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Larvicidal Activity of 96% Ethanol Extract of Arabica Coffee Fruit Peel (*Coffea arabica* L.) on Mortality of Housefly Larvae (*Musca domestica*)

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ABSTRACT

Houseflies (*M. domestica*) are vectors of disease in humans and animals, so vegetable insecticides are needed that can be used to control *M. domestica* populations, one of which is Arabica coffee fruit peel extract. The purpose of this study was to determine the effect of giving various concentrations of 96% ethanol extract of Arabica coffee fruit peel on the mortality of *M. domestica* larvae, as well as calculating the LC_{50} and LT_{50} values of *M. domestica* larval mortality. The research design was a completely randomized design (CRD) using 20 third instar larvae of *M. domestica*. The concentration variations of Arabica coffee fruit peel extract tested were 0.05%, 0.1%, 0.5%, 1% and 0% as control with 3 replicates using the feeding assay method. Larval mortality was observed for 48 hours (hour 0, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48) post extract. Data were analyzed by descriptive and inferential analysis using Kruskal-Wallis test and probit analysis of LC_{50} and LT_{50}. The results showed a significance value >0.05, meaning that there was no significant difference in the mortality of third instar larvae of *M. domestica* between treatments. The LC_{50} value obtained was 0.01%. LT_{50} values at concentrations of 0.05%, 0.1%, 0.5%, and 1% were 274.52 hours, 134.90 hours, 532.20 hours and 0 hours, respectively. The conclusion of this study is that 96% ethanol extract of Arabica coffee fruit peel has no effect on the mortality of *M. domestica* larvae, the concentration of Arabica coffee fruit peel extract that causes the fastest death of *M. domestica* larvae is 0.1%, and LT_{50} at extract concentrations of 0.05%, 0.1%, 0.5% is 274.52 hours, 134.90 hours, and 532.20 hours, at 0% and 1% concentrations have no LT_{50} value.

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1. Introduction

The housefly (*Musca domestica*) is a member of the order Diptera whose population is widespread throughout the world. This fly accounts for 98% of the total types of flies that are often found in residential areas so it is called the house fly. House flies are often found in hospitals, restaurants, garbage bins, markets, and even in livestock pens (in animal feces). Houseflies are one of the mechanical vectors that become pathogen-carrying agents from the perch that cause diseases in

humans and animals (Khamesipour et al., 2018). Houseflies carry more than 130 pathogens in the form of parasitic worms, bacteria, fungi, and viruses (Khamesipour et al., 2018).

The cosmopolitan nature of this fly makes it easy to transmit pathogens quickly. Diseases transmitted by houseflies include diarrhea, typhoid, myiasis, cholera, shigelloasis, salmonellosis, tuberculosis, leprosy, hepatitis, and anthrax (Kassiri et al., 2012). Research conducted by Manalu et al. (2013) states that 63.30% of diarrhea cases in toddlers are caused by pathogens transmitted by houseflies, besides that it can also cause discomfort in a place. The number of problems caused by houseflies is the reason for controlling the population of houseflies using insecticides (Wudianto, 2010).

Insecticides that are commonly used in fly population control are synthetic insecticides. The use of synthetic insecticides can ultimately have negative impacts such as threatening human health, disrupting the lives of other living things, causing environmental pollution in water, soil, or air, and causing resistance to houseflies, namely propoxur, permethrin, and pyrethroids (Wiharyono et al., 2019). Plants that can be used as larvicides are plant species that contain tannins, flavonoids, saponins, essential oils, and alkaloids (Kartini & Pratiwi, 2020), one of which is the fruit peel of arabica coffee (*Coffea arabica* L.) (Masruri et al., 2019). Mauliyana & Harlita's research (2021) states that saponins and alkaloids are stomach poisons because they can cause corrosion in the digestive tract so that feeding activity in larvae is inhibited. Flavonoids on the other hand can weaken the nervous system and inhibit breathing, resulting in larval death (Basundari et al., 2018).

Arabica coffee peel extract is known to be used as a larvicide for *Anopheles* sp. larvae and caterpillar control in pakcoy plants (Mauliyana & Harlita, 2021). Insecticides for adult houseflies can temporarily use Miana leaves (*Coleus blumei*) and bintaro fruit extract (*Cerbera odollam*) (Surahmaida & Umarudin, 2019). Saponin and flavonoid compounds from white guava leaves are known to be able to become housefly larvicides (Nurhayati & Sukesi, 2018). Research related to larvicides needs to be carried out to determine the potential of Arabica coffee fruit peel as a vegetable insecticide that can be used to kill pathogenic vectors of houseflies.

2. Methods

2.1. Preparation of Ethanol Extract of Arabica Coffee Fruit Peel

The preparation of Arabica coffee fruit peel extract refers to the method of Harahap et al. (2021). Arabica coffee fruit peel were washed thoroughly, then dumped as much as 5 kg, and dried in a laboratory room for 4 days. The dried Arabica coffee fruit peel was then weighed again to determine its dry weight, then pulverized using a blender until it became dried peel. This dried peel was then weighed as much as 800 grams and then put into a dark bottle of maceration vessel, then macerated with the ratio of dried peel and 96% ethanol solvent (1: 4). The maceration process of Arabica coffee fruit peel was carried out for 6 days and stirred every day. This stirring functions so that the dried peel does not settle, the surface area of the dried peel can be extracted optimally so that chemical compounds can be drawn by the solvent optimally. Dried peel that has been macerated is then filtered using filter paper, so that extract is formed. The extract is then concentrated using a rotary evaporator at 42^{o}C which forms a semi-viscous extract, then concentrated again using a waterbath at 40^{o}C. Extract of Arabica coffee fruit peel was then diluted using distilled water to form an extract solution with a concentration of 0.05%; 0.1%; 0.5%; and 1%.

2.2. Preparation of 2% Arabica Coffee Fruit Peel Extract Stock Solution

Arabica coffee extract was put as much as 2 grams into a 100 mL beaker glass. Aquabidest was then added to the beaker glass until it reached the limit of 100 mL, and homogenized using a magnetic stirrer.

2.3. Dilution of Arabica Coffee Fruit Peel Extract 0%; 0.05%; 0.1%; 0.5%; and 1%

The 0% extract concentration was made by putting 100 mL of aquabidest into a beaker glass, without adding Arabica coffee fruit peel extract and used as a negative control. The concentration of 0.05% extract was made by putting 2.5 mL of extract stock solution into a glass beaker, then adding aquabidest as much as 97.5 mL (derived from 2 grams of extract), and homogenized. The

concentration of 0.1% extract was made by putting 5 mL of extract stock solution into a glass beaker, then adding aquabidest as much as 95 mL, and homogenized. The 0.5% extract concentration was made by putting 25 mL of extract stock solution into a glass beaker, then adding 75 mL of aquabidest, and homogenizing. The 1% extract concentration was made by putting 50 mL of extract stock solution into a glass beaker, then adding aquabidest as much as 50 mL (derived from 2 grams of extract), and homogenized.

2.4. Preparation of Instar III Larval Feed for Feeding Assay Model Extract Testing

Milk powder was weighed as much as 10 grams and sand as fly larvae media weighed 35 grams, then put into a plastic container for testing. The extract solution with each concentration is put into the container as much as 2 mL, then stirred until it blends with the feed and larval media. The dose was then used for one test container containing 20 fly larvae.

2.5. Housefly Instar III Larvae Extract Testing

The testing of extracts against instar III larvae of houseflies refers to the method of Nurhayati (2016). The third instar larvae of houseflies used for testing each concentration were 20. The design model used was RAL (Completely Randomized Design) using 5 concentrations (0%; 0.05%; 0.1%; 0.5%, and 1%) and each concentration was repeated 3 times as in (Table 1), so that 15 Petri dishes were needed and a total of 300 instar III larvae were used.

Table 1. Experimental randomization model with completely randomized design (CRD)

P1	P2	P3	P4	P5
P4	P3	P5	P2	P1
P3	P5	P4	P1	P3

Notes:

P1= Petri dish containing feed and media for instar III larvae + 2 ml of 0% extract solution (no Arabica coffee fruit peel extract or only 2 ml of aquabidest).

P2= Petri dish containing food and media for instar III larvae + 2 ml of 0.05% Arabica coffee rind extract solution.

P3= Petri dish containing feed and media for third instar larvae + 2 ml of 0.1% Arabica coffee rind extract solution.

P4= Petri dish containing food and media for third instar larvae + 2 ml of 0.5% Arabica coffee fruit peel extract solution.

P5= Petri dish containing feed and media for instar III larvae + 2 ml of 1% Arabica coffee fruit peel extract solution.

Each petri dish was labeled according to the concentration of the extract, then 20 instar III housefly larvae were put into each petri dish containing feed, media, and extract solution, after which the petri dish was closed using gauze. Observation of the mortality of third instar larvae was carried out for 48 hours, namely at the 0th, 4th, 8th, 12th, 16th, 20th, 24th, 28th, 32nd, 36th, 40th, 44th, and 48th hours after administration of the extract. The number of third instar larvae mortality was recorded and processed using regression test and probit analysis to determine the LC_{50} and LT_{50} values.

2.6. Data Analysis

Data analysis used in this study was descriptive and inferential analysis. Descriptive analysis was used to describe data on the number of larval deaths and explain the results of Nurhayati's (2016) research, including compounds in Arabica coffee fruit peel extract that have the potential to cause death in larvae. Inferential analysis was used to calculate the difference in the average number of deaths in each treatment. Inferential analysis begins with a normality test of the number of deaths of instar III larvae of *M. domestica* using Kolmogorov-Smirnov analysis. The resulting data weren't normally distributed, then continued with non-parametric tests using the Kruskal-Wallis test. The data was also tested using a non-parametric linear regression test, namely the Kernel test. The next analysis is probit analysis which is used to determine the LC_{50} (Lethal Concentration) and LT_{50} (Lethal Time) values of third instar larval mortality of *M. domestica*.

3. Results and Discussion

The results in this study were calculated through the average larval mortality in percentage (%). The results in this study show the concentration that causes death in housefly larvae (*M. domestica*) along with the LT_{50} (Lethal Time) value. The morphology of *M. domestica* larvae between dead and alive has a difference. Living *M. domestica* larvae are characterized by having a yellowish-white body color, can move, and are active (Figure 1a). The dead *M. domestica* larvae are characterized by a body that does not move, all parts of the body are black, and the body is stiff (Figure 1b). The body of the dead larvae underwent a slow color change that became blacker over time during the 48 hours of observation.

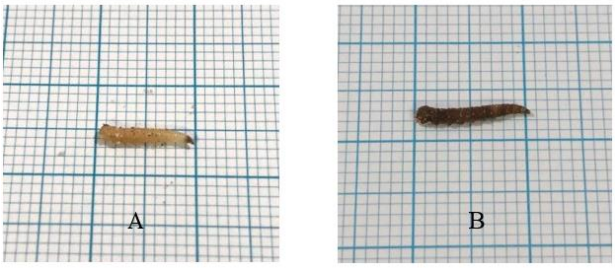

Figure 1. Third instar larvae of M. domestica; (A) live and (B) dead larvae (Personal documentation, 2023)

The discoloration of dead instar III *M. domestica* larvae is referred to as melanization. This refers to Boucias & Pendland (1998) which states that melanization is an insect defense mechanism against foreign compounds that enter the body and can inhibit enzymes in the insect body. Rustam & Tarigan (2021) state that larvae that experience melanization will show symptoms of slowed movement, reduced appetite, and wrinkled skin surface. Dono et al. (2006) stated that the body of larvae that experience melanization will turn brown to black. These symptoms occurred in the third instar larvae of *M. domestica* that died during the study. This melanization process is a sign that the death of *M. domestica* instar III larvae in this study was caused by toxins from secondary metabolites in Arabica coffee fruit peel extract consumed by the larvae.

3.1. Effect of Ethanol Extract of Arabica Coffee Fruit Peel (*C. arabica*) on Mortality of Instar III Larvae of Housefly (*M. domestica*)

The highest mortality rate of third instar larvae of *M. domestica* for 48 hours was 1 larva, which was found in treatments P2, P3, and P4. Meanwhile, in treatments P1 and P5 there was no mortality of third instar larvae of *M. domestica* (Table 2).

Tabel 2. Mortality of third instar larvae of *M. domestica* treated with coffee arabica (*C. arabica*) fruit peel extract for 48 hours.

Treatment	Larva (individual)	Larval mortality in each replication (individual)			Average percent larva mortality (%)
		I	II	III	
P1	20	0	0	0	0
P2	20	0	1	0	0,017
P3	20	0	1	0	0,017
P4	20	0	0	1	0,017
P5	20	0	0	0	0

Notes:
P1= concentration 0%
P2= concentration 0,05%
P3= concentration 0,1%
P4= concentration 0,5%
P5= concentration 1%

Secondary metabolites of Arabica coffee fruit peel that cause death in instar III larvae of *M. domestica* include alkaloids, flavonoids, tannins, and saponins (Mauliyana & Harlita, 2021). Alkaloids act as stomach poisons to larvae. Alkaloid compounds consumed by larvae will cause the taste receptors in the larval mouth area to be inhibited. This causes the larvae to fail to get a taste stimulus so that they are unable to recognize their food and cause the larvae to die of starvation (Chowański et al., 2016). Alkaloids that enter the digestive organs will then be circulated with the

blood and affect the larval nervous system by inhibiting the impulse delivery system to the muscles so that the larvae gradually die (Chowański et al., 2016). The presence of alkaloids as a stomach poison in this study is evidenced during the observation of dead larvae experiencing changes in the color of the abdomen on the ventral part, from yellowish white to black. The ventral part of the larva is the organ of food digestion (Velasquez et al., 2013). Alkaloids also inhibit cardiac contractile activity in insects, and if viewed from the molecular side, alkaloids can increase the activity of reactive oxygen species (ROS).

High levels of ROS can cause oxidative stress in cells resulting in disruption of the mitochondrial membrane and protein damage (Chowański et al., 2016). Flavonoid compounds in coffee fruit peel extract can also be toxic to larvae by attacking the respiratory system of *M. domestica* larvae through their spiracles located in the posterior abdomen and then entering the larval body, causing the spiracle nerves to be damaged and weakened. This also causes the larvae to be unable to breathe and die. Flavonoids also have a bitter taste so larvae tend to refuse to eat them. Saponin compounds are also stomach poisons because they reduce the wall membrane of the digestive tract so that the digestive organs become corrosive (Wardani et al., 2010). Saponins that have entered the larval digestive tract will also inhibit the process of food uptake (Chaeib, 2010). Saponins in Arabica coffee fruit peel extract can degrade the cuticular cell membrane of *M. domestica* larvae (Chaeib et al., 2007).

The mortality data of the third instar larvae of *M. domestica* were then further analyzed using statistical analysis. First, the data were tested for normality using the normality test, but the results obtained were not normally distributed (sig. <0.05), so then the data were tested with the Kruskall-Wallis non-parametric test. The results of the Kruskal-Wallis test showed a sig value. > 0.05, which means there is no significant difference in the number of third instar larval mortality of *M. domestica* in all treatments (Table 3).

Table 3. Kruskal-Wallis test value

	Total mortality
Kruskal-Wallis H	2,333
Df	4
Asymp. Sig.	0,675

The mortality data for third instar larvae of M. domestica were subjected to non-parametric regression using the Kernel test. The obtained regression equation was Y = 200-7.40X, indicating that the concentration of Arabica coffee fruit peel extract does not affect the larvae's mortality. This is illustrated in Figure 2 and detailed in Table 4.

Table 4. Kernel test results of mortality of third instar larvae of *M. domestica*

Coefficients					
	Unstandardized Coefficients		**Standardized Coefficients**	**T**	**Sig.**
	B	**Std. Error**	**Beta**		
Concentration	-7.401E-18	.078	.000	.000	1.000
(Constant)	.200	.260		.769	.456

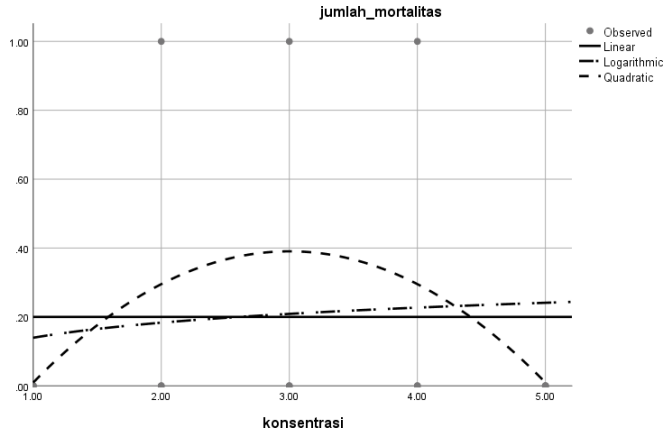

Figure 2. Mortality graphic of house flies

3.2. Extract Concentration Causing the Fastest Death of Housefly (*M. domestica*) Larvae

Data on the time of death of third instar larvae of *M. domestica* can be seen in (Table 5). The fastest death of third instar larvae during 48 hours after the application of Arabica coffee fruit peel extract was found in P3 at the 12th hour. The number of third instar larvae deaths at that hour was 1. The next death of third instar larvae of *M. domestica* was in P2 at the 28th hour as much as 1 tail, then in P4 at the 32nd hour as much as 1 tail.

Table 5. Data on larval mortality time at each extract concentration

Treatment	Cumulative number of deaths at hour -												
	0	**4**	**8**	**12**	**16**	**20**	**24**	**28**	**32**	**36**	**40**	**44**	**48**
P1	0	0	0	0	0	0	0	0	0	0	0	0	0
P2	0	0	0	0	0	0	0	1	1	1	1	1	1
P3	0	0	0	1	1	1	1	1	1	1	1	1	1
P4	0	0	0	0	0	0	0	0	1	1	1	1	1
P5	0	0	0	0	0	0	0	0	0	0	0	0	0

Notes:
P1= concentration 0%
P2= concentration 0,05%
P3= concentration 0,1%
P4= concentration 0,5%
P5= concentration 1%

The mortality data of third instar larvae of M. domestica were analyzed for probit values using Excel, resulting in a regression equation y = -1.7596x + 8.1061 with r^2 = 0.5173 (Table 6). Jelita et al. (2020) explained that the y parameter represents the probit number, while the x parameter represents the log concentration of Arabica coffee fruit peel ethanol extract. To find the LC50 value, a y value of 5 was input into the equation, yielding an x value of 1.77 (Figure 3). The LC50 value in this study is 0.01%. Previous research on insecticide tests using white guava leaf ethanol extracts against M. domestica larvae found an LC50 value of 0.028%, and Anisah & Sukesi (2017) reported an LC50 value of 0.4287% using betel leaf extract.

Table 6. Regression equation and probit LC50 of arabica coffee fruit peel extract against third instar larvae of *M. domestica*

Probit regression equation	**y = ax + b** **5 = -1.7596x + 8.1061**
X	1.77
LC_{50} (antilog x)	0,01%

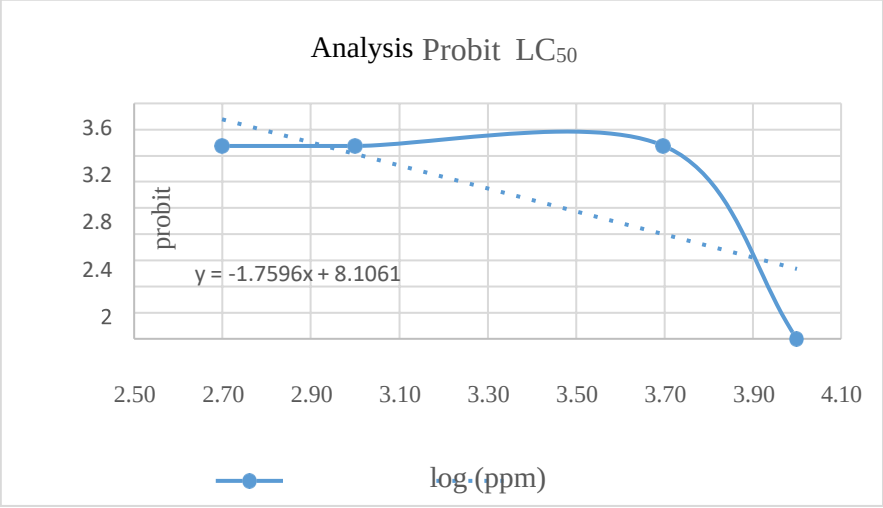

Figure 3. Probit regression equation graph of LC_{50} of arabica coffee fruit peel extract on the mortality of third instar larvae of *M. domestica*

The LC_{50} value is the concentration of an extract that can cause 50% death of *M. domestica* larvae (Jelita et al., 2020). The LC_{50} value in this study was obtained at 0.01%, meaning that the Arabica coffee fruit peel extract at that concentration should be able to kill 50% of the third instar larvae population tested. However, the results in this study do not match the LC_{50} results obtained.

Chlorogenic acid is a compound that dominates the peel of Arabica coffee fruit. This compound is a phenolic compound formed from a combination of quinic, caffeic, p-coumaric, and ferulic acids. These compounds also act as antioxidants and repellents, just like caffeine. Caffeine is one of the compounds that act as antioxidants (Ameca et al., 2018). The two dominant compounds (caffeine and chlorogenic acid) cause a decrease in the toxic properties of Arabica coffee skin extract against *M. domestica* instar III larvae. This is also reinforced in the research of Zarnita et al. (2022) and Prayogi (2019), reporting that chlorogenic acid in coffee fruit peels only acts as an attractant for Hypothenemus hampeii species on cocoa plants, but does not cause death in trapped insects.

Translated with DeepL.com (free version). This is consistent with the research because Arabica coffee fruit peels are not able to kill insects. Similar to chlorogenic acid, caffeine compounds found in Arabica coffee fruit peel extract are only able to repel insects and indirectly reduce the level of insect reproduction (Kim et al., 2006). The low toxicity of Arabica coffee rind extract to *M. domestica* larvae is also evidenced by the length of time for larval mortality as indicated by the LT_{50} value.

3.3. Lethal Time (LT_{50}) Mortality of Instar III Larvae of Housefly *(M. domestica)*

The results of the LT_{50} probit analysis showed the results of the probit regression equation and the LT_{50} values of each concentration of 0.05%; 0.1%; and 0.5% were 274.52 hours; 134.90 hours; and 532.20 hours respectively as shown in (Table 7). While at 1% concentration there was no LT_{50} because there was no death of third instar larvae of *M. domestica* during the observation hours of the study. The LT_{50} value is the time required for a certain concentration of a substance or extract to kill 50% of the test animal population. The lower LT_{50} value will cause the infection of a substance or extract to test animals faster as well (Nurhaifah & Sukesi, 2015).

Table 7. Regression equation and probit LT_{50} of each concentration of arabica coffee fruit peel extract against *M. domestica* larvae

	0,05%	**0,1%**	**0,5%**	**1%**
Regression equation	Y = 3.3876x –3.2609	Y = 3.4644x –2.3792	Y = 2.9403x –2.9138	0
LT50	274.52 hours	134.90 hours	532.20 hours	-

The LT_{50} results in the table above show that the 0.5% concentration of Arabica coffee fruit peel extract has a high LT_{50} value, which is 532.20 hours or ± 22 days. The LT_{50} value is greater than other concentrations of Arabica coffee fruit peel extract because based on observations during the study, the first death of *M. domestica* instar III larvae at a concentration of 0.5% is longer than other concentrations, namely at the 36th hour after administration of the extract. Meanwhile, the first death of third instar larvae of *M. domestica* at concentrations of 0.05% and 0.1% occurred at the 32nd and 16th hours after extract application, respectively. The LT_{50} value exceeds the life limit of the third instar larval phase of *M. domestica*. This is reinforced by research by Ardiansyah (2019), that the third instar larval phase of *M. domestica* will develop into pupae within 72 - 96 hours, so that these conditions cause the larval feeding activity to stop and puparium is formed and then turns into a pupa in soil or sand media.

4. Conclusion

The 96% ethanol extract of Arabica coffee fruit peel at concentrations of 0.05%; 0.1%; 0.5%; and 1% had no effect on the mortality of housefly larvae (*M. domestica*). The concentration that was able to cause the death of housefly larvae (*M. domestica*) as early as 48 hours after adding the ethanol extract of Arabica coffee (*C. arabica*) fruit peel was 0.1%. The LT_{50} value of mortality of third instar larvae of houseflies (*M. domestica*) at concentrations of 0.05%; 0.1%; and 0.5% were 274.52 hours; 134.90 hours; and 532.20 hours, respectively, while the concentrations of 0% and 1% had no LT_{50} value because there was no death of third instar larvae of *M. domestica*.

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