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Determination of LC_{50} Value of *Nicotiana tabacum L.* Extract Against *Tenebrio molitor* and *Zophobas morio* Larvae

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Abstract. Tobacco has lots of active compounds that can be used as raw material for making natural insecticides. *Tenebrio molitor* and *Zophobas morio* are examples of agricultural pests. The purpose of this study was to determine LC_{50} values of tobacco extract on *Tenebrio molitor* and *Zophobas morio*. Previous research showed that tobacco extract had neurotoxin activity and nicotine was the highest content contained in the tobacco leaves. Tobacco extract was obtained by the Extended Heat Reflux Extraction method with ethanol solvent. LC_{50} values of tobacco extract for *Tenebrio molitor* and *Zophobas morio* were 21.1 mg/ml and 71.1 mg/ml, respectively.

Keywords: Tobacco, Extended Heat Reflux Extraction, LC50, Tenebrio molitor, Zophobas morio

INTRODUCTION

Majority of the population in agricultural countries work as farmers who rely heavily on agricultural products as their primary income. In agricultural sector, pest invasion is one of the frequently encountered issues. Therefore, pest control is necessary to increase the productivity of the sector. *Tenebrio molitor* and *Zophobas morio* are two examples of the agricultural pests that consume crops, especially in the storage. To kill these, natural pesticides must be applied because they leave lesser residues than chemical pesticides. Based on the previous research, tobacco can be utilized as natural pesticides due to its nicotine content [1].

Tobacco is a tropical plant that contains activated compounds which are suitable for making pesticides. Tobacco leaves consist of phenolic compounds, terpenoids, flavonoids, steroids, alkaloids, saponins, and tannins [2]. These compounds exhibit antibacterial activity [3] and insecticidal activity. As an insecticide, tobacco extract can be used as mosquito repellent [4] and larvacide against *Aedes aegypti* larvae [5]. Alkaloids are the compounds that act as toxin in tobacco. The main toxin of the tobacco is nicotine which has been used since at least the 15th century [6]. As a neurotoxin, nicotine is capable of creating imbalance on the central nervous system, the peripheral arrangement of ganglia and the peripheral autonomic nervous system ganglia [7]. These compounds can be obtained from tobacco

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leaves by using extraction method. In this research, the LC_{50} for both *Tenebrio molitor* and *Zophobas morio* larvae were determined by using tobacco extract as the sample.

METHODS

Preparation

Nicotiana tabacum L. leaves used in this research were obtained from Ponorogo. 1000 grams of leaf powder were extracted using Extended Heat Reflux Extraction (EHRE) with 95% ethanol. This technique used the allihn condenser with a height of 30 cm, 500 ml two-neck boiling flask, a heating mantle, and a rotary evaporator. *Tenebrio molitor* and *Zophobas morio* larvae used in this research were approximately three-months-old.

Extended Heat Reflux Extraction

Extended Heat Reflux Extraction (EHRE) method was carried out for 10 times in this research. Each time, 100 grams of tobacco leaf powder and 500 ml of 95% ethanol solutions were placed inside the two-neck boiling flask and stirred using magnetic stirrer. Water was used as the chiller that was circulated within the allihn condenser. Each extraction cycle runs for about 6 hours at 70°C for more optimum result [8]. The subsequent stage after EHRE was vacuum filtration to separate the extract from the tobacco residues.

Evaporation of Extract

After the completion of extraction followed by vacuum filtration, the extract was evaporated using a rotary evaporator to remove the solvent. The rotary evaporator was set at 50°C and 40 rpm. The evaporation run for about 1.5 hours and proceeded with heating the extract in a water bath at 70°C until the extract thickened.

LC_{50} Test

Tenebrio molitor and *Zophobas morio* were tested with tobacco extract. 10 species of both *Tenebrio molitor* and *Zophobas morio* were put into glass container and sprayed with diluted tobacco extract with certain concentrations (±1ml). The mortality of the species was checked for 24 hours. The concentrations of the extract were 15 mg/ml, 20 mg/ml, 25mg/ml, and 30mg/ml for *Tenebrio molitor* and 60mg/ml, 80 mg/ml, 100 mg/ml, and 120mg/ml for *Zophobas morio.*

RESULTS

An extract yield of 23.16 % with water content of 22.96% was obtained by EHRE method. From the value of the water content, it could be concluded that the extract required further evaporation in order to enable preservation for longer duration and to prevent the fungal growth in the extract because the optimum water content in the extract was below 10% according to the previous research [9]. The contents of the tobacco extracts after semiquantitative analysis by GC-MS were shown in Table 1. Based on the results, nicotine was identified as the dominant compound in the tobacco extract (33.08%).

TABLE 1. *Nicotiana tabacum L.* Extract Composition Analysis by GC-MS

Retention Time	Quality	Compounds	Content (%)
18.374	93	Nicotine	2.74
18.588	97	Nicotine	7.58
19.139	97	Nicotine	9.52
19.236	97	Nicotine	1.45
19.691	97	Nicotine	10.53
19.960	97	Nicotine	1.26
20.904	95	Pyridine, 3-(3,4-dihydro-2H-pyrrol-5yl)-	6.62
24.938	96	2,3'-Dipyridyl	4.66
28.303	98	Cotinine	1.85
29.165	80	1-Pyrrolidinecarboxaldehyde,2-(3-pyridinyl)-	6.25
29.572	93	1-Acetyl-2-Pyridinyl-2,3,4,5-Tetrahydropyrrole	1.77
30.523	99	Hexadecanoic Acid	5.34
31.585	94	Cyclopentadecanone, 2-hydroxy-	1.34
41.287	92	Stigmastan-3,5-diene	3.69
46.893	99	gamma-Sitosterol	30.89

LC_{50} test was the next stage after extraction and analysis of the extract composition by GC-MS. The data of mortality of both *Tenebrio molitor* and *Zophobas morio* larvae were shown in Table 2 and Table 3.

TABLE 2. Mortality of *Tenebrio molitor*

Concentration (mg/ml)	Mortality Test 1 (specimen)	Mortality Test 2 (Specimen)	Standard Deviation
0	0	0	0
Concentration (mg/ml)	**Mortality Test 1 (specimen)**	**Mortality Test 2 (Specimen)**	**Standard Deviation**
15	2	2	0
20	5	4	0.707
25	6	6	0
30	8	9	0.707

TABLE 3. Mortality of *Zophobas morio*

Concentration (mg/ml)	Mortality Test 1 (Specimen)	Mortality Test 2 (Specimen)	Standard Deviation
0	0	0	0
60	2	2	0
80	5	6	0.707
100	9	8	0.707
120	9	10	0.707

From these data, the LC_{50} could be estimated with the probit analysis. Estimation of LC_{50} was computed using Analysis tool-pack of Microsoft Excel with a confidence level of 95 % and the alpha value of 0.05. Figure 1 and Figure 2 presented the graphical correlation between the Log Concentration and Probit Number of both *Tenebrio molitor* and *Zophobas morio*.

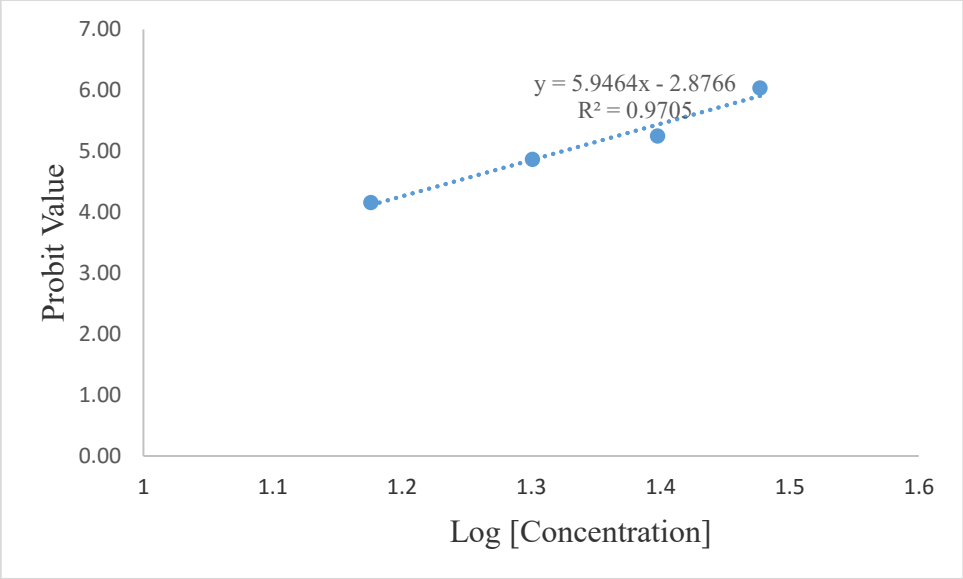

FIGURE 1. Probit Value vs Log Concentration of *Tenebrio molitor* Mortality

The data in Figure 1 had a p-value of 0.0149 ($P \leq 0.05$), which indicated the reliability of the data and the concentration of the extract affected the mortality of the pests. The graph had a line function of Y=5.95X-2.88. To obtain the LC_{50} value, the Y value was substituted with 5 (probit value for 50% mortality) then compute the antilog function using the result of X. Based on the calculation, LC_{50} value of the tobacco extract was 21.1 mg/ml for *Tenebrio molitor*.

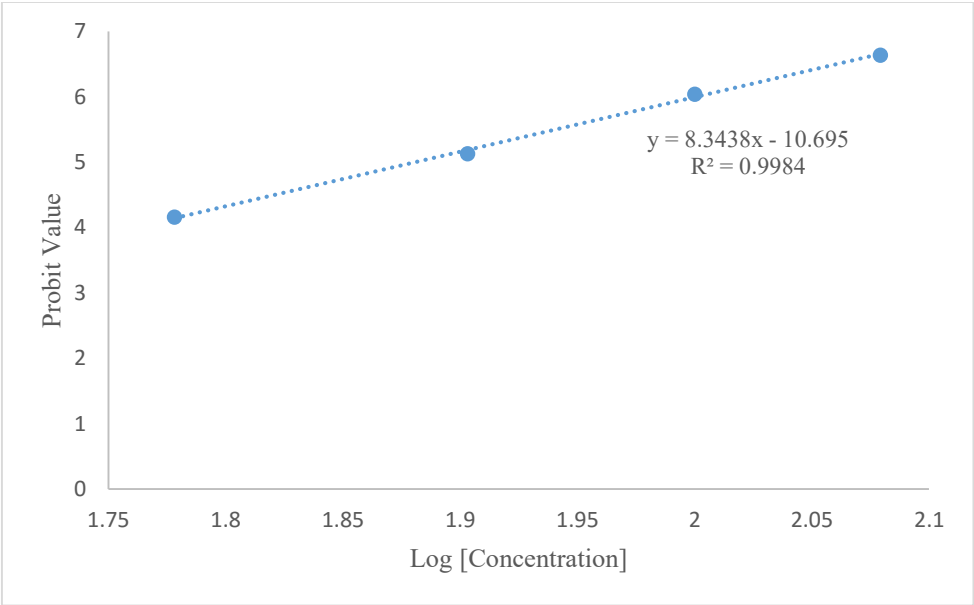

FIGURE 2. Probit Value vs Log Concentration of *Zophobas morio* Mortality

The data in Figure 2 had p-value of 0.0008 ($P \leq 0.05$), so it could be concluded that these results were also reliable and the concentration of the extract had an effect against the mortality of the pests. The graph had a line function of Y=8.344X-10,695. Using the same way of calculating LC_{50} as for the data in Figure 1, LC_{50} value of the tobacco extract was 71.1 mg/ml for *Zophobas morio*.

The tobacco extract killed the *Tenebrio molitor and Zophobas morio* in two ways, such as neurotoxin and digestive toxin. The effect of neurotoxin was observed from the immediate paralysis of the pest bodies after being sprayed with

tobacco extract. The effect of digestive toxin was visible after 24 hours of contact with the pests whereby the abdomen of the pest blackened [10].

RESULTS AND DISCUSSIONS

There was not any previous research which investigated the effect of tobacco extract against *Tenebrio molitor* and *Zophobas morio.* Therefore in this discussion, the LC_{50} value of tobacco extract was compared with the LC_{50} values of the other plants extract in order to evaluate the effectiveness. Table 4 below listed the LC_{50} values from various extracts against *Tenebrio molitor* larvae.

TABLE 4. LC_{50} Value Comparison of Various Raw Materials

Raw Material	**Food Material**	**Pest**	**LC_{50}**	**Researcher**
Nicotiana tabacum L. Extract (Tobacco)	-	*Tenebrio molitor*	21.1 mg/ml	Sentosa, 2019
Raw Material	**Food Material**	**Pest**	**LC_{50}**	**Researcher**
Capsicum annuum L Extract (Bell pepper)	+	*Tenebrio molitor*	1 mg/ml; also able to kill 95-100% of population	Ngo et al., 2017 [11]
Melia azedarach L. and *Pithecellobium jiringa* Extracts (Chinaberry and Djenkol)	+	*Tenebrio molitor*	900 mg/ml; also able to kill 82.5% of population	Arifin, 2014 [12]
Allium sativum Oil (Garlic)	+	*Tenebrio molitor*	7.7 – 13 mg/ml	Plata Rudea et al., 2017 [13]
Cedrus deodara Oil (Himalayan Cedar)	-	*Tenebrio molitor*	34 mg/ml	Buneri et al., 2017 [14]

From the research comparison in Table 4, it could be concluded that tobacco extract was relatively good as a natural pesticide. However, the tobacco had a higher value of LC_{50} compared to bell pepper and garlic.

Capsaicin and dihydrocapsaicin were the active compounds of the bell pepper. Bell pepper extract caused mortality of the pest by affecting the larval growth and retarding the larva [15]. Even though the bell pepper extract had lower value of LC_{50}, the tobacco extract was more effective in killing the pest because tobacco extract acted as neurotoxin which paralyzed the pest in a shorter time. On the other hand. The active compounds contained in garlic oil were the sulfide compounds including *diallyl sulfide, diallyl disulfide* dan *diallyl trisulfide*. Similar to bell pepper, garlic oil was able to kill larva by affecting the larval growth and retarding the larva. Furthermore, garlic oil could cause hormonal disbalance, specifically neuroendocrine hormone and juvenile hormone [16], that resulted in retardation of the larva. Unlike bell pepper and garlic, tobacco was not consumable material so its application as natural pesticide would be more beneficial and economically less competitive.

CONCLUSIONS

The results of this research showed that *Nicotiana tabacum L.* extract had insecticidal activity against *Tenebrio molitor* and *Zophobas morio*. The LC_{50} values were 21.1 mg/ml and 71.1 mg/ml for *Tenebrio molitor* and *Zophobas morio*, respectively. The tobacco extract acted as neurotoxin and digestive toxin because the extract was capable of paralyzing the pest as soon as the extract was sprayed onto the pest and the abdomen of the pest was blackened after 24 hours of being contacted with the extract.

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