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EVALUATION OF ADULTICIDAL EFFICACY OF CYPERMETHRIN, TETRAMETHRIN, AND PIPERONYL BUTOXIDE IN HOUSEFLY (MUSCA DOMESTICA)

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Abstract

Introduction: *Houseflies serve as vectors of diseases in animals and humans. Chemical pesticides are a practical way of eliminating insects; however, resistance to insecticides has been reported. This study aims to evaluate the adulticidal efficacy of cypermethrin, tetramethrin, and piperonyl butoxide in housefly.* ***Methods:*** *Adult flies were cultured within net enclosures using completely randomized design with four replications each treatment. Treatment A was water and served as the negative control, treatment B was cypermethrin + tetramethrin + piperonyl butoxide 32% EC and served as the positive control, and treatment C was cypermethrin + tetramethrin + piperonyl butoxide 48% EC. The number of dead houseflies were counted 15, 30, 60, and 120 minutes post- application of treatments. The results were analyzed through analysis of variance ($P \leq 0.05$) followed by Tukey's HSD (honestly significant difference).* ***Results and Discussion:*** *The values for the cumulative mean and percentage of killed houseflies were all significantly different from each other, with treatment C showing the highest values, followed by treatment B, then treatment A. The maximum adulticidal effect of treatment C was apparent within 15 minutes post-application, while the maximum adulticidal effect of treatment B was observed within 30 minutes after application.* ***Conclusion:*** *Results suggest that treatment C was a more effective adulticidal than other groups. Using cypermethrin + tetramethrin + piperonyl butoxide 48% EC can be the solution to the problem, but it should be noted that future resistance may occur. Increased concentrations may also imply environmental, human, and animal health hazards.*

INTRODUCTION

Major non-biting nuisance insects that occur worldwide, houseflies (*Musca domestica*) are a very common pests in poultry farms (1). Furthermore, due to their behavior and life cycle, they inevitably become a threat to biosecurity, which is the reason for their economic importance in poultry farms (2). Adult houseflies commonly serve as mechanical vectors of pathogenic bacteria of more than 100 other causative agents of diseases like (3) in the family Enterobacteriaceae including *Salmonella* sp. and *Escherichia coli* strain O157:H7 (4), and *Campylobacter* sp. (5) which affect both animal and human, leading to the occurrence of food-borne illnesses (3).

Houseflies can obtain pathogenic bacteria from feed, food, waste, and manure. Moreover, studies show that houseflies contribute to the prevalence of antimicrobial resistance in a flock by spreading antimicrobial-resistant bacteria (6). Aside from bacteria, houseflies can carry other types of pathogens such as viruses like Newcastle disease virus which can cause high mortality in a flock, leading to economic devastation in a poultry farm (7). Houseflies can also carry helminth ova, protozoan cysts and trophozoites which can cause human gastrointestinal diseases (8-9). Therefore, adult houseflies play a big role in both food security and food safety. Pest control must be given importance in farms.

Control of housefly requires a number of different measures, such as chemical methods, including the use of insecticides (4,10). Insecticides are used daily to kill vectors that pose threats to livestock production and public health and are deemed as the most practical method to

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control pests. The use of pesticides significantly reduces the occurrence of health problems due to pests (11).

Pyrethroids such as cypermethrin and tetramethrin are synthetic analogs of pyrethrum, a natural substance that is derived from dried chrysanthemum flowers (*Tanacetum cinerariaefolium*) (12). They serve as insecticidal sprays and dusting powders, both domestically and in the agricultural sector (13).

Pyrethroids, in general, are insecticides that produce neurological signs and have a rapid knockdown effect. Pyrethroids are modern insecticides structurally similar to natural pyrethrins which is an active insecticidal compounds due to their photostability; pyrethrins have a reduced photostability and are more biodegradable (14-15), hence proving their reliable performance. Chemically, esters of chrysanthemic acid (ethyl 2,2-dimethyl-3-(1-isobutenyl) cyclopropane-1-carboxylate) (16) they took 19% of the total global market of insecticides in 2019. They are known to be effective in killing insects in many orders including Diptera, of which houseflies are a part (17). Plant compounds have been used in agriculture as insecticides that are not highly toxic to mammals and are biodegradable.

In commercial formulas, pyrethroids are commonly combined with piperonyl butoxide (PBO) which acts as a metabolic synergist that improves efficacy (18). PBO is a semi-synthetic synergist for natural pyrethrum and synthetic pyrethroid insecticides in phytochemicals and biocides derived from natural safrole and its mechanism of action is through inhibition of the mixed function oxidase, thus decreasing the oxidative breakdown of pyrethrum, synthetic pyrethroids, and other pesticides (19). By itself, it has low insecticidal activity, but boosts the efficacy of the active ingredient, therefore allowing decreased amounts of pesticide to be used (15).

The effective concentration (EC) of cypermethrin, tetramethrin, and piperonyl butoxide in insecticide products found in the market is 32% such as Flykill® which has been deemed effective by buyers. It remains an ideal insecticide for treating nets and other fabrics owing to the low cost, longer residual activity and low mammalian toxicity. However, resistance to a combination of pyrethroids and PBO as insecticides has been reported (17,20). In this study, 48% effective EC of cypermethrin, tetramethrin, and PBO were tested to determine if it has greater efficacy than the products currently available in the market. This study aims to evaluate the adulticidal efficacy of cypermethrin, tetramethrin, and piperonyl butoxide in houseflies (*Musca domestica*).

METHODS

The study was conducted in June 2023 at a private facility in Bay, Laguna, and the Department of Basic Veterinary Sciences, College of Veterinary Medicine, University of the Philippines Los Banos.

Housefly Culture

Six 1.829 m x 0.914 m x 1.829 m net enclosures (Figure 1) located out doors were used to culture houseflies. The net enclosures were exposed to a temperature of 32 to 38°C for optimal housefly growth. The experiment was conducted using a completely randomized experimental design (CRD).

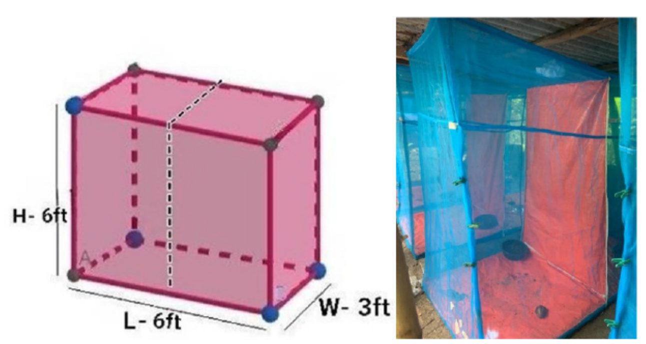

Figure 1. Sample Design of the Net Enclosure with Measurements 1.829 m (6 ft.) x 0.914 m (3 ft.) x 1.829 m (6 ft.)

The six enclosures were equally divided into two using a plastic tarpaulin. Each of the three treatments had four replicates, which resulted in 12 enclosures. Each replicate contained approximately 700 larvae, cultured on 500 g of feces obtained from a broiler farm post-harvest, which totaled around 8,400 larvae and six kg of feces, placed on small plastic bins. Water was regularly sprayed once every day onto the feces to create a high-moisture environment favored for houseflies' growth. Each replicate housed the larvae until they developed into adult flies seven days after, which signaled the start of the experiment. There were three treatments as shown in Table 1.

Table 1. Treatment Groups and Their Corresponding Components

Treatment Group	Component
Treatment A	Control (water)
Treatment B	Cypermethrin 12% + Tetramethrin 4% + PBO 16% (Flykill®)
Treatment C	Cypermethrin 18% + Tetramethrin 6% + PBO 24%

PBO = piperonyl butoxide

Per manufacturer recommendations, treatment B had a dosage rate of 1 mL per 70 mL of water and treatment C had dosage rate of 1 mL per 105 mL. The application rate was 100 mL/m^2 on approximately 700 flies within the net enclosure

Pesticide Application

A commercially available handheld water sprayer with pump action trigger, made of plastic, with a capacity of 500 mL was used to distribute the control and insecticides within replicates. The same type of sprayer was used in all treatments. It dispensed about 0.5 mL per spray. The water and insecticides were manually sprayed inside, on all panels, of the enclosure to ensure proper distribution. The housefly was deemed dead once it fell on the ground, motionless, with no signs of life such as buzzing or attempts to fly. The housefly should remain unresponsive to any stimulus such as contact with any object. A dead fly also had its legs and wings folded close to its body, both with no movement. The dead houseflies within the net enclosure were tallied at 15, 30, 60, and 120 minutes after the application of the treatments. The surviving houseflies were left to die inside the enclosure after the experiment.

Statistical Analysis

The data collected were tabulated and statistically analyzed via one-way analysis of variance (ANOVA) at p-value ≤ 0.05 through the R Studio for Windows developed by Posit PBC, based in Massachusetts, United States of America. After, the Tukey's HSD (honestly significant difference) test was used for post-hoc analysis.

RESULTS

Houseflies are common pests found in poultry farms that bring nuisance as well as a number of diseases to both humans and animals, and thus this study focuses specifically on them. Flies come in contact with surfaces rich in microbes such as garbage and manure, and become contaminated. In poultry farms, other than being a nuisance to farm staff, houseflies can reduce egg production. In this study 48% EC of cypermethrin, tetramethrin, and piperonyl butoxide (PBO) was compared to a negative control and positive control groups with regard to the number of dead flies after administration. The initial number of adult houseflies was not controlled, and their differences were due to randomness. The houseflies that dropped on the ground, motionless, were the only ones counted. The result of the mean of dead houseflies per treatment, post-application at various time intervals is shown in Table 2.

Table 2. The Mean of Dead Houseflies Post-Application at Various Time Intervals

Time	Treatment A	Treatment B	Treatment C
15 mins	1.25^{c}	458.5^{b}	698^{a}
30 mins	0^{c}	14.5^{a}	4.25^{b}
60 mins	0^{b}	2.75^{a}	0^{b}
120 mins	0	0	0

abc superscript corresponds to different letters within rows whose values are significantly different at P-value <0.05

Tukey's range test indicates that the values from all treatments in the 15-minute mark are significantly different from each other, with treatment C killing the most flies, followed by treatment B, then treatment A. All values in the 30-minute mark are significantly different from each other as well, with treatment B killing most flies, followed by treatment C, then treatment A. Treatment B killed the most flies at the 60-minute mark, while treatments A and C did not kill any; the values of treatments A and C are not significantly different from each other, while the value of treatment B is significantly different to both treatments A and C. Table 3 shows the cumulative mean number of dead houseflies per treatment post-application at various time intervals.

Table 3. The Cumulative Mean Number of Dead Houseflies Post-Application of Each Treatment

Time	Treatment A	Treatment B	Treatment C
15 mins	0.125^{c}	458.5^{b}	689^{a}
30 mins	0.125^{c}	473^{b}	639.25^{a}
60 mins	0.125^{c}	475.75^{b}	639.25^{a}
120 mins	0.125^{c}	475.75^{b}	639.25^{a}

abc superscript corresponds to different letters within rows whose values are significantly different at P-value <0.05

All of the values from each treatment are significantly different from each other; the highest values are found under treatment C, followed by treatment B, then treatment A. Treatment C consistently killed the most dead houseflies, followed by treatment B, then treatment A, which has the same average of dead houseflies across all time intervals. On average, for treatment A, most houseflies were killed in 15 minutes, and the average number of houseflies beyond 15 minutes was left unchanged. The trend for treatment B, on the other hand, is the opposite, as the least number of dead houseflies were tallied at the 15 minutes mark, while the highest was during the 60 to 120 minutes mark, showing that the houseflies were gradually killed. Table 4 exhibits the percentage of flies killed out of the 700 estimated number of larvae cultured in each replicate, at various time intervals.

Table 4. The percentage of Dead Houseflies Post-Application of Each Treatment

Time	Treatment A	Treatment B	Treatment C
15 mins	0.1775^{c}	65.5^{b}	98.4275^{a}
30 mins	0.1775^{c}	67.57^{b}	99.035^{a}
60 mins	0.1775^{c}	67.965^{b}	99.035^{a}
120 mins	0.1775^{c}	67.965^{b}	99.03^{a}

abc superscript corresponds to different letters within rows whose values are significantly different at P-value <0.05

All of the values in each time interval are significantly different according to Tukey's range test. The pattern visible across time intervals is that treatment C killed the most flies, followed by treatment B, then treatment A. In general, cypermethrin, tetramethrin, and PBO 48% EC was the most effective in killing adult houseflies, reaching its full adulticidal efficacy 30 minutes post-administration.

DISCUSSION

Musca domestica, part of the order Diptera and family Muscidae, is a synanthropic organism. It can be found ubiquitously in rural and urban areas and various types of animal farms. Flies come in contact with surfaces rich in microbes such as garbage and manure then become contaminated. With the abundance of houseflies in a farm, it is possible that antimicrobial-resistant bacteria are present, hence promoting antimicrobial resistance in a flock (2). Various application methods can be used to control *Musca domestica* in poultry production, such as biological agents (21); baits and traps (4); ultraviolet light, delta traps, and electrocuting grids (22), sprays, dust, impregnated strips, aerosol, fumigants and insecticides (10). Reduction of moisture levels in farms is also an indispensable part of cultural control as larvae tend to prefer areas with 50-75% moisture. Water management includes monitoring pipe leakages and content of water containers, providing proper airflow through tunnel ventilation, and maintaining waterers (4).

Chemical insecticides act as toxins on different bodily systems of the housefly. Insecticides are popularly used in poultry management because of their low cost and are fast-acting. Insecticides are categorized as larvicidal and adulticidal. Larvicidal insecticides include insect growth regulators and are only used during immature phases of houseflies. Insecticides including malathion, diazinon, dichlorvos, and cypermethrin, are common agents that show efficacy against houseflies (23). Adulticidal insecticides, on the other hand, are effective against adult houseflies. These can be applied as a spray, bait, and fog (10).

In this study, 48% EC of cypermethrin, tetramethrin, and piperonyl butoxide was compared to a negative control and positive control groups with regard to the number of dead flies after administration. The initial number of adult houseflies was not controlled, and their differences were due to randomness. The houseflies that dropped on the ground, motionless, were the only ones counted. The experiment involved three treatment groups: treatment A being water which served as the negative control, treatment B being tetramethrin, cypermethrin, and PBO (32% EC) which served as the positive control, and treatment C being tetramethrin, cypermethrin, and PBO (48% EC). Each of the three treatments had four replicates each. The number of dead houseflies were counted every 15, 30, 60, and 120 minutes after the application of treatments.

There is a wide margin between the results of treatment A to both treatments B and C, and the widest margin is seen between treatments A and C. All deaths under treatment A occurred within the first 15 minutes of administration. This can be due to the fact that water does not cause any form of toxicity to houseflies. The dead houseflies in treatment A can be due to the houseflies previously injured before spraying, the slim chance that they got injured while spraying, or other external factors.

The raw number, mean number, and percentage of killed houseflies due to treatment B is lower than that of treatment C. Both treatments B and C reached their full efficacy within 30 minutes post-administration. Treatment C was able to eliminate around 30% more insects than treatment B. Hence, the maximum effect of treatment C is higher than treatment B. This is due to the fact that treatment C has a higher concentration. The concentration of the drug at the target receptor site affects the action of the drug. Ideally, there is a direct relationship between the concentration of the drug and the measured effect of the drug. Following the same principle, since treatment C has higher EC than treatment B, a higher number and percentage of flies at all time intervals were killed when treatment C was administered. Since treatment C was able to produce a higher maximum response than treatment B, it can be deemed that treatment C has higher efficacy than treatment B. In the same light, treatment C killed most of the houseflies within 15 minutes, thus exhibiting a higher knockdown effect than treatment B.

This can be explained by the growing resistance of insects to pyrethroids because they have been used for decades for the benefit of both agricultural and public health sectors; long- term use of these active ingredients has led to resistance over time (20). Resistance was first recorded in 1951 by Busvine. Recently, researchers have been studying the mechanism behind the resistance to pyrethroids. Insects have managed to develop new mechanisms to combat the insecticidal activity of pyrethroids, namely the formation of the knockdown resistance (*kdr*), alterations in the enzymes that detoxify insecticides, and reduced uptake of insecticides. Pyrethroids target the central nervous system and work on voltage-gated sodium channels (VGSC). Upon binding of the pyrethroid molecule on the α subunit of the channel, slowed gating transitions as well as persistent and irreversible opening of the channel occur (23). Hence, increased amounts of sodium ions enter the nerve cells, resulting in neuronal hyperexcitability and permanent depolarization. Additionally, pyrethroids also target calcium channels, nicotinic acetylcholine receptors, intercellular gap junctions, and GABA-gated chloride channels.

The main resistance mechanism against pyrethroids is *kdr*. The voltage-gated sodium channels (VGSC) are heavily involved in the mode of action of pyrethroids as it serves as the primary target site. Studies have shown that the change of leucine residue to phenylalanine in VGSC causes kdr mutation, which ultimately decreased sensitivity of houseflies to pyrethroids. In houseflies and other species of insects such as *Anopheles* sp., the mutation in the sodium channel gene is due to a change of leucine to phenylalanine (L1014F) (17). This specific mechanism was observed in China and Pakistan where insect populations in multiple parts of the country were found to exhibit such action, which is mainly attributed to L1014F formation in VGSCs (17,20). Pyrethroids containing natural pyrethrins have a problem with instability when exposed to light, air, and heat (23).

In contrast to pyrethroids, resistance to PBO has not occurred in insects. In fact, some studies reported that adding PBO to insecticides can hinder insect resistance. This is due to its ability to inhibit metabolic-resistant enzymes responsible for the detoxification of insecticides (24). There are three main enzyme groups including glutathione S-transferases (GSTs), cytochrome p450s and esterase's (ESTs) that are responsible for metabolic resistance to the main classes of insecticide applied against insects (25).

The results of the experiment provide evidence that 32% EC of cypermethrin, tetramethrin, and PBO may not be enough to produce the rapid, 100% knockdown effect that consumers want due to resistance, and suggests that advancing to 48% EC is a possible solution to the problem since it produced a rapid and almost 100% knockdown effect on approximately 700 insects in each replicate in 30 minutes or less. In this combination, cypermethrin and tetramethrin are the knockdown agents, and PBO is the synergist. Combining knockdown agents and synergists can prevent easy insecticide resistance compared to using only a single ingredient. Insecticides are presumed to produce a rapid knockdown effect on insects and are deemed effective by customers once they see its quick action against pests. PBO combined with pyrethroids produces such effects, that is why this combination has been used in pest control for years (14,26).

However, increasing effective concentrations also means increasing its toxicity. There have been reports of human toxicity due to pyrethroids combined with piperonyl butoxide. A study found administering doses lower than the supposed doses can cause acute dermal toxicity (27). Pyrethroids are probably human developmental neurotoxicants and adverse impacts of pyrethroid exposure on neurodevelopment are likely at exposure levels occurring in the general population. Preventive measures to reduce exposure among pregnant women and children are warranted. In addition, inhalation of cypermethrin, tetramethrin, and PBO may cause cardiotoxic effects in humans (28).

PBO inhibits mixed-function oxidase enzymes or cytochrome p450 enzymes which are responsible for the metabolic detoxification of various insecticides and toxins (18). Cytochrome p450 is one of the main functional enzymes in insecticide metabolism, responsible for the synthesis hormone metabolism (e.g. ecdysone and juvenile hormone), stress response, and toxin catabolism in insects (24,26). Inhibition of the enzymes impedes toxin metabolism and excretion. A combination of PBO and pyrethrins results in a more rapid knockdown effect, higher mortality, and long-lasting residual action. PBO poses minimal health risks to humans and birds, but can also be toxic to fresh and saltwater fish (29). Overall, the combination of cypermethrin, tetramethrin, and piperonyl butoxide, in certain doses, is not a threat to human health.

Tetramethrin, being a Type I pyrethroid, induces a neurological syndrome via their effects on the central and peripheral nervous systems along with signs such as incoordination, tremors, seizures, and death. Cypermethrin, a Type II pyrethroid, produces a choreoathetosis syndrome wherein hyperactivity, tremors and incoordination happens, leading to writhing movements (30).

According to the literature, pyrethroids have a short residual effect as compared to other insecticides but have great knockdown effects, especially in higher concentrations, as evidenced by this study (20). Pyrethroids pose low toxicity on mammals unless consumed in high amounts and exposed to for a long time. In contrast to organophosphates, pyrethroids cause low fatality in humans and pose low environmental risk (29).

This is beneficial in the sense that consumers will be satisfied with its quick action against insects, but repeated spraying might be required for the elimination of flies, causing increased consumption of insecticides. Moreover, increasing concentration does not mark the drug safe from resistance, but rather encourages it in the long run, whereas insecticides that have a long residual effect can be of harm to the environment as they persist for a longer amount of time and can also be toxic to other organisms, including birds, fish, beneficial insects, and non-target plants, as well as air, water, soil, and crops (11). Insecticides reach bodies of water through seepage and wind; therefore, increased concentrations and residual activity can be detrimental to the aquatic ecosystem (31). Consequently, marine life can be exposed to danger, including fish. Cypermethrin, specifically, is recorded to cause harmful effects on exposed fish of different species, resulting in death (32). In the environment, pesticides, including insecticides, are subjected to photodegradation and hydrolysis, as well as other factors like temperature, pH, and oxygen, hence are prone to breakdown, making it difficult for to survive past 100 days in soil (33). There are positive and negative effects coming from different formulations; achieving the right balance between these pros and cons is ideal to create a safe environment for both humans and animals. Overall, the combination of cypermethrin, tetramethrin, and piperonyl butoxide, in certain doses, are not threats to human health.

CONCLUSION

Results of the experiment showed that treatment C showed a significant increase in adulticidal efficacy in houseflies as compared to the positive and negative controls, both in the average number of flies killed across all time intervals and the percentage of flies killed. This suggests that cypermethrin, tetramethrin, and PBO 48% EC has a significantly greater insecticidal activity against adult flies as compared to the commercially available cypermethrin, tetramethrin, and PBO 32% EC, and is effective in eliminating houseflies in a short amount of time. Overall, the combination of cypermethrin, tetramethrin, and piperonyl butoxide, in certain doses, are not threats to human health. It is recommended to conduct further studies to determine the residual time of the cypermethrin, tetramethrin, and PBO combination, ideally one with a longer experiment duration. Perhaps another chemical which has minimal toxicity but has long residual effect can be added to the formulation to achieve long residual duration. Lastly, it is recommended to determine the type and degree of toxicity 48% EC can have on chickens or swine, if ever present.

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