

# Research Article

# Bio Oil of Waste Tobacco Stem: Extraction, Physicochemical Properties, and Its Biological Activities

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#### Abstract

Tobacco has been cultivated as a commercial crop by Indonesian farmers for a long time. The content of tobacco waste makes 24% of the weight of tobacco leaf. In 2014, tobacco plantation produced approximately 39,902.88 tonnage waste. There is a potential use of tobacco stem for botanical pesticide. In Indonesia, the use of tobacco waste for botanical pesticide is practiced by Tani Punik Mitra Farmers group. The active ingredients in nicotine are believed to support the effectiveness of its use in the field. Pyrolysis is another technology that can be used to make a pesticide from tobacco stem. The pyrolysis process shall result in bio oil that has many benefits as antimicrobial, antifungal, insecticide, and insect repellent. Bio oil obtained from tobacco waste can perform as an insecticide. The insecticide properties of bio oil can be seen from its chemical contents. Bio oil from tobacco leaves can cause death to Colorado Potato Beetle (CPB) Leptinotarsa decemlineata L. (Coleoptera: Chrysomelidae) in 24 hours after treatment. Mostly, bio oil acts as a contact poison, stomach poison, antifeedant, and insect repellent. It is possible because of the acetic acid contained in it, which can cause damage to the permeability of insect cuticle. The application of bio oil from conara wood can repel mosquitoes from the species of Culex pipiens and Aedes togoi. Bio oil originated from May organic materials can be used as an insecticide for controlling pests. Bio oil plays more role as a contact poison rather than stomach poison, antifeedant, and repellent. The acetic acid in bio oil damages the permeability of insect cuticles that result in their death. Bio oil originated from May organic materials can be used as an antimicrobial and botanical pesticide. Keywords: Depression; Stress; Agarwood; Adrenaline; Cortisol; Vitamin

B; TNF- $\alpha$ ; IL-1 $\beta$ ; COX-2

# Introduction

Tobacco has been cultivated as a commercial crop by Indonesian farmers for a long time. Farmers prefer to cultivate tobacco since it is easy to grow, and it has a high value in a certain period. Pressures on tobacco crops could not halt the strong interest of farmers to grow tobacco. In 2015, the people's tobacco plantation area measured 101.11 ha with 172.53 tonnages production [1]. From the data, we can see a high potential of tobacco waste, which mostly is not used. The content of tobacco waste makes 24% of the weight of tobacco leaf [2]. In 2014, tobacco plantation produced approximately 39,902.88 tonnage waste, or 109.32 tonnage/ day. Hence, there is a very high potential for tobacco waste to be further processed.

The use of tobacco waste as a botanical pesticide has been widely practiced but mostly still using tobacco leaves instead of other components of tobacco waste. The use of another component like tobacco stem is rare. Besides, the limited information that can be found on processing tobacco waste into a botanical pesticide has prohibited its development. Such a fact shows that there is a potential use of tobacco stem for botanical pesticide. We can found botanical pesticides produced from various kinds of plants and by various technologies. Some of the technologies used to extract active materials for a botanical pesticide consist of distillation, maceration, and pyrolysis. Maceration and pyrolysis are samples of technologies for making a botanical pesticide from tobacco stem.

Maceration in making botanical pesticide involves soaking tobacco stem in water. The use of this method was developed in China by soaking 150 Kg–300 kg of 30 cm long tobacco stems in an organic solvent such as ethanol, n-Hexane, and acetone [3].

The extracted tobacco can be effectively used for 10 days-14 days. The tobacco extract is sprayed on land with a comparison of 1:4 [4]. The botanical pesticide made from this method usually contains active ingredients such as nicotine, nicotine sulfate, and other nicotine substances. Such ingredients perform as a contact poison, stomach poison, and fumigation. Nicotine is an organic compound normally found in the tobacco plant, especially on tobacco leaves. This compound can be easily transported into the network of a tobacco plant, and soluble in water and organic solvents. Nicotine kills insects by affecting the ganglia through their central nervous system. In a small amount, nicotine will cause trans-synaptic conduction, while in a high amount, it will cause blocking conduction. It is due to the nicotine ion absorbed through the nerve thread that is lethal for the targeted organism [5].

In Indonesia, the use of tobacco waste for botanical pesticide is practiced by Tani Punik Mitra Farmers group. The group utilizes tobacco stem as a botanical pesticide. The result is nearly as satisfying as a chemical pesticide [6]. With its superiorities, the active ingredients in nicotine are believed to support the effectiveness of its use in the field. In addition to its active ingredients, its relatively simple process makes botanical pesticide through maceration much more preferable for farmers since it only requires simple technologies, which can be ideally applied by farmers groups.

#### **Bio Oil**

Pyrolysis is another technology that can be used to make a botanical pesticide from tobacco stem. This advanced technology has been widely developed nowadays. The pyrolysis process shall result in bio oil that has many benefits as antimicrobial, antifungal, insecticide, and insect repellent. The pyrolysis process may produce bio oil with different contents depending on the material, age of the material, and various organic compositions contained in such material [7]. Bio oil from tobacco stem waste, bio oil from rapeseed, bio oil from tomato plant [8,9] (Figures 1-4).



Figure 1: Bio oil from tobacco stem waste.



Figure 2: Bio oil from rapeseed.



Figure 3: Bio oil from tomato plant.



**Figure 4:** Chemical structure of a  $\gamma$ - butyrolactone compound [20].

Bio oil derived from different organic materials can be used as botanical pesticide because it has several bioactive compounds such as phenol, acetic acid, ketone, aldehyde, and some other compounds. Each organic material has different chemical compounds. Bio oil made from tobacco stem at the temperature of 350°C shall be dominated by main component from the ketone group. Pyrolysis at the temperature of 400°C is dominated by acetic acid while pyrolysis at the temperature of 500°C is dominated by ketone [2]. Bio oil from tobacco leaves has phenol acting as pesticide [10]. Eight of the ten highest compounds are from the phenol groups.

The pyrolysis technique can be used in processing organic waste into many beneficial materials. Bio oil for insecticide material has been derived from grape seeds, birch wood, tomato waste, and tobacco. The use of tobacco as pesticide is potential to be developed as botanical pesticide. S Endang, 2012. stated that pyrolysis at the temperature of 500°C done to leaf vein and petiole can produce 39.74% and 36.76% bio oil (Tables 1 and 2), respectively [2]. This is not much different from the study conducted by [7], which stated that a 5 seconds tobacco leaf pyrolysis at the temperature of 500°C shall produce 43.4% bio oil. The maximum result can be obtained with an effective pyrolysis plant, heat of machine, additional inert material, and the basic material used for the pyrolysis.

 Table 1: Composition results of tobacco stem pyrolysis on various temperatures.

Pyrolysis tempera- ture (oC)	Results (%)			
	Bio oil	Biochar	Gas	
250	29.06	47.77	23.16	
300	27.79	37.33	34.89	
350	38.06	34.30	27.63	
400	36.06	32.07	31.86	
450	39.00	30.08	30.92	
500	39.74	29.61	30.65	

 Table 2: Composition results of tobacco stalk on various temperatures.

Pyrolysis tempera- ture (oC)	Result (%)			
	Bio oil	Biochar	Gas	
250	16.25	76.71	7.04	
300	26.63	57.26	16.11	
350	29.58	50.32	20.09	
400	33.12	42.05	24.83	

Table 3: GC-MS analysis results on bio oil from tobacco stalk.

450	33.46	38.83	27.71
500	36.76	37.40	25.85

#### **Extraction of Tobacco Stems Waste**

The tobacco stem was collected from tobacco fields. Two kilograms of tobacco stem were dried to obtain a moisture content of approximately 15%, and then the tobacco stems were cut into small pieces approximately 2 cm-4 cm in length. This material was put into closed pyrolysis equipment, and heated up to 450°C. The smoke was destilated to obtain the tobacco bio oil.

### Physicochemical Properties of Bio oil of Waste Tobacco Stem

Bio oil obtained from tobacco waste can perform as an insecticide. The insecticide properties of bio oil can be seen from its chemical contents as shown in Table 3. The table shows that the Gas Chromatography-Mass Spectrometry (GC-MS) analysis performed on the bio oil from tobacco leaf pyrolysis at various temperatures has proven its potential to be used as an insecticide. Pyrolysis performed on tobacco leaves at 350°C shows the main component with the highest compound concentration from the ketone group. At the temperature of 400°C with acetic acid as the highest compound, and at the temperature of 500°C shows the main component with the highest compound concentration from the ketone group [2,11]. Table 4 exhibits the results of a study by [7] that the bio oil contained pesticide from the phenol group.

No.	Compound Name	Compound Formula	BM	Percentage (%)		
				350°C	400°C	500°C
1.	2-Propanone, 1-hydroxya acetol	C <sub>3</sub> H <sub>6</sub> O <sub>2</sub>	74	13.23		23.74
2.	2-propanone (CAS) acetone	C <sub>3</sub> H <sub>6</sub> O	58	25		
3.	2-butanone (CAS) methyl ethyl ketone	C <sub>4</sub> H <sub>6</sub> O	72	3.79		
4.	2-propanone, 1-acetol acetate	C <sub>5</sub> H <sub>8</sub> O	116	5.11		8.66
5.	2-butanone	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>	88	1.91		
6.	2-pentanone	C <sub>5</sub> H <sub>20</sub> O	86	0.53		
7.	Acetic acid	C <sub>2</sub> H <sub>4</sub> O <sub>2</sub>	60		21.77	

Table 4: Analysis results of GC-MS on bio oil from tobacco leaf pyrolysis.

Area	Peak No.	Chemical Name	Concentration (mg/mL)
14,203	1	Phenol	1.378
14,064	4	2-methylphenol	1.340
7,757	6	4-Ethylphenol	0.726
6,631	3	2-,ethylphenol indole	0.646
3,836	9	d-limonene	0.514
2,48	2	2,4-dimethylphenol	0.288

## **Bio Oil of Waste Tobacco Stem and its Biological** Activities

The chemical content of bio oil from phenol, acetic acid, propanone, and acetone groups is potential to be used as insecticide and it is correlated with death to the targeted insect pest. As can be seen from graphic 1, bio oil extracted from tobacco leaf pyrolysis at the temperature of  $350^{\circ}$ C,  $400^{\circ}$ C,  $450^{\circ}$ C,  $500^{\circ}$ C, and  $550^{\circ}$ C with a concentration of 375 mg/mL can cause death to Colorado Potato Beetle (CPB) *Leptinotarsa decemlineata* L. (Coleoptera: Chrysomelidae) in 24 hours after treatment of 87.5; 87.5; 94; 93; and 94%, respectively. 48 hours after the treatment, bio oil from all treatments was capable to cause death to *L. decemlineata* of 100% [10].

Much research has proven that bio oil shows positive results when used as a botanical pesticide. Examples of the existing uses of bio oil as botanical pesticide come from vitex pubescens timber of 5% that can cause death to *Coptotermes curvignatus* of 100% [12] bio oil from coconut shell can cause death to *Nilaparvata lugens* of 100%, [13], bio oil from timber can cause death to *Musca domestica* of 72% [14], bio oil from birch timber can cause death to *Myzus persicae* of 95% [15] and bio oil from tomato and tobacco leaves can cause death to *Leptinotarsa decemlineata* of 80% and 94%, respectively [10,16].

Bio oil has a flexible and synergistic effect when combined with a chemical pesticide. Some studies reported that the application of bio oil with carbosulfan increases the mortality rate of the targeted pest [17]. Besides carbosulfan, the application of bio oil from bamboo combined with chemical pesticides with active materials of hexaflumuron and imidacloprid can control the growth of *S. litura* instar 1 and 2 as well as *Empoasca flavescens* on tea and cotton. A combination between bio oil and hexaflumuron can control *S. litura* in seven days after the application with only a half of the recommended dosage for chemical pesticide while a mixture of bio oil with imidacloprid can control *E.* flavescent on day 1, 3, 7, and 11 after the application with only a half recommended dosage for imidacloprid [18].

Mostly, bio oil acts as a contact poison, stomach poison, antifeedant, and insect repellent. The bio oil property as contact poison is possible because of the acetic acid contained in it, which can cause damage to the permeability of insect cuticle that can result in death [17]. Bio oil insecticide property as stomach poison was found in its application to M. domestica larvae that increased mortality rate through soak method [19].

Bio oil activity as antifeedant was found in a study by [20] showing that an application of bio oil from the urban waste of 1% has antifeedant property towards *Spodoptera litura* of 80.65% in 24 hours after treatment. It is because bio oil contains a  $\gamma$ -butirolakton compound that is suggested to have antifeedant property. This compound is formed from lactone. Lactone has been long known as a compound with antifeedant properties to various types of insect pests including *S. litura*. Reports show other lactone compound

with antifeedant properties to *S. litura*, such as salanno butyrolactone, desacetyl salanno butyrolactone, 12-hydroxy oleanolic lactone, and pectolinarigenin [21,22], Bio oil is soluble in different organic solvents such as n-hexane, methanol, and ethanol. Bio oil from urban waste performed best when dissolved in methanol with 80.65% antifeedant property, followed by ethanol with 28.57% antifeedant property [20].

In addition to its antifeedant property, bio oil is excellent for repellent. The application of bio oil from conara wood can repel mosquitoes from the species of *Culex pipiens* and *Aedes togoi* of 100% and 95.9%, respectively. The bio oil was repellent for seven hours after the treatment [23]. Repellent is necessary to drive away pests that can damage plants. It is much more affordable rather than controlling or eliminating pests that already inhabit the plant growth. Even though it is known that the bio oil has repellent properties, further study is necessary to figure out the chemical compound in it that acts as the repellent, and how to develop it. Such repellent property in bio oil can support its wider use for a botanical pesticide [23].

Moreover, the development of bio oil to kill pests is potential because it also performs as an antifeedant and repellent with a synergistic effect when applied together with chemical insecticide. Bio oil is excellent for prolonged use as a botanical pesticide due to its superiorities. A botanical pesticide must be excellent with superior properties to replace chemical pesticides [24]. A botanical pesticide is excellent to prevent pest growth so that it is not only superior for increasing the mortality rate of the pest but also for specifically restraining its growth. A botanical pesticide that can specifically restrain pests and that is safe for natural enemies will create a sustainable ecosystem in agriculture and prevent pest invasion caused by pest resistance resulting from a chemical insecticide.

Other studies supported the insecticide activities of bio oil from tobacco stem, claiming that bio oil originated from may organic materials can be used as an insecticide for controlling pests. Several applications of bio oil as botanical pesticide utilized bio oil from vitex pubescens timber of 5% that can cause death to *Coptotermes curvignatus* of 100% [12], bio oil from coconut shell can cause death to *Nilaparvata lugens* of 100%, [13], bio oil from kiln-dried wood can cause death to *Musca domestica* of 72% [14], a mixture of bio oil from a neem tree, citronella grass, and peanut can cause death to Culex quinquefasciatus of 92% [25], bio oil from birch wood can cause death to *Myzus persicae* of 95% [15], and bio oil from tomato and tobacco leaves can cause death to *Leptinotarsa decemlineata* of 80% and 94%, respectively [10,16].

Bio oil plays more role as a contact poison rather than stomach poison, antifeedant, and repellent although it showed a relatively high mortality rate through the soak method, namely above 80%. The performance of bio oil as a contact poison is following a study performed by [26] reporting that the application of bio oil from coconut shell by spraying method with concentrations of 2% and 10% can cause death to Odontotermes sp. Termites and Ferrisia virgata mealybug of 85 and 96%, respectively. The contact poison activity in bio oil is mostly caused by the acetic acid contained in it. The acetic acid damages the permeability of insect cuticles that result in their death [17]. Another research also suggested that bio oil works better through contact than by soak or indirect methods. Bio oil from co-conut shell has a lower lethal concentration (LC) on brown planthopper when applied directly or through spraying compared with indirect or soak methods [13].

Bio oil has antifeedant property towards *Anticarsia gemmatalis* and *Pseudoplusia includens* insects. Bio oil made *Anticarsia gemmatalis* and *Pseudoplusia includens* to have less ability to feed on leaves than the control group of 100% and 94.71%, respectively [27]. The antifeedant property of bio oil makes it more excellent to be used as an insecticide. The antifeedant property of Bio oil is expected to reduce damages on plants and prevent the growth of *S. litura* pest in addition to its abilities to kill the targeted pest.

#### Conclusion

The antifeedant activity of bio oil from tobacco stem waste is proven by a study conducted by stating that the application of bio oil from the urban waste of 1% showed antifeedant percentage towards *S. litura* of 80.65% in 24 hours after treatment. The  $\gamma$ -butyrolactone compound found in bio oil is purported to contribute to its antifeedant property. This compound contains lactone core. Lactone is known for its antifeedant property towards various insect pests including *S. litura*. Other lactone compounds with antifeedant properties to *S. litura* include salanno butyrolactone, desacetyl salanno butyrolactone, 12-hydroxy oleanolic lactone, and pectolinarigenin. The antifeedant property shown by bio oil from tobacco stem waste suggested that it may contain a compound that plays the role as antifeedant to insect pests.

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