract with EtOH, aquadest, and NADES solvents on the mortality of fall armyworm (7-day testing time) and comparison with controls. Note: Negative control = control variable, where the treatment was carried out without a mixture of cigarette butt waste extracts; positive control = control variable, neem oil.

Figure 2 shows that the commercial bioinsecticide, namely neem oil, can kill the armyworms for seven days. This makes it an effective bioinsecticide. However, the mortality rate from the cigarette butt bioinsecticides was higher, which makes them a more effective bioinsecticide in killing these pests. Moreover, producing bioinsecticides from cigarette butts uses waste, so this approach can reduce the total cigarette waste in Indonesia. When the results of the bioinsecticide efficacy test were compared with the negative control, the mortality rate of armyworms tested with the bioinsecticides was higher. These results prove that the compounds extracted from cigarette butts play a role in killing fall armyworms despite using different types of solvents.

Tobacco extract bioinsecticide can kill armyworms effectively; a concentration of 25% could kill 5 armyworms in 20 hours (Sakadzo et al., 2020).

3.3. Analysis of the Content of the Cigarette Butt Bioinsecticide

Aquadest-extracted bioinsecticide was taken for GC/MS analysis because that solvent did not affect the lives of the fall armyworms. Analysis of the compounds contained in the cigarette butt bioinsecticide was carried out using the GC/MS instrument. The five compounds with the highest peak area percentage can be seen in Table 1.

Table 1 Results of GC/MS analysis of five compounds with the highest peak area percentage in aquadest-extracted bioinsecticide

Retention time (minutes)	Area %	Compound	Function
29.52	22.67	16-Hentriacontanone	*Neurotoxic* against insects (Abdelgaleil et al., 2009)
16.63	15.83	9,12-Octadecadienoic Acid	Pesticides and antimicrobial (Gomathy and Rathinam, 2017).
20.82	12.47	n-Hexadecanoic Acid	Pesticides and nematicides (Gomathy and Rathinam, 2017).
21.97	10.53	1,15-Pentadecanediol	Antibacterial, antifungal, and antiviral (Hase et al., 2017)
19.78	10.09	2,3-Dimethyl-5,6-diphenyl-1,7-dihydrodipyrrolo pyridine	Insecticides (Tomizawa and Casida, 2005).

The results of GC/MS analysis showed that there were several compounds that could kill the fall armyworm. The compounds were detected through their retention time. That is the time it takes for a particular compound to travel through the GC/MS column after it has been injected. Each compound spends a different amount of time according to its chemical composition, hence the different retention times (Etxebarria et al., 2009). A ketone function group, 16-Hentriacontanone, was detected with the highest peak area. It can act as an insecticide that directly attacks the central nervous system, namely acetylcholinesterase (Abdelgaleil et al., 2009). The second and third highest content was fatty acids, which were 9,12-Octadecadienoic acid and n-Hexadecanoic acid. Fatty acids can cause the contents of cells to leak, so the insects dehydrated and die (Mohamad et al., 2013). Pyridine compound (2,3-Dimethyl-5,6-diphenyl-1,7-dihydrodipyrrolo pyridine) was detected. If it is consumed by insects, it can cause death by paralysis (Tomizawa and Casida, 2005).

3.4. Analysis of the Inhibition Activity of the Cigarette Butt Bioinsecticide Against the Acetylcholinesterase Receptor

The 9-(3-Iodobenzylamino)-1,2,3,4-Tetrahydroacridine control ligand compound, which is an acetylcholinesterase receptor inhibitor, was docked to determine the active site of the receptor. The RMSD value and the docking score were 0.454 Å and -12.9 kcal/mol, respectively. After identifying the active side of the receptor, the bioinsecticide compounds were docked. The three compounds with the highest docking score were 16-Hentriacontanone, 9,12-Octadecadienoic Acid, and 2,3-Dimethyl-5,6-diphenyl-1,7-dihydrodipyrrolo pyridine. The results were analyzed for interaction with the receptors; the results can be seen in Table 2.

The 16-Hentriacontanone compound had a 100% similarity with the control ligand, and it produced a hydrogen bond with an important residue in the target inhibition, His480. On the hydrophobic side, 16-Hentriacontanone interacts with important residues of Trp83, Trp472, Tyr370, Tyr374, and Tyr71.

Table 2 Similarity of interaction between bioinsecticide compounds with control ligand

Compound (ligand)	Docking score (kcal/mol)	Similarity of interactions with control ligand	Similarity (%)
9-(3-Iodobenzylamino)-1,2,3,4-Tetrahydroacridine (Control Ligand)	-12.9	Trp83, Leu479, Trp472, Tyr370, Tyr374, Tyr71, Gly150, Gly149, Tyr162, Glu237	100
16-Hentriacontanone	-7.7	Trp83, Leu479, Trp472, Tyr370, Tyr374, Tyr71, Gly150, Gly149, Tyr162, Glu237	100
9,12-Octadecadienoic Acid	-7.7	Trp83, Trp472, Tyr370, Tyr374, Tyr71, Gly149, Gly150, Tyr162	80
2,3-Dimethyl-5,6-diphenyl-1,7-dihydrodipyrrolo pyridine	-10.3	Tyr374, Tyr370, Trp83, Tyr71	40

As with other compounds, such as 9,12-Octadecadienoic Acid, producing hydrophobic bonds with important residues Trp83, Trp472, Tyr370, Tyr374, and Tyr71 and 2,3-Dimethyl-5,6-diphenyl-1,7-dihydrodipyrrolo pyridine produces hydrophobic bonds with important residues Tyr370, Tyr374, Tyr83, and Tyr71. The presence of bonds with important residues of the acetylcholinesterase target receptor signified that all the test compounds interacted with the residues that play an important role in inhibiting acetylcholinesterase, which can kill the fall armyworms.

4. Conclusions

The results of the present study show that different solvents vary the yield of the cigarette butt extract. Moreover, the mortality rates of the armyworms showed that the compounds in the cigarette butts were effective at killing the armyworms. Ethanol 96% solvent was the most effective in dissolving the cigarette butt bioinsecticides because it produced the highest extract yield with an average of 27.2±2.0%. Cigarette butt bioinsecticide with 96% ethanol solvent was the most effective because it had the highest mortality rate for the fall armyworm with 60% mortality by the fourth day. The by GC/MS instrument identified several compounds in cigarette butt bioinsecticide. Moreover, the bonding of important residues of acetylcholinesterase receptors by the cigarette butt bioinsecticide compounds indicates the inhibitory activity of acetylcholinesterase inhibitors that can kill the fall armyworms. For further studies, the following activities are recommended: look for an effective method for drying NADES solvent to obtain crude extract then calculating the yield, use another type of mixture to make NADES solvent, vary the conditions of the extraction operation, vary the concentrations of bioinsecticide to determine the most effective dose for killing armyworms, and analyze the extract with 96% ethanol and NADES solvents using GC/MS.

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