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Original Research Article

The Comparative Study of Arabica Used Coffee Grounds and Temephos in Controlling the *Aedes aegypti* Larvae

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ABSTRACT

Aedes aegypti (L.) is the most common mosquito transmitting Dengue viruses which cause mortality to Thai population and worldwide. Used coffee grounds still contain a significant amount of caffeine and have been reported to inhibit the growth of mosquito larvae. Arabica coffee has been famously grown and consumed as beverage drink in northern parts of Thailand. The used coffee grounds each day in this area should be applied to control the mosquitos instead of throwing away as waste. None of the studies has compared the efficacy in controlling the mosquito larvae among types of roasted used coffee ground before. This study was aimed to evaluate the efficacy of three types of used roasted Arabica coffee grounds (light, medium, and dark) with different concentrations (50 mg/mL, 100 mg/mL, and 125 mg/mL) and standard mosquito larvacide, Temephos (0.01mg/mL) on mortality of the third instar of *Aedes aegypti* larvae. Factorial experiment arranged in complete randomized design (CRD) under laboratory was conducted. Results showed that among three types and three concentrations of roasted used coffee grounds, the light roasted used coffee grounds at the concentration of 125 mg/mL was the best in inhibiting the larvae survival. However, the temephos (0.01 mg/mL) could kill all the larvae within 10 minutes whereas the highest concentration (125 mg/mL) of light roasted used coffee ground could cause 100% mortality in the period of 24 hours after the larvae were exposed to it. The light roasted coffee presents the highest caffeine content compared to the medium or dark roasted. The amount of caffeine left in the light roasted used coffee ground could possibly have contributed to the higher efficacy in causing mortality of the mosquito larvae than other types of roasting. Therefore, light roasted used coffee grounds could be used to slow down the increase of *A. aegypti* populations when the outbreaks of *Aedes aegypti* borne diseases have not yet occurred

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INTRODUCTION

Aedes aegypti is one of the vectors transmitting diseases to humans. Mosquitoes are one of the deadliest animals in the world. Aedes aegypti is the primary transmitter of the four viruses that have had the greatest impact on human health, the viruses causing yellow fever, dengue fever, chikungunya, and Zika fever (Jayme et al. 2019). Coffee has become a famous beverage nowadays. However, there are some waste products from making coffee such as coffee grounds. A lot of research found that the used coffee grounds still contained a lot of important compounds (Gruczyńska et al. 2018). Research study revealed that coffee grounds can inhibit the growth of mosquito larvae (Aditama and Stiepu 2019; Miranda et al. 2021). Research of used coffee grounds for the mortality test of *Aedes aegypti* had been conducted by Miranda et al. 2021) and found that coffee grounds can kill Aedes larvae. The important compound that showed negative effects for mosquito larval growth is caffeine. And the larval instars which had been influenced the most were the 3rd going to the 4th instar. Once the 3rd instar larvae were exposed to the caffeine, the larvae would not be able to develop to 4th instar and the mosquito larvae would not be able to complete the metamorphosis (Aditama and Sitepu 2019). During the process of producing coffee beans (*Coffea arabica*), there are 3 types of roasting such as light roasting, medium roasting, and dark roasting. The amount of caffeine left in the coffee beans after undergoing the roasting processes should not be the same. According to that, the effect of each used coffee ground under different roasting types may have different effects in controlling the growth of mosquito larvae. The conventional method of inhibiting the mosquito larval growth is using the chemical substance called temephos (Sutiningsih et al. 2017). However, using temephos can be expensive and cause the larvae to develop resistance. Thus, this study was conducted to investigate the efficacy of arabica used coffee grounds among different types of roasting compared to the temephos which has been used conventionally.

MATERIALS AND METHODS

Sample Collection

The used coffee grounds (Coffea arabica) were supported by Alexta coffee shop, Chiangrai. There were 3 types of roasting such as light roasting, medium roasting, and dark roasting. The 3rd instar of larvae were supported by Vector Borne Disease Control Center 1.3 Chiang Rai Thailand

Coffee Drying Process

This process was done by bringing 3 types of roasted coffee grounds including light roasted, medium roasted and dark roasted coffee grounds into the incubator at 60°C overnight to make the coffee dried completely

Preliminary of Experiment

In order to investigate the larvicidal activity of used coffee grounds, the preliminary experiment was conducted. Factorial experiment was arranged in complete randomized design (CRD). Three types of used roasted coffee grounds were weighed to be 2.5, 5.0, 10.0, and 12.5 g and then put each mentioned amount in each disposal tea filter bag. After that each tea filter bag was soaked in 100 mL of distilled water for 6 hours in order to get all the roasted used coffee grounds to dissolve as much as possible and to make each concentration to be approximately 25, 50, 100, and 125 mg/mL respectively. After that 40 mL of each coffee concentration was transferred into a plastic plate (3 plates for different concentrations of each roasted coffee ground). Forty milliliters of temephos at standard concentration of 1% was used as a positive control whereas 40 mL of water was used as a negative control. Twenty larvae of A. aegypti were released into each plastic cup containing different concentrations of used roasted coffee ground including both control treatments. The mortality of larvae among these concentrations and control treatments was recorded at 24 hours for 7 days.

Larvicidal Activities between the Best Roasted Used Coffee Ground and Temephos

After preliminary, the light roasted used coffee ground showed the most efficacy to kill the larvae. According to that the experiment was focused mainly on comparisons of the light roasted used coffee grounds and the standard larvicide, temephos. Factorial experiment arranged in CRD was used for the experimental design same as previously mentioned. The light roasted used coffee grounds were prepared at concentrations 50, 100 and 125 mg/mL respectively. After that the volume of 40 mL of each coffee concentration was poured into a plastic cup (3 replicates for different concentrations of light roasted coffee). The 40 mL of temephos at concentration of 1% was used as positive control. The water was again used as negative control. Twenty individuals of 3rd instar larvae were released into the plastic cups containing these different concentrations of used coffee ground and the plastic cup containing the 1% Temephos and water. The mortality of larvae among these concentrations of coffee ground and 1% Temephos was recorded at every 24 hours for 7 days

Statistical Analysis

Data was analyzed by analysis of variance (ANOVA). Mean mortality differences among treatments were compared by Duncan test. In all analyses, $P < 0.05$ will be taken to indicate statistical significance

RESULTS

The light roasted used coffee ground showed significantly higher mortality rate than other types of roasting in preliminary experiments (Figure 1). There was no difference in mortality rate between medium roasted and dark roasted used coffee grounds (Figure 1). The light roasted coffee ground was chosen to conduct further analysis. When considered at concentrations, the light roasted used coffee ground at 125 mg/mL showed significant highest efficacy in causing almost 100% of mortality rate within 24 hours of exposure (Figure 2). Within 2 days, the concentrations of 50 mg/mL and 100 mg/mL started to increase in the efficacy in killing the larva. By 5th day, all concentrations showed 100% of larval mortality (Figure2). When the efficacy of light roasted used coffee grounds was compared with commercial product, Temephos at the conventional dosage of 0.01 mg/mL, the result revealed that Temephos had significant faster acting and higher efficacy in killing

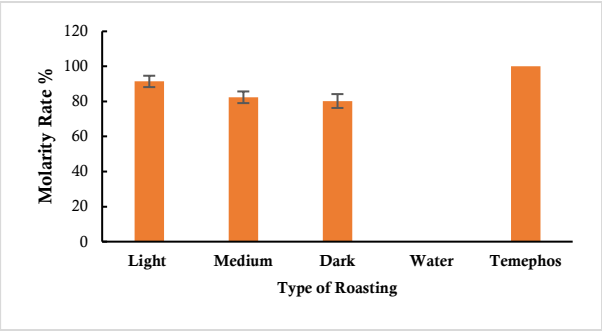

Figure 1. Preliminary the mortality rate among types of roasted used coffee grounds compared to stand standard larvicidal (Temephos). No significant difference among all tested coffee grounds ($p>0.05$).

the larva than the light roasted coffee grounds at every concentration (Figure 2). Temephos acted within the first 10 minutes after the larva was exposed to it and it could cause mortality rate to be more than 50% and it could kill all the larvae (100%) within 40 minutes. All concentrations of light roasted used coffee grounds started to have the effect to kill the larva at 24 hours (day 1) (Figure 2). And the light roasted used coffee ground at 125 mg/mL could cause 100% mortality rate. The concentrations of 50 mg/mL and 100 mg/mL started to increase and caused the mortality rate to be about 90% on the second day. On the 5th day, all concentrations of light roasted used coffee ground resulted in 100% of mortality rate (Figure 2).

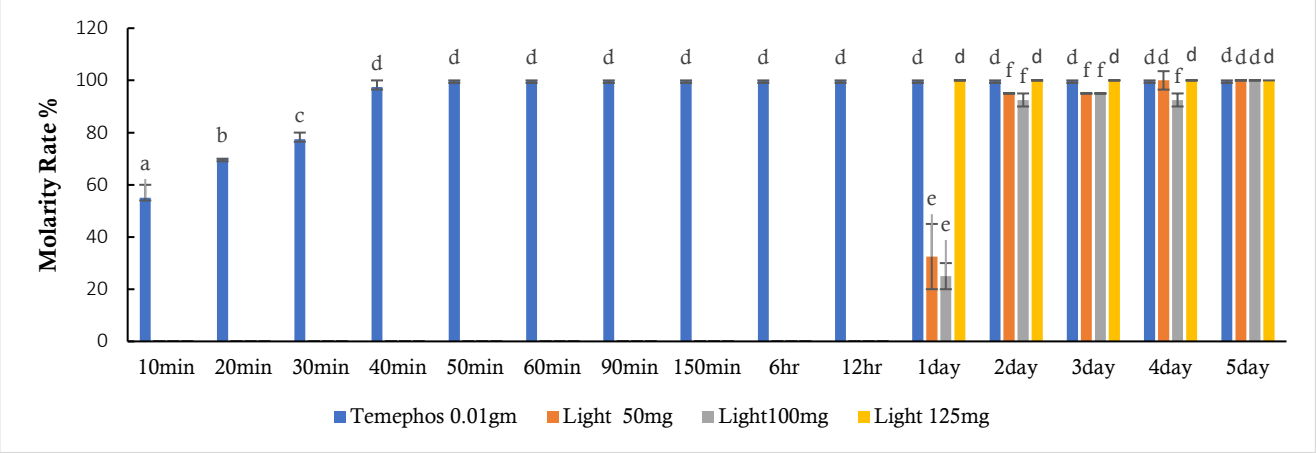

Figure 2. Mortality rate of light roasted used coffee ground VS Temephos. Different letters indicate different levels of significance ($p<0.05$).

DISCUSSION

The mortality rate of Aedes aegypti larvae was compared among different concentrations of coffee ground and commercial product, Temephos. Different types of roasted coffees showed different efficacies in causing mortality to the A. aegypti larva. From the preliminary of our study, the light roasted used coffee grounds worked best in killing mosquito larva (90% of mortality rate) compared to other types of coffee ground roasting (Figure1). However, the medium roasted and the dark roasted used coffee grounds had the same efficacy in causing mortality rate which was about 80%. Coffee grounds have been reported to be toxic to Aedes aegypti larvae, especially the 3rd instar (Aditama and Sitepu 2019). Former studies revealed that coffee grounds contained bioactive compounds such as alkaloids, flavonoids, folifenol, saponins, triterpoid and tannins (Pratita et al. 2017; Socala et al. 2020; Alamri et al. 2020). The reasons why mortality occurs to the mosquito larva because alkaloid compounds, flavonoids and tannins inhibit parasympathetic nerves in the nervous system of insects (Anindita Tri Kusuma 2017). Moreover, the bitter taste of the coffee grounds can cause irritation when the larvae ingest it because these compounds reduce the activity of digestive enzymes and inhibit the absorption of food when consumed by the larvae and cause the larvae to die slowly (Anindita Tri Kusuma 2017). Caffeine (1, 3, 7-trimethylxanthine), a component of coffee, tea and other widely consumed beverages, has been used in toxicological studies of several organisms. Results have revealed deleterious effects on the nervous system (Alasmari 2020; Iriondo-DeHond et al. 2020), on the sensitization of DNA to damage and on the delayed entry of cells into mitosis and on other aspects of cell division (Deplanque et al. 2000; Redazione di Mypersonaltrainer 2021), on the development of organisms (Hussein et al. 2022), on fertility (Wesselink et al. 2016), and on chromatin structure (Chung 2021). Only small amount of caffeine (CAF) can also increase the production of chromosomal damage induced by chemicals or ionizing radiation (Kushwaha et al. 2021; Chung 2021). In relation to mutagenicity, results for the same organisms (mosquitos) in literature are occasionally antagonistic (Aditama and Sitepu 2019). For the house fly, Musca domestica, caffeine was found to inhibit the growth of house fly larva by interfering with the puparium formation because caffeine affects the hormone controlling responses in house fly. House fly pupal mortality was found when larvae are treated with higher concentrations of caffeine (Penningto et al. 2017). Differences in coffee green bean composition, roasting conditions, and extraction procedures adopted for the coffee beverages preparation result in great diversity of the chemical composition of the final product (Sualeh et al. 2020), which could account for differences in the biological activities of coffee brews. Light roasted coffee is a light brown color and has no oil on the surface of the beans. These coffees typically have a crisp acidity, a mellow body, and bright flavors. The light roasted coffees are roasted in order to preserve the unique characteristics of the coffee bean. Light roasted coffee contains the highest overall content of caffeine among all coffees (Alqarni et al. 2018). The quantity of caffeine was significantly affected by the

roasting degree and light roasted coffee (6.42 mg g-1) presented higher caffeine levels than medium (5.77 mg g-1) and dark roasted (2.63 mg g-1) beans (Król et al. 2020). This could explain that why our result showed that the light roasted coffee showed higher efficacy in killing mosquito larvae better than medium and dark roasted coffee. Because the light roasted coffee contained more amount of caffeine which plays important role in causing negative effects to the larva, the higher mortality rate of the larvae was demonstrated in the light roasted coffee than other types of coffee roasted ones. During our experiment, we had limited number of mosquito larvae. When the result showed that the light roasted coffee performed good trend in controlling the mosquito larvae, we chose only light roasted coffee to further analyze to investigate the most optimal concentration to cause the highest mortality of the larvae. The concentrations chosen in the study were verified until the result showed significant in causing mortality to the larvae. At preliminary experiment, we chose the concentrations to be 25 mg/ml to 50 mg/mL because the study done by Laranja et al (2003) proposed that at concentration of 1.0 mg/ml and 50 mg/mL of coffee grounds completely blocked the development of Aedes aegypti in the early stage. However, our preliminary result demonstrated that the concentration of 25 mg/mL to 45 mg/mL did not show any differences in killing the larvae compared to the control treatment (water). Therefore, we adjusted the concentrations to be 50 mg/mL, 100 mg/mL, and 125 mg/mL respectively. And our result showed that the concentration of 125 mg/mL seemed to work best in killing the larvae. However, when tested with the conventional temephos at 0.01 mg/mL, the result showed that temephos had much higher efficacy to kill the larvae and acted much faster (within 10 minutes) than the 125 mg/mL of light roasted coffee ground solution. Temephos, an organophosphate larvicide, has been used for dengue vector control in Thailand since 1950 (Overgaard et al. 2018). The efficacy test of temephos sand granules was performed in 1972, and this larvicide has since been recommended for use in Aedes aegypti control (Bang et al. 1972; George et al. 2015). This larvicide is acceptable for public use and believed to be an effective larval control agent at present. However, a high incidence of the mosquitos developing resistance to this chemical has been reported in many provinces of Thailand such as Roi Et, Bangkok, Nakhon Sawan, and Nakhon Ratchasima (Sumarnrote et al. 2017; Amelia-Yap et al. 2018; Doum et al. 2020). Unlike adulticide, the use of nontoxic to human larvicide like temephos leads to unawareness of resistance development because of improper application schemes. The slow release pattern of temephos sand granules over time has been noted under field conditions (Williams et al. 2019) because of its low solubility in water. This character could facilitate selection for tolerance up to resistance levels in nature on exposure to insecticide at below-mortality doses (Margus et al. 2019). To overcome the problem of insect vectors developing resistance to insecticides is to rotate the applications of effective insecticides to avoid the selection pressure for the developing the resistant genes (Dusfour et al. 2019). Used coffee grounds can be an alternative way to avoid the developing resistance of mosquito larvae but more experiment is needed to adjust the dosage of used coffee grounds to be able to have equivalent efficacy to the conventional larvicidal products

CONCLUSIONS

Used coffee grounds have the ability to increase mortality of A. aegypti larvae. There was different significant efficacy to cause mortality among different types of roasting. The light roasted performed best efficacy in killing the larvae. The high concentration of the light roasted used coffee grounds showed higher efficacy of inhibiting the survival of the larvae. However, the efficacy of used coffee grounds is still a lot less compared to the conventional Temephos. Used coffee grounds can be used to prevent the increasing of the larvae at the time that the numbers of the larvae are not many and the outbreak of the disease carried by this A. aegypti vector still not occurring. Further study is needed to investigate the best dosage and concentration of the used coffee ground solution to make it become almost equivalent to the conventional larvicidal products. Finally, used coffee grounds can be value added by developing their properties to control the insect vectors.

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