IOP Conference Series: Earth and Environmental Science

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To cite this article: H N Andjani *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **353** 012047

Acute Oral Toxicity Test of *Nicotiana tabacum L.* Bio-Oil Against Female Winstar Rats

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Abstract. Tobacco plants are notably known for its pesticidal properties, particularly due to its nicotine content. In this study, *Nicotiana tabacum L.* bio-oil was obtained using pyrolysis technique. The safety of the bio-oil to be used as bioinsecticide was analyzed through acute oral toxicity test by administering 5000 mg bio-oil/kg body weight of female winstar rats that were analogous to humans. It was concluded that the bio-oil was not toxic due to absence of mortality and no significant change in the body weight and behavior of the rats.

1. Introduction

The use of synthetic pesticides for pest control is often associated with health and environmental issues due to its tendency to leave long-term residues that are harmful for living things. This issue can be overcome by substituting the chemical-based pesticides with natural sources that contain pesticidal properties. In Indonesia, plant species with pesticidal proporties were estimated around 2400 plant species belonging to 235 families [1]. A prominent example of pesticidal plant is tobacco. Several application of tobacco include fungicide [2], insecticide [3], mosquito repellent [4] and larvacide [5]. Previous research examined the secondary metabolites that were present in tobacco plants such as alkaloids, flavonoids, phenols, steroids, terpenoids, saponins and tannins [6]. Alkaloids compounds, particularly nicotine, are the dominant compounds that effectively work as toxin.

While it is crucial to determine the effectiveness of pesticides on the target pests, it is equally important to check its safety on humans before the pesticide is utilized. This can be accomplished by biologically testing the product against Winstar rats that are analogous to humans in order to evaluate their toxicity and possible side effects on humans. The outcome provides information about the

biological activity of the tested material that can be observed from the behavioral changes, the number of deaths, abnormalities in organ function or other physiological disorders. Based on these results, acute oral toxicity of the product can be evaluated. This type of test was selected because some pesticides residues are odorless or not clearly visible, thus the people might be unaware of being exposed to the pesticides by unintentionally consuming them. Moreover, oral poisoning typically occurs when the people do not clean the crops properly in prior to consumption.

Acute oral toxicity test aims to investigate the toxicity of a pesticide when it directly enters the digestive tract. Administration of test substances, not more than 2-3% of body weight, can be done through food, capsules or carrier solutions delivered by special syringe. In this study, tobacco bio-oil was obtained by pyrolysis technique and its active compounds were characterized by GC-MS to identify which compounds dominantly exhibited pesticidal activity. The later stage was acute oral toxicity test to examine its safety if used by humans.

2. Methods

This research methodologies were divided into three major steps such as preparation of raw material, pyrolysis and acute oral toxicity test. The details were described in the following subchapters.

2.1. Sample Preparation

Nicotiana tabacum L. var. Virginia (tobacco) leaves used in this study was originated from several farmers at the end of harvesting period. The leaves were dried and chopped by the farmers prior to this study. In the laboratory, the leaves were first washed using clean water to remove dirt and other impurities. The leaves were dried under sunlight followed by using an oven or furnace at a temperature of 60^{o}C for complete drying. The leaves were grinded using blender and were filtered to obtain a homogeneous size of around 60 μm.

2.2. Pyrolysis

Pyrolysis were carried out on dried tobacco leaves to produce bio-oil as reported previously [2]. The reactor used was a cylindrical reactor made of stainless steel with a diameter of 8 cm and a length of 40 cm. The reactor was heated in the furnace at 500^{o}C. Nitrogen gas that acted as the inert gas was used for 250 grams of dried tobacco leaves. The gaseous product of pyrolysis was condensed to obtain concentrated bio-oil. Later, the bio-oil was characterized using GC-MS analysis to determine its composition.

2.3. Acute Oral Toxicity Test

The procedures of acute oral toxicity test was in accordance to OECD Guidelines for Testing of Chemicals No. 401 [7]. The body weight of 2-to-3-months-old female Winstar rats was 130-140 g. Before testing, the rats were fasted for 24 hours. 5000 mg bio-oil/kg body weight was injected on 5 female Winstar rats. The result of observation at 0 hours was used as a control observation. Observations of behavior changes and physiological reactions were carried out at 30 minutes, 1 hour, 2 hours, 4 hours and 24 hours after treatment. Mortality, change in body weight and behavior were analyzed for 14 days. On the 14th day, the visceral organs of living rats were observed macroscopically.

3. Results and Discussion

The results of this research comprised the bio-oil yield obtained from pyrolisis and analysis of mortality, behavioral and body weight change on the female Winstar rats to evaluate the acute oral toxicity of the bio-oil.

3.1. Bio-Oil Yield

Bio-oil is a thick brown liquid condensate obtained from the gaseous product of pyrolysis. The optimum temperature for pyrolysis to produce higher yield of tobacco bio-oil was reported at 500^{o}C [8]. At this temperature, the increase in the gaseous product resulted in high formation of liquid condensate. In this

research, the obtained yield was equal to 73.37 g or equivalent to 35%. In addition to bio-oil, pyrolysis also produced char as the leftover from the burning of tobacco leaves. This char was physically black solid with a strong odor. Characteristics of char produced by the two pyrolysis processes had no physical differences. The result of characterization by GC-MS was shown in the following table.

Table 1. Tobacco Bio-Oil Composition

No.	RT (Minute)	Compound Name	Peak Area (%)
1	7.9	p-Xylene	0.8
2	8.1	Styrene	2.2
3	8.8	Phenol	5.0
4	9.4	d-Limonene	4.1
5	12.2	Indole	3.9
6	12.7	Nicotine	37.5
7	13.0	7-Methylindole	1.7
8	13.8	Nicotine	2.4
9	16.3	Neophytadiene	10.6
10	18.6	Phytol	1.21

From the result shown in Table 1, the most dominant compound was found to be nicotine which accounted for 39.9% peak area. This alkaloid compound contributed in pesticidal activity due to its high toxicity even at low dosage. Xylene derivatives were typically used as a solvent in the production of pesticides. However, it was categorized as hazardous air pollutant by Environmental Protection Agency (EPA) in which both acute and chronic exposure to xylene could result in adverse effects on human health [9]. Phenolic compounds derived from plant extracts also contributed in insecticidal activity. D-limonene produced moderate toxicity against several insect species without causing mutagenic or genotoxic effect [10]. Indole showed minimum toxicity at low concentration which was suitable to be applied as pest attractant [3]. Phytol and its metabolites acted as both insecticidal and repellent agents for various pests including rice grain moth [11], white fly [12], sumac flea beetles [13]. Phytol in combination with neophytadiene produced high insecticidal activity against Colorado potato beetle [14].

The insecticidal activity of the bio-oil by testing it against three different types of insect species such as ants, whip scorpions and centipedes. Concluded from the result, the time required to cause mortality was 2 minutes for ants, 4 minutes for whip scorpions and 7 minutes for centipedes [3]. Immediately after the bio-oil was sprayed, all of the tested insects demonstrated agresive movement then they were gradually immobilized and died. The mortality of the insects was caused by the toxicity mechanism of the bio-oil through respiratory system, skin contact and digestive tract [15]. The microparticles of the sprayed bio-oil was inhaled and later absorbed through the trachea and the skin layer of the insects. The entry of the bio-oil also occurred through the digestive tract as the residues of the sprayed bio-oil within the container was directly consumed by the insects. In addition, the body weight of the insect might have affect the lethal dose of the bio-oil. For the same quantity of bio-oil administered into the body of the insects, the ants with the lightest body weight reacted more readily towards the bio-oil because the ants required lower lethal dose in comparison to the two remaining insects.

3.2. Mortality Analysis

The tobacco bio-oil was tested against female Winstar rats. From the results of observation after oral testing at a dose of 5000 mg/kg bw, there was no mortality of the Winstar rats. The result indicated that the bio-oil was safe to use at the given dosage.

3.3. Behavioral Change Analysis

The behavior of Winstar rats showed a decrease in platform activity and motor activity at the 30th minutes after administration of the test sample with dose of 5000 mg/kg bw, but it was still within the

normal range. During 24 hours of observation, no straub tail elevation, piloerection, ptosis, lacrimation, catalepsy, salivation, tremor, seizures and writhing were detected. Moreover, all Winstar rats showed normal response on the pineal reflex, corneal reflex, and breathing. Other parameters such as reflex, hanging, retablism, flexion, hafner, defecation and urination did not show any change after administration of the test sample. Grooming activity slightly decreased after administration of the test preparation, but still considered normal. The following figure revealed the macroscopic observation of organs on the 14th day of testing. Based on the observation, it was concluded that no organ abnormalities were found in all of the Winstar rats.

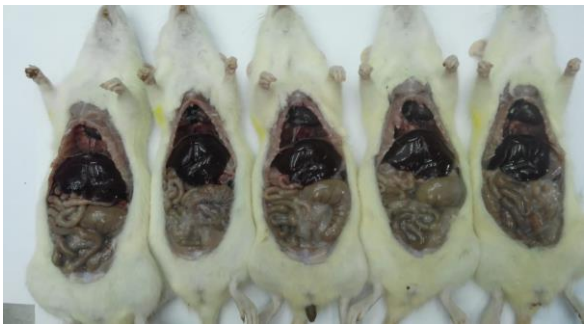

Figure 1. Macroscopic Observation of Visceral Organs of Winstar Rats

3.4. Body Weight Change Analysis

The results of body weight profile for 14 days were shown in Figure 2 and Figure 3. During the testing period, the body weight profile of the Winstar rats persistently increased. The decrease in body weight only occurred on the 13th day. Changes in the body weight were comparable to the control group. This proved the oral administration of the test sample at a dose of 5000 mg/kg bw did not affect the body weight of the Winstar rats.

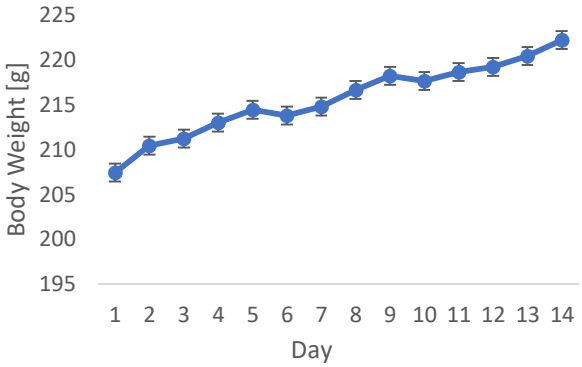

Figure 2. Body Weight Profile of Control Group Rats After Oral Administration of Sample

From the result, the difference in the average body weight of rats in control and treated group was 2.5%. Overall, the graphic pattern was steady throughout the experimental period. The body weight slightly increased after each day except on the 13th day where it decreased by 3.2 g. In conclusion, the result indicated that the tobacco bio-oil at dose of 5000 mg/kg bw did not significantly affect the body weight of rats. The results were comparable and followed the general trend in control group. Thus, the possible oral lethal dose for tobacco was over 5000 mg/kg bw.

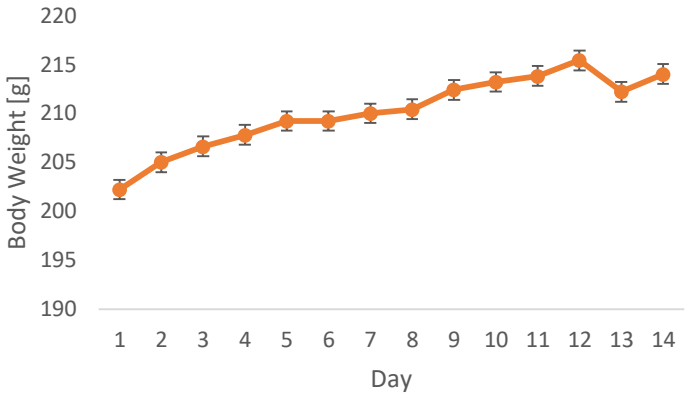

Figure 3. Body Weight Profile of Treated Group Rats After Oral Administration of Sample

Previous research also examined the acute oral toxicity of other plant extracts with pesticidal property such as neem and Java tea leaf [16,17]. The result yielded similar result as this study in which no toxicological sign was found at dose of 5000 mg/kg. As for the use of chemicals, toxicological signs on rats were shown after administering ethyl carbamates (ethyl-4-bromophenyl-carbamate and ethyl-4-chlorophenyl-carbamate) at dose of 300-2000 mg/kg which resulted in various levels of immobility, prostration, hypothermia, depression of spontaneous and provoked behavior, and paralysis with extension of hind quarters [18]. On the other hand, oral administration of 172.95 mg/kg and 207.50 mg/kg endosulfan and cypermethrin at 1:1 ratio caused acute cholinergic symptoms on rats including occasional pawing, burrowing chewing, licking, salivation, coarse whole body tremors, writhing, hyperactivity to sound and touch, abnormal gait asnd development of hind limb extensor tone. Moreover, mild histopathological changes in liver and kidney were shown in rats that were treated with dose of 207.50 mg/kg bw [19].

Table 2. Acute Oral Toxicity of Java Tea Leaf and Neem Extracts

Researcher	Raw Material	Given Dosage	Response
Yusuf *et al*. (2012)	Neem Extract	5000 mg/kg	No toxicological sign
Raj *et al.* (2013)	Endosulfan and Cypermethrin (1:1)	172.95 mg/kg and 207.50 mg/kg	Acute cholinergic symptoms and mild histopathological changes in liver and kidney were shown
Prado-Ochoa e*t al.* (2014)	Ethyl Carbamates	300-2000 mg/kg	Behavioral changes were shown
Pariyani *et al*. (2015)	Java Tea Leaf Extract	5000 mg/kg	No toxicological sign

4. Conclusion

From the result of acute oral toxicity test against female winstar rats, *Nicotiana tabacum L.* bio-oil administered at dosage of 5000 mg/kg bw was not toxic due to absence of mortality and no significant change in the body weight and behavior. The result implied that the tobacco bio-oil was safe to be used by humans as bioinsecticide.

References

[1] Kardinan A 2002 *Pestisida Nabati*, *Ramuan dan Aplikasi* (Jakarta: Penebar Swadaya).
[2] Jufri A, Rachmadiva, Gozan M and Suyono E A 2018 Formulation, stability test and in vitro penetration test of emulgel from tobacco leaves extract *J. Young Pharm.* **10** 69–72.
[3] Gozan M, Yasman P. Wulan and Dawitri E 2014 Tobacco Leaves Pyrolysis for Repellent Active Compound Production *Int. J. Appl. Eng. Res.* **9** 9739–9749.

[4] Jufri M, Irmayanti E and Gozan M 2016 Formulation of Tobacco Based Mosquito Repellent to Avoid Dengue Fever *Int. J. PharmTech Res.* **9** 140–145.
[5] Ekapratiwi Y, Rachmadiva, Virgine K, Fauzantoro A, Gozan M and Jufri M 2019 The effect of tobacco extracts based biolarvicide emulsion formulation against Aedes aegypti larvae *AIP Conf. Proc.* **2092** 03009.
[6] Sharma Y, Dua D and Srivastava N 2016 Antibacterial Activity, Phytochemical Screening and Antioxidant Activity of Stem of *Nicotiana tabacum Int. J. Pharm. Sci. Res.* **7** 1156–1167.
[7] OECD 1987 Test No. 401: Acute Oral Toxicity *OECD Guidelines for the Testing of Chemicals Section 4* (Paris: OECD Publishing).
[8] Booker C J, Bedmutha R, Vogel T, Gloor A, Xu R, Ferrante L, Yeung K K C, Scott I M, Conn K L, Berruti F and Briens C 2010 Experimental Investigations into the Insecticidal, Fungicidal, and Bactericidal Properties of Pyrolysis Bio-oil from Tobacco Leaves Using a Fluidized Bed Pilot Plant *Ind. Eng. Chem. Res.* **49** 0074–10079.
[9] Mohammadyan M and Baharfar Y 2015 Control of workers' exposure to xylene in a pesticide production factory *Int. J. Occup. Environ. Health*. **21** 121–126.
[10] Karr L L and Coats J R 1988 Insecticidal Properties of d-Limonene. *J. Pestic. Sci.* **13** 287–290. doi:10.1584/jpestics.13.287
[11] Adjalian E, Sessou P, Odjo T, Figueredo G, Kossou D, Avlessi F, Menut C and Sohounhloué D 2015 Chemical Composition and Insecticidal and Repellent Effect of Essential Oils of Two Premna Species against Sitotroga cerealella *J. Insect* 1–6. doi:10.1155/2015/319045
[12] Ruiz-Sanchez E, Cruz-Estrada A, Gamboa-Angulo M and Bórges-Argáez R 2013 Insecticidal effects of plant extracts on immature whitefly Bemisia tabaci Genn. (Hemiptera: Aleyroideae). *Electron. J. Biotechnol.* **16** doi:10.2225/vol16-issue1-fulltext-6
[13] Vencl F V and Morton T C 1998 The shield defense of the sumac flea beetle, Blepharida rhois (Chrysomelidae: Alticinae) *Chemoecology.* **8** 25–32.
[14] Cáceres L A, McGarvey B D, Briens C, Berruti F, Yeung K K and Scott I M 2015 Insecticidal properties of pyrolysis bio-oil from greenhouse tomato residue biomass *J. Anal. Appl. Pyrolysis.* **112** 333–340. doi:10.1016/j.jaap.2015.01.003
[15] Ghosh M 1995 *Concepts of insect control* (New Delhi: New Age International Limited).
[16] Yusuf S, Himmi S K, Tarmadi D, Zulfiana D, Ismayati M and Setyowati A 2012 Development of Stored Product Pest Control Technology Using Biopesticide Based on Neem (*Azadirachta indica. A. Juss*) *Pangan.* **21** 211–219.
[17] Raj J, Mohineesh, Ray R, Dogra T D and Raina A 2013 Acute oral toxicity and histopathological study of combination of endosulfan and cypermethrin in wistar rats *Toxicol. Int.* **20** 61–67.
[18] Prado-Ochoa M G, Gutiérrez-Amezquita R A, Abrego-Reyes V H, Velázquez-Sánchez A M, Muñoz-Guzmán M A, Ramírez-Noguera P, Angeles E and Alba-Hurtado F 2014 Assessment of acute oral and dermal toxicity of 2 ethyl-carbamates with activity against Rhipicephalus microplus in rats *BioMed. Res. Int.* p 956456.
[19] Pariyani R, Ismail I S, Azam A A, Abas F, Shaari K and Sulaiman M R 2015 Phytochemical Screening and Acute Oral Toxicity Study of Java Tea Leaf Extracts. *BioMed. Res. Int.* p 742420.

Acknowledgments
The authors gratefully acknowledge the research funding from *Hibah Publikasi Terindeks Internasional Untuk Tugas Akhir Mahasiswa UI* (PITTA) 2019 and the research facilities provided by Universitas Indonesia.