



## Larvicidal Efficacy of Abamectin–Pyriproxyfen Mixtures Against *Musca domestica* in a Poultry Farm: A Field Evaluation

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### ABSTRACT

The house fly, *Musca domestica*, is a notorious veterinary pest that has developed resistance to insecticides. One approach to address this concern is by mixing insecticides to overcome resistance and accelerate pest management. This study was conducted to evaluate the larvicidal efficacy of the abamectin and pyriproxyfen binary mixture against *M. domestica* larvae, based on its ability to reduce larval populations and inhibit pupal formation. The study was conducted in the UPLB VTH Experimental Poultry House. Forty-five boxes, each containing 500 larvae, were distributed across five treatment groups, with nine replicates per treatment. The treatments consisted of distilled water (T1), low-concentration pyriproxyfen (T2), high-concentration pyriproxyfen (T3), low-concentration abamectin-pyriproxyfen binary mixture (T4), and high-concentration abamectin-pyriproxyfen binary mixture (T5). The treatments were applied to each box on days 0 and 7, and the number of live larvae and pupae was recorded on days 7 and 14. On day 7, T5 achieved the highest larval reduction of 88.49%, which was significantly higher than the other treatments. By day 14, T3, T4, and T5 exhibited comparable larval reduction with no significant differences among them. In terms of day 7 pupal inhibition, T5 had the highest inhibition at 95.75%, followed closely by T3 and T4, with no significant difference among the three. By day 14, T5 again had the highest effect at 95.26%, with T3 and T4 also showing similarly strong results. The results of this study highlight the potential of the abamectin-pyriproxyfen binary mixture as a tool in managing house fly populations. However, the lack of significant differences between the binary mixture and the high-concentration pyriproxyfen suggested that the enhanced efficacy was not substantial.

**Keywords:** Abamectin, binary insecticide mixture, house fly control, larvicidal efficacy, pyriproxyfen.

## INTRODUCTION

The poultry industry continues to be a vital component of the Philippine agricultural sector,

accounting for 15.7% of the country's total value of production in agriculture and fisheries (Philippine Statistics Authority, 2023). With

chicken meat's affordability and the resurgence of African Swine Fever (ASF) cases, it continues to gain popularity among consumers (Acosta, 2022). From small-scale backyard farming to large-scale commercial operations, the industry has become an attractive livelihood for many. The rising consumer preference has resulted in a corresponding increase in production. While the industry is steadily recovering from the negative growth rate in chicken production from 2019 to 2021, some of the challenges for optimal poultry operations persist up to this day. However, intensified production systems have also amplified persistent challenges, particularly pest infestations.

One of the major concerns of the poultry industry in the Philippines is house fly (*Musca domestica*) infestation. This species is of great importance to public health because it plays an important role in transmitting diseases as a mechanical vector of several pathogens, such as bacteria and viruses (Issa, 2019). House flies are normally seen around human settlements due to their synanthropic nature. Aside from being a public health risk, large populations may cause a nuisance to the surrounding community, leading to poor public relations of the operating farm (Licup et al., 2024). Because of this, the development of effective insecticides is critical for the control and management of house flies.

The house fly is one of the most common species of fly all over the world. It serves as the mechanical vector of different pathogenic organisms that may cause several animal and human diseases. Among these pathogens, the most isolated are bacteria and fungi (Deakpe et al., 2018). These flies are usually the culprits of disease outbreaks, especially those associated with *Campylobacter*, *Chlamydia*, *Enterococcus*, *Escherichia*, *Salmonella*, and *Shigella*, to name a few. Large fly populations may congregate in poultry farms, specifically in poultry manure. This problem must be addressed as it poses a risk to public health and comfort in nearby communities (Sanchez- Arroyo & Capinera, 2020).

Effective control of *M. domestica* is therefore essential in poultry production systems. The control methods can be classified

as cultural, biological, and chemical. Cultural control involves modifying the environment to become less favorable for the flies, such as sanitation and manure management. Another method is biological control, which refers to the introduction of natural predators such as parasitic wasps to suppress the fly population. The final component of integrated pest management is the use of chemical control, in the form of insecticides. These chemicals can be categorized as adulticides and larvicides. Adulticides are available in baits, sprays, and surface treatment, while larvicides are available as spray and feed-troughs (Iqbal et al., 2014; Igarashi et al., 2020).

The indiscriminate use and heavy reliance on chemical control can lead to the rapid development of house fly insecticide resistance. One example is the excessive use of pyriproxyfen as a feed through in the 1980s, which has resulted in rapid development of resistance in just a few years (Geden & Devine, 2012). Additionally, a recent study by Khan, (2024) has revealed that *M. domestica* has shown resistance to propoxur, dichlorvos, permethrin, deltamethrin, and beta-cypermethrin. This growing resistance significantly reduces the efficacy of commonly used insecticides and poses a major challenge to sustainable fly control.

To mitigate resistance development, poultry farms commonly adopt insecticide rotation strategies, where compounds with different modes of action are used alternately to reduce selection pressure on fly populations. While this approach can delay resistance, it may not always provide consistent control, particularly in high-density production systems with continuous fly breeding.

An alternative strategy is the use of insecticide mixtures, which combine compounds with different modes of action to enhance efficacy and potentially reduce resistance development. For example, abamectin is a potent insecticide from the family of avermectin compounds, and it is used for various pests, including cockroaches, house flies, mites, and parasitic worms. This compound prevents the

transmission of electrical activity in the muscles and nerves by interfering with the gamma-aminobutyric acid (GABA) receptors. Specifically, it amplifies the glutamate effects on the GABA-gated chloride channel, glutamate-gated chloride channel, and histamine-gated chloride channel, which ultimately leads to the paralysis of the target (Batiha et al., 2020, Nassar, 2016). In Rousw and Wright's (1986) study, abamectin was found to be effective on six insecticide-resistant strains of *M. domestica*. On the other hand, pyriproxyfen is a pesticide compound classified as an insect growth regulator (IGR) which has shown high effectiveness against dipterans such as house flies. Its mode of action involves mimicking the hormone responsible for suppressing the pupa to adult development of the insect (Geden & Devine, 2012).

Therefore, this study aims to evaluate and compare the larvicidal efficacy of an abamectin + pyriproxyfen binary mixture with pyriproxyfen alone against *Musca domestica* in a poultry farm setting.

## METHODS

### Manure Box

The manure boxes measuring 12 x 12 x 9 inches, were assigned to five treatment groups, with nine replicates each. A total of 500 larvae were placed in each box to establish the baseline population. A net cover was placed on top of each box as a measure to prevent egg deposition by other flies and to provide accurate counts of newly emerged larvae and pupa throughout the experiment.

### Larvicide

The treatments utilized in this study consisted of distilled water for the negative control, 10% pyriproxyfen emulsifiable concentrate (EC) in 1:100 and 1:50 EC: distilled water concentrations for the positive control, and the binary mixture of 5% abamectin and 15% pyriproxyfen EC in 1:200 and 1:100 EC:distilled water concentrations for the experimental control. Each manure box was sprayed with 35 mL of its assigned treatment per application.

## Experimental Design

A completely randomized design (CRD) was employed in this study, consisting of five treatment groups with nine replicates per treatment (total n = 45 experimental units). Replicates were randomly assigned to treatments to minimize bias and ensure independence among experimental units. This replication level is consistent with similar semi-field studies evaluating larvicidal efficacy in poultry environments.

**Table 1.** Treatments used for the experimental trial

Group	Treatment
T1	Control (distilled water)
T2	10% pyriproxyfen (1:100)
T3	10% pyriproxyfen (1:50)
T4	5% Abamectin and 15% Pyriproxyfen (1:200)
T5	5% Abamectin and 15% Pyriproxyfen (1:100)

## Environmental Conditions

The experiment was conducted inside a poultry house under semi-field conditions, where environmental parameters were not artificially controlled but monitored throughout the study period. Ambient temperature and relative humidity were recorded daily, with temperatures ranging from approximately 30–35 °C and relative humidity ranging from 60–80%, reflecting typical tropical poultry production conditions. These environmental conditions are conducive to rapid development of *M. domestica* and representative of real farm settings.

## Sample Collection

On day 0, each replicate was evenly sprayed with 35 mL of the assigned treatment and left undisturbed for 7 days until the first data collection. After 7 days, the number of live larvae and pupae was manually counted. A larva is considered dead or affected if no movement is seen on initial observation or upon probing using thumb forceps. Otherwise, they are considered alive or unaffected if movement is observed or it proceeds to the pupal stage. The number of pupae was also counted separately from the

larval counts. Additionally, the pupae and remains of dead larvae from the first collection were discarded after counting. The data obtained served as the result for day 7. Following data collection, each manure box was sprayed again with its assigned treatment and left undisturbed for another 7 days. The second data collection was conducted 7 days after the second application of treatment. The data obtained for the second collection served as the result for day 14.

The following parameters were recorded in every data collection:

- Number of live larvae observed per treatment.
- Number of pupae observed per treatment.
- Percentage of larvae reduction from each box

formula for Day 7

$$\% \text{ of larvae reduction day 7} = \frac{500 - (\text{larvae day 7} + \text{pupae day 7})}{500} \times 100$$

formula for Day 14

$$\% \text{ of larvae reduction day 14} = \frac{500 - (\text{larvae day 14} + \text{pupae day 7} + \text{pupae day 14})}{500} \times 100$$

- Percentage of pupae inhibition from each box

formula for Day 7

$$\% \text{ of pupae inhibition day 7} = \frac{500 - \text{pupae day 7}}{500} \times 100$$

formula for Day 14

$$\% \text{ of pupae inhibition day 14} = \frac{500 - (\text{pupae day 7} + \text{pupae day 14})}{500} \times 100$$

### Statistical Analysis

The data collected from the field trial were analyzed using ANOVA with a post hoc Tukey's Honestly Significant Difference (HSD) test. The statistical differences in every treatment group was considered significant if  $p < 0.05$ .

### Correction of % Mortality

To account for the natural mortality, Abbott's formula (Abbott, 1925) was used to correct the calculated percent larval mortality in the treatment groups.

## RESULTS AND DISCUSSION

The consistent demand for poultry products has spurred the steady growth of the Philippine poultry industry. The expansion of poultry

operations creates favorable conditions for house fly infestations, as these pests are attracted to organic substrates such as manure. House flies pose a significant threat to biosecurity as they are known vectors of pathogens such as *Salmonella* and *Campylobacter*. *Musca domestica* infestations are a public health concern that may ultimately lead to the closure of poultry operations. Chemical control methods are commonly used to manage infestations, but full efficacy is yet to be achieved. Pyriproxyfen is an example of an insect growth regulator used as a chemical control in the poultry industry. Abamectin, on the other hand, is a novel insecticide used against the larvae of *M. domestica*.

According to Taillebois et al. (2022), the use of binary mixtures may provide an effective approach to the rapid development of insecticide resistance. Combining two insecticides with synergistic effect reduces the amount needed for both components to produce comparable or enhanced results, thereby minimizing environmental impact, decreasing production costs, and delaying the development of insecticide resistance. As of now, there is limited data about the effect of abamectin-pyriproxyfen binary mixture on *M. domestica* larvae. This study investigated the efficacy of pyriproxyfen and the binary mixture of abamectin and pyriproxyfen on house fly larvae in a poultry farm at higher and lower concentrations over a 7 and 14-day period.

**Table 2.** Effect of the different treatments on larval reduction (%).

Interval	T1	T2	T3	T4	T5
Day 7	6.98 d	57.78c	75.63 b	76.88 b	88.49 a
Day 14	55.4 7c	69.37 b	85.38 a	84.89 a	88.41 a

Means with different alphabet among rows are significantly different ( $p < 0.05$ ) (T1 - distilled water; T2- pyriproxyfen low concentration; T3 - pyriproxyfen high concentration; T4 - abamectin-pyriproxyfen low concentration; T5 - abamectin-pyriproxyfen high concentration)

The average larval reduction across groups is shown in Table 2. To account for the natural mortality, Abbott's formula (Abbott, 1925) was

used to correct the average larval reduction percent of the treatments using the results from the control group. The data on day 7 showed that the high concentration abamectin-pyriproxyfen (T5) exhibited the most significant larval reduction among all treatments with an average reduction of 88.49%. The lower concentration abamectin-pyriproxyfen binary mixture (T4) exhibited a slightly higher reduction of 76.88% compared to 75.63% from the high concentration pyriproxyfen (T3), but the difference was not statistically significant. Both T4 and T3 however, achieved significantly higher reduction than the 57.78% reduction observed in the low concentration pyriproxyfen (T2). The control group treated with distilled water (T1) exhibited the least reduction among the treatments.

On day 14, larval reduction improved across all groups. Notably, the control group achieved 55.46% reduction from the 6.98% reduction on day 7. The relatively high larval reduction observed in the control group is likely attributable to natural developmental processes, including pupation and mortality under manure-based conditions, rather than treatment effects. In manure ecosystems, larval survival is influenced by factors such as density-dependent competition, microbial activity, and environmental conditions, which can result in substantial baseline reductions over time.

Despite this improvement, all the larvicide-treated groups still exhibited significantly higher larval reductions. Based on the results, the higher concentration of abamectin-pyriproxyfen binary mixture showed the most substantial efficacy against house fly larvae. The efficacy of abamectin against *M. domestica* larvae was investigated in a study by El Sheriff et al. (2022), wherein the insecticides abamectin, chlorfenapyr, and lambda-cyhalothrin, and their binary mixtures were evaluated under laboratory conditions. Abamectin was only next to chlorfenapyr in terms of individual performance; however, when combined with chlorfenapyr, it became the second most potent among the treatments, outperforming abamectin when used alone. In contrast, the mixture of abamectin and lambda-cyhalothrin was not recommended due to their antagonistic effect, as it exhibited the

least efficacy among the treatments. On the other hand, the IGR pyriproxyfen has been widely used in house fly control programs, but limited evidence is available regarding its synergistic activity with other chemicals against house fly larvae.

However, its potential as a component in binary mixtures was observed against *Aedes aegypti* larvae, wherein its combination with spinosad exhibited a synergistic activity on both larvicidal effect and adult emergence inhibition (Darriet & Corbel, 2006). At present, no published study has focused on the synergism between abamectin and pyriproxyfen. The results of this study suggest that the binary mixture was effective in reducing house fly larvae populations. The consistently high reduction observed from the higher concentration of the mixture indicates a positive interaction between the two components. However, further studies are needed to confirm the precise nature of this interaction.

**Table 3.** Effect of the different treatments on pupal inhibition (%).

Interval	T1	T2	T3	T4	T5
Day 7	75.20c	90.17b	94.57a	94.86a	95.75a
Day 14	68.11c	87.35b	93.93a	93.93a	95.26a

Means with different alphabets among rows are significantly different ( $p < 0.05$ ) (T1 - distilled water; T2- pyriproxyfen low concentration; T3 - pyriproxyfen high concentration; T4 - abamectin-pyriproxyfen low concentration; T5 - abamectin-pyriproxyfen high concentration)

Pupal inhibition was also assessed to provide a more comprehensive evaluation on the larvicide's efficacy over time. Table 3 shows the pupal inhibition observed across all groups at day 7, with T5 showing the highest inhibition followed closely by T4 and T3, all of which were significantly higher than T2. The trend remained consistent at day 14, with T5 maintaining the highest pupal inhibition and T1 remaining the lowest inhibition. Treatments T3, T4, and T5 showed significantly higher pupal inhibition compared to T2, while T2 remained significantly higher than T1. The decrease in percentage inhibition on day 14 is due to the cumulative calculation of the number of larvae that failed to pupate on day 7 and day 14. The results for

pupal inhibition indicate that both concentrations of the binary mixture were on par with the high concentration of pyriproxyfen.

The results of this study revealed that T5 consistently outperformed other treatments in terms of average larval reduction and pupal inhibition across both time points. However, since the differences were not statistically significant, particularly on day 14, where T3, T4, and T5 did not significantly differ, the results of the binary mixture do not reflect substantially greater efficacy. Therefore, the inclusion of abamectin in the binary mixture should be subjected to further investigation. While the observed performance of the binary mixture on larval reduction and pupal inhibition suggests that it could still be a valuable tool for integrated pest management, the effective concentration should be determined first to avoid environmental buildup and possible risk to non-target organisms.

Furthermore, future studies should also consider establishing a standardized threshold for the efficacy of house fly larvicide products. The 80% criterion developed by World Health Organization (WHO) for mosquito larvicides may serve as a reference until house fly- specific standards are established (Control of Neglected Tropical Diseases (NTD), 2005).

Khatun et al. (2015) documented the effectiveness of abamectin alone or in combination with emamectin benzoate, lambda-cyhalothrin, and lufenuron on *Bactrocera cucurbitae*. It was determined that abamectin alone can significantly reduce fruit infestation to 16.81% as compared to the control group. When used in combination with lambda-cyhalothrin, the amount of fruit infestation reduced to 13.40%. Therefore, the study concluded that the abamectin and lambda-cyhalothrin combination is an effective strategy to mitigate the emergence of fruit flies. Rashid et al. (2012) demonstrated that abamectin and spinosad is more effective in controlling the maturation of *L. sativae* compared to cyromazine. It was also determined that the combination of abamectin and oil can have a stronger insecticidal effect than abamectin alone due to its high LC50, or

the concentration of the substance that can cause 50% of deaths in a population.

A study by Harris et al. (2013) assessed the effect of pyriproxyfen on *Anopheles arabiensis* on fecundity. Results showed a reduction in fertility of the pesticide when female mosquitoes were blood-fed a day before exposure to pyriproxyfen. Another study by Chandel et al. (2016), determined the effect of the auto dissemination strategy of pyriproxyfen on *Aedes albopictus* which resulted in 29.7% to 30.8% mortality of pupae.

As of today, there are still minimal studies of the effect of abamectin and pyriproxyfen combination on flies. One study by Salehzadeh et al. (2020) studied the effect of abamectin, dinotefuran, imidacloprid, and pyriproxyfen-abamectin combination on *Blattella germanica*. The samples were more attracted to the abamectin and pyriproxyfen-abamectin combination and this was ingested in larger amounts as compared to the other insecticides. However, the dinotefuran and imidacloprid baits had a higher mortality rate compared to the insecticide containing abamectin.

## CONCLUSIONS

This study demonstrated the efficacy of the binary mixture of abamectin and pyriproxyfen against *M. domestica* larvae under poultry farm conditions, showing consistently high larval reduction and pupal inhibition at both observation periods. Although T5 showed the highest average efficacy, no significant differences were detected among T3, T4, and T5, indicating that the improvement was not statistically substantial. Future research should evaluate abamectin alone, test varying mixture concentrations to determine the optimal dose, and establish standardized efficacy thresholds for house fly larvicides. Laboratory-based studies with uniform larval stages and controlled environmental conditions are also recommended to reduce variability and improve experimental precision.

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