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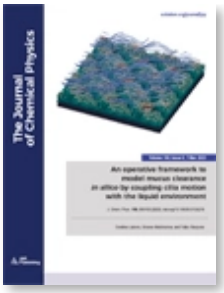

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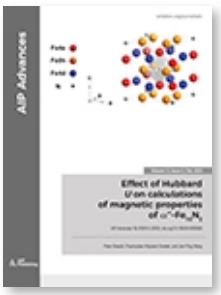

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Published Online: 10 December 2019

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Conference date: 22–24 July 2019
Location: Padang, Indonesia
ISBN: 978-0-7354-1944-5
Editors: Kenny Lischer, Tomy Abuzairi, Siti Fauziyah Rahman and Misri Gozan
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Determination of LC_{50} value of *Nicotiana tabacum L.* extract against *Gryllus bimaculatus* imago and *Galleria mellonella* larvae

Cite as: AIP Conference Proceedings **2193**, 030024 (2019); https://doi.org/10.1063/1.5139361
Published Online: 10 December 2019

Hannah Natasha Andjani, Yogi Sentosa, Kori Yati, et al.

Determination of LC_{50} Value of *Nicotiana tabacum L.* Extract against *Gryllus bimaculatus* Imago and *Galleria mellonella* Larvae

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Abstract. Replacement of synthetic pesticide with natural pesticide is highly demanding because it is more environmentally friendly. Tobacco is majorly exploited for cigarettes production. Therefore, it needs to be developed for other alternative products, one of which is insecticide due to nicotine content and other toxic compounds. This research aimed to determine the effectiveness of tobacco as an insecticide for *Galleria mellonella* and *Gryllus bimaculatus*. The raw material used in this study was the leaf extract of *Nicotiana tabacum L., var. Virginia* that was obtained by Ethanolic Heat Reflux Extraction (EHRE) technique. After testing on the insects, LC_{50} values were 36.6 mg/ml for *Galleria mellonella* and 38.5 mg/ml for *Gryllus bimaculatus*.

Keywords: Nicotiana tabacum L., var. Virginia, Galleria mellonella, Gryllus bimaculatus, ethanolic heat reflux extraction*,* $LC_{50.}$

INTRODUCTION

The existence of pests is a significant threat to the livestock and agriculture industries. Among farmers, the use of pesticides improves the production of agricultural crops and increases farm profits. Although the use of synthetic pesticides is often seen as an effective solution to combat the invasion by pests, it is often associated with health and environmental issues if used excessively in the long term. In fact, there are abundant natural sources with pesticidal properties that are more environmentally-friendly, one of which is tobacco.

Tobacco has long been utilized as the raw material for a variety of application including fungicide [1], mosquito repellent [2,3,4], larvacidal [5] and anti-biofilm agent [6]. The secondary metabolites that are present in tobacco plants include alkaloids, flavonoids, phenols, steroids, terpenoids, saponins and tannins [7]. For pesticidal purpose, alkaloids are found to be the dominant compounds that act as toxin. A notable example of alkaloid compound with neurotoxicity is nicotine. Previous research investigated the active compounds in tobacco which exhibited pesticidal activity such as nicotine, d-limonene, indole, and pyridine [8,9,10]. Although previous researchers had experimented the tobacco extract against several pests, the effectiveness of tobacco against crickets (*Gryllus bimaculatus*) and wax moths (*Galleria mellonella*) has not been investigated yet. *Galleria mellonella* and *Gryllus bimaculatus* are two disastrous insects that infect beeswax combs and vegetable plants. The attack by the wax moth is one of the root causes for the decline in the population of the honeybee colonies *Apis mellifera* [11]. On the other hand, the field

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AIP Conf. Proc. 2193, 030024-1–030024-7; https://doi.org/10.1063/1.5139361
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crickets *Gryllus bimaculatus* are detrimental for both agricultural and non-agricultural sectors. Several damages were found in vegetation, woodpiles, fabrics, paper, wood, and rubber. The objective of this research was to determine the lethal concentration of tobacco extract that caused mortality of *Gryllus bimaculatus* and *Galleria mellonella* by determination of LC_{50}. The active compounds contained in the extract were characterized by GC-MS to identify which compound exhibited insecticidal activity. The selected method of extraction in this study was Ethanolic Heat Reflux Extraction due to its ability to produce high yield of nicotine [12].

METHODS

Preparation

The tobacco used in this research was Virginia tobacco leaves originating from Ponorogo, East Java. The tobacco leaves that had been dried under sunlight were grinded using a blender and sieved using sieve mesh 200 to obtain fine powder with uniform size. The tobacco leaf powder was further dried using memmert oven at a temperature of 70 °C for one hour.

Ethanolic Heat Reflux Extraction

100 grams of dried tobacco leaf powder was mixed with 500 ml of 95% ethanol in a three-neck extraction flask. The flask was heated with hot plate magnetic stirrer set at 1500 rpm until the inside temperature reached ± 70 ° C. Ethanol vapor resulted during the extraction process was condensed within the Allihn condenser using water that was circulated as a coolant. The EHRE was carried out for 6 hours.

The Concentration of Tobacco Extract

The subsequent stage after extraction was vacuum filtration. The extracted tobacco was filtrated with the help of a vacuum pump to separate the filtrate from the tobacco residue. The filter media used were Whatmann filter No. 3 (diameter 125 mm, pore size 6 μm) and proceeded with membrane filter (diameter 50 mm, pore size 5 μm) to ensure the filtrate is free from residue. The filtrate was then filled into the evaporator flask to carry out rotary evaporation. The vacuum rotary evaporator unit was set at a water bath temperature of 50 °C until the remaining ethanol in the extract is completely evaporated, leaving only the tobacco extract. Further concentration using a water bath at the temperature of 80 °C until the concentrated tobacco extract was visibly thick.

LC_{50} Analysis

Tobacco extract was tested against *Gryllus bimaculatus* and *Galleria mellonella*. The ages of the insects used in this research were instar III for *Galleria mellonella* and ± 1.5 months for *Gryllus bimaculatus.* Ten species of *Gryllus bimaculatus* and *Galleria mellonella* were placed inside two separate glass containers and sprayed with ±1 ml of the extract with predetermined concentration. The extract concentrations were 15, 30, 45 and 60 mg/ml for *Gryllus bimaculatus*. For *Galleria mellonella*, the extract concentrations were 40, 60, 80 and 100 mg/ml. The mortality of the species was examined for 24 hours.

RESULTS AND DISCUSSION

Extraction Yield

Based on the previous research, the maximum duration for EHRE was identified to be 6 hours. This was because the organic compounds, including nicotine, would decompose faster if the extraction was conducted for more than 6 hours [13,14]. After the completion of EHRE, the extract yield was equal to 23.16% with the water content of 16.43%. Theoretically, the water content should be maintained at a value below 10% to prevent the possibility of fungal growth and to preserve the quality of the extract [15]. In this research, the water content of the extract could have been lowered by extending the duration for evaporation. The next step was the characterization of the extract

by GC-MS, and the results were shown in Table 1. Each of the components was compared with the results from the previous studies to determine which of the identified compounds contributed to the pesticidal activity.

TABLE 1. Characterization of Tobacco Extract by GC-MS

No.	Retention Time (Minute)	Compound Name	Peak Area (%)	Pesticidal Activity
1	3.37	3-methyl-Pyridine	1.85	Insecticide, herbicide [2]
2	6.40	(R)-(+)-Limonene	0.66	Insecticide [2]
3	6.79	2-methyl-Phenol	0.49	Fungicide, herbicide [16,17]
4	7.86	4-Hydroxypyridine	0.81	Insecticide, herbicide [2]
5	12.05	1H-Indole	1.37	Pesticide [2]
6	12.76	3-(1-methyl-2-pyrrolidinyl)-Pyridine (Nicotine)	49.18	Pesticide, insecticide [13,18]
7	13.59	3-(2-pyrrolidinyl)-Pyridine (Nornicotine)	0.74	Pesticide, insecticide [13,18]
8	15.06	Cotinine	1.82	Pesticide, insecticide [13,18]
9	28.21	Hexadecanoid acid, methyl ester	1.80	Pesticide, nematicide [19,20]
10	28.66	Palmitic Acid	2.01	Insecticide [21,22]
11	28.73	Hexadecanoid acid, ethyl ester	2.06	Pesticide, nematicide [19,20]
12	29.14	Methyl 10-trans,12-cis-octadecadienoate	0.62	Insecticide [23]
13	29.19	11-Octadecanoic acid, methyl ester	2.43	-
14	29.38	Methyl 9-cis,11-trans-octadecadienoate	0.72	Insecticide [23]
15	29.41	Methyl 9-Octadecenoate	2.64	Insecticide [23]
16	29.56	Methyl stearate	0.47	-
17	29.76	9,12-Octadecadienoic acid	21.27	Insecticide [23]
18	31.46	Methyl 20-methyl-heneicosanoate	0.91	-
19	31.63	13-Docosenoic acid	1.86	-
20	31.77	Ethyl pentadecanoate	0.53	-
21	32.73	15-Tetracosenoic acid, methyl ester	1.89	Nematicide, pesticide [24]
22	34.14	22-Tricosenoic acid	1.91	Insecticide [20]
23	35.72	Stigmasta-3,5-diene	1.96	Insecticide [25]

Out of 23 overall compounds identified in the tobacco extract, 18 of them were proven to have pesticidal effect according to the previous studies. Furthermore, 13 out of 18 pesticidal compounds contributed to insecticidal activity. Two of the most dominant compounds found in the tobacco extract were nicotine and 9,12-octadecadienoic acid in which both of them had insecticidal properties. On the other hand, two of the least dominant compounds were methyl stearate and 2-methyl-phenol. 2-methyl-phenol acted as herbicide and fungicide with neurotoxicological properties [16] while methyl stearate acted as anthelmintics for nematodes [26]. The results indicated the suitability of tobacco extract as natural pesticide.

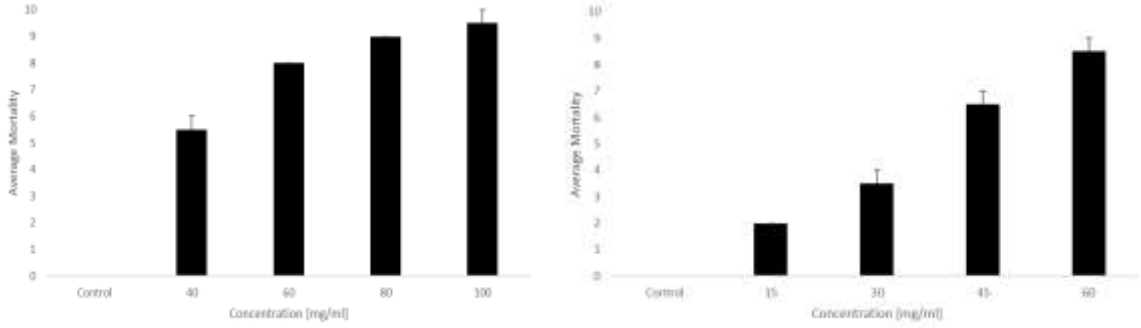
Insecticidal Activity

Analysis of insecticidal activity produced by the tobacco extract was conducted in duplicates simultaneously to ensure equal condition for both insects while investigating whether or not there was any significant difference in the outcome. Physical comparison of the insects before and after treatment was visualized in Figure 1 and Figure 2. The effect of extract penetration into the bodies of the insects was visible from the darkening of skin color for *Galleria mellonella* and immediate hyperactive movement for *Gryllus bimaculatus*. After some time of exposure, the insects gradually paralyzed and died due to prolonged exposure to tobacco extract beyond their tolerable limit.

FIGURE 1. *Gryllus bimaculatus* and *Galleria mellonella* Before Treatment

FIGURE 2. *Gryllus bimaculatus* and *Galleria mellonella* After Treatment

The average mortality of *Gryllus bimaculatus* and *Galleria mellonella* was examined for 24 hours after spraying each extract concentration. As shown graphically in Figure 3a and Figure 3b, the mortality of both insects increased with the increase of extract concentration. The mortality was caused by the poisoning mechanism of tobacco extract through respiratory system, skin contact and digestive tract [27]. Based on the result, higher mortality of *Galleria mellonella* larvae was obtained at faster rate compared to *Gryllus bimaculatus.* Such phenomenon might indicate that *Galleria mellonella* larvae had weaker resistivity towards the toxicity of the extract, so it only required low concentration to cause high mortality. Moreover, the entry of the extract was observed to be more intense because the larvae could not mobilize as fast as *Gryllus bimaculatus,* so they were unable to escape well. This caused them to consume more of the extract residues within the container. Due to its soft and thin cuticle, the extract became more readily absorbed into their body.



(a) (b)

FIGURE 3. Average Mortality of (a) *Galleria mellonella* (b) *Gryllus bimaculatus*

The data comprising the effect of extract concentration on the mortality of the insects were subjected to analysis of variance. The parameters that were analyzed were the standard deviation and the coefficient variant as mentioned in Table 2.

TABLE 2. LC_{50} Analysis of *Galleria Mellonella* and *Gryllus Bimaculatus*

Insect Species	Concentration [mg/ml]	Average Mortality	Standard Deviation	Coefficient Variation	LC_{50} [mg/ml]
Galleria mellonella	Control	0%	0.00	-	36.6
	40	55%	0.50	0.09	
	60	80%	0.00	0.00	
	80	90%	0.00	0.00	
	100	95%	0.50	0.05	
Gryllus bimaculatus	Control	0%	0.00	-	38.5
	15	20%	0.00	0.00	
	30	35%	0.50	0.14	
	45	65%	0.50	0.08	
	60	85%	0.50	0.06	

Standard deviation of 0.5 was identified at concentration of 40 mg/ml and 100 mg/ml for Galleria mellonella and at concentration of 30 mg/ml, 45 mg/ml and 60 mg/ml for Gryllus bimaculatus. The value indicated a relatively low variation in the result which corresponded to the precision of data and absence of drastic difference of data with one another. Interpretation of Standard Deviation Index [28] was used as a guideline for further evaluation of data. Based on the guideline, the standard deviation values in this research were acceptable. Comparing the precision of data should not be limited to only using standard deviation but also the coefficient of variation for better accuracy. Coefficient of variation is a ratio of standard deviation to the mean. Distributions with a coefficient of variation to be less than one are considered to be low-variance. The values of the coefficient of variation shown previously in Table 4.4 were far below 1, which represented accuracy of data.

Previous research examined the insecticidal activity of several plant oils against the last larval instar of *Galleria mellonella* as shown in Table 3 [29]. The plant oils were extracted by water-distillation process. Each of the concentration of plant oil was added with 1 ml of acetone and let to evaporate for about 3-5 minutes. The result showed that the test materials exhibited 50% mortality of the tested insect population at a very low value of lethal concentration. The active compound found in essential oils was found to be monoterpenes, comprising about 90% of the overall components. Other study suggested that treatment with essential oils resulted in visible symtoms similar to those produced by organophosphate and carbamate insecticides which suggested a neurotoxic effect [30]. In comparison to the result in this research, the tested materials were more effective as shown by significantly lower values of LC_{50}. The possible reason might be associated with the selected method of extraction and addition of acetone which affected the quantity of active compound extracted.

TABLE 3. LC_{50} Analysis of Plant Oils Against *Galleria Mellonella*

Raw Material	Duration [hours]	LC_{50} [mg/ml]
Clove Oil (*Eugenia aromatica L.*)	24 hours	0.00345
Lemongrass Oil (*Cymbopogon citratus*)	24 hours	0.00434
Basil Oil (*Ocimum basilicum L.*)	24 hours	0.00988
Spearmint Oil (*Mintha viridis L.*)	24 hours	0.003467
Thyme Oil (*Thymus vulgaris L.*)	24 hours	0.004367

For the case of *Gryllus bimaculatus*, the was a limited number of research which evaluated the effectiveness of natural pesticide through the determination of mortality and LC_{50}. Gwokyalya & Altuntaş (2019) investigated the insecticidal effect of boric acid on *Gryllus bimaculatus,* and the value of LC_{50} was found to be 0.3201 mg/ml [31]. The result was far more effective than the tobacco extract, causing high mortality with low lethal concentration.

CONCLUSION

Nicotiana tabacum L., var. Virginia leaf extract obtained from Ethanolic Heat Reflux Extraction (EHRE) had shown insecticidal activity against *Gryllus bimaculatus* imago and *Galleria mellonella* larvae. The values of LC_{50} were 38.5 mg/ml for *Gryllus bimaculatus* and 36.6 mg/ml for *Galleria mellonella.*

ACKNOWLEDGMENTS

Authors gratefully thank the financial support by DRPM Dirjen Penguatan Riset dan Pengembangan Kemenristek Dikti through *Penelitian Dasar Unggulan Perguruan Tinggi* (PDUPT) 2019 (contract Nr. 21/AKM/PNT/2019) and the research facilities provided by Universitas Indonesia.

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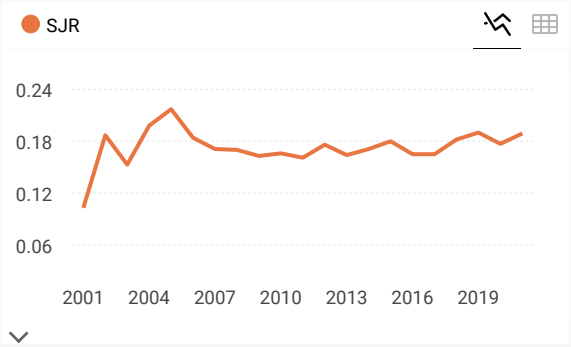

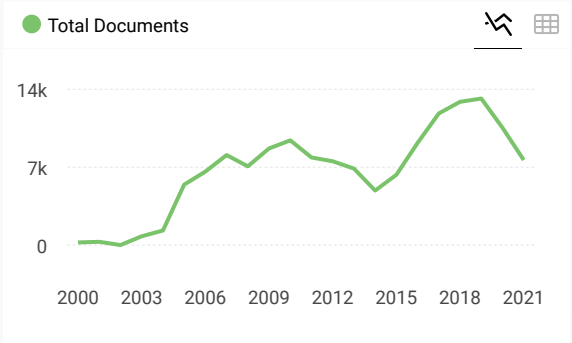

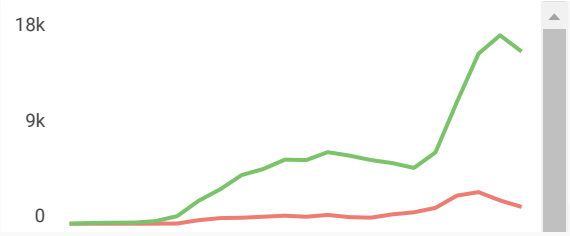

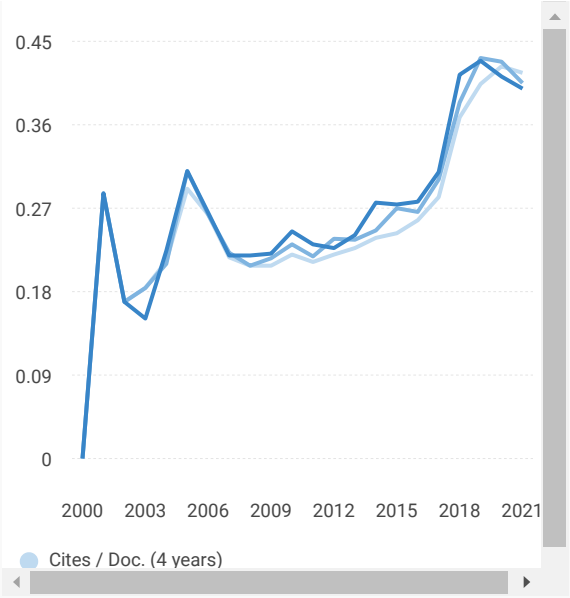

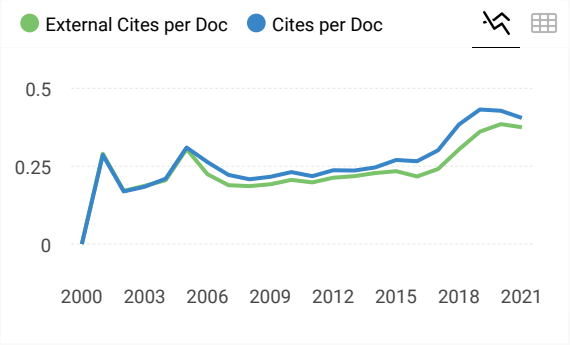

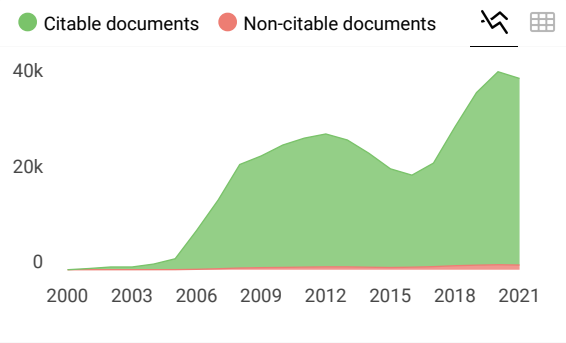

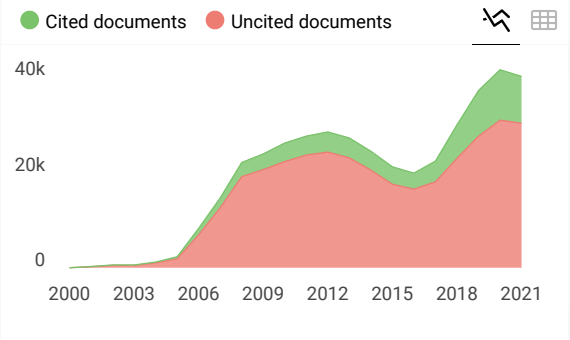

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