

Original article (Orijinal araştırma)

Insect growth regulators as chemosterilants: a study on house fly, *Musca domestica* L., 1758 (Diptera: Muscidae) populations in Türkiye¹

Kemosterilant olarak böcek büyüme düzenleyicileri: Türkiye'deki ev sineği, *Musca domestica* L., 1758 (Diptera: Muscidae) popülasyonları üzerine bir çalışma

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Abstract

The house fly, *Musca domestica* L., 1758 (Diptera: Muscidae), is a public health pest commonly found on animal farms, manure heaps, and garbage dumps. In agricultural and livestock areas, house flies are frequently exposed to pesticides used against various pests, which leads to the development of insecticide resistance. This resistance complicates their control and has prompted researchers and insecticide manufacturers to explore alternative control strategies and methods. This study examines the effects of insect growth regulators (IGRs), specifically diflubenzuron and pyriproxyfen, used as larvicides, on egg yield, egg-laying index, and egg-to-adult transition rates in six different house fly populations. These populations were collected and cultured from five Turkish provinces (Antalya, Bursa, Edirne, Gaziantep, and İzmir) between June 2020 and August 2021, as well as from a susceptible population provided by the World Health Organization (WHO). The adult house flies were fed sugar solutions (40%) containing either 5% or 10% concentrations of diflubenzuron and pyriproxyfen. Our findings show an 80% reduction in egg yield and a 90% reduction in adult emergence rates across all populations compared to the control group. Although exposure to IGRs significantly decreased egg area indices, it did not affect the egg to adult transition rate.

Keywords: Chemosterilant, diflubenzuron, *Musca domestica*, pyriproxyfen

Öz

Ev sineği, *Musca domestica* L., 1758 (Diptera: Muscidae), hayvan çiftliklerinde, gübre yığınlarında ve çöp alanlarında yaygın olarak bulunan bir halk sağlığı zararlısıdır. Tarım ve hayvancılık alanlarında, ev sinekleri çeşitli zararlılara yönelik kullanılan pestisitlere sıkça maruz kalmakta ve bu durum insektisit direncinin gelişmesine yol açmaktadır. Gelişen direnç, bu zararlının kontrolünü zorlaştırmakta ve araştırmacılar ile insektisit üreticilerini alternatif mücadele stratejileri ve yöntemleri aramaya yönlendirmektedir. Bu çalışma, larvasit olarak kullanılan böcek gelişim düzenleyicilerin (diflubenzuron ve pyriproxyfen) altı farklı ev sineği popülasyonunda yumurta verimi, yumurtlama indeksi ve yumurtadan ergin döneme geçiş oranı üzerindeki etkilerini incelemektedir. Bu popülasyonlar, Haziran 2020 ve Ağustos 2021 tarihleri arasında Türkiye'nin beş ilinden (Antalya, Bursa, Edirne, Gaziantep ve İzmir) toplanmış ve kültüre alınmış popülasyonlar ile Dünya Sağlık Örgütü (WHO) duyarlı popülasyonundan oluşmaktadır. Ergin ev sinekleri, %40 şeker içeren ve %5 ya da %10 oranında diflubenzuron ve pyriproxyfen içeren çözeltilerle beslenmiştir. Bulgularımız, kontrol grubuna kıyasla tüm popülasyonlarda yumurta veriminde %80 ve ergin hale geçme oranlarında %90 azalma olduğunu göstermektedir. Böcek gelişim düzenleyicilere maruz kalma, yumurta alan indeksini anlamlı düzeyde azaltmış olsa da yumurtadan ergine geçiş oranını etkilememiştir.

Anahtar sözcükler: Kemosterilant, diflubenzuron, *Musca domestica*, pyriproxyfen

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Introduction

The house fly *Musca domestica* L., 1758 (Diptera: Muscidae) is a widespread vector of numerous pathogens, including viruses, bacteria, and fungi in human-inhabited environments. House flies are found in manure heaps, garbage dumps, and livestock-raising areas (Sudagidan et al., 2022). This insect transmits pathogens that can cause significant diseases, such as cholera, dysentery, hepatitis, and tuberculosis, in humans and animals. In agricultural settings, such as barns and poultry houses, high densities of house flies can also negatively impact the production of meat, eggs, and milk (Cheng & Kesler, 1961). Furthermore, house flies contribute to the spread of *Escherichia coli* Escherich (Enterobacteriaceae: Enterobacteriales) bacteria, which can infect both animals and humans and potentially promote antibiotic resistance (Bakry et al., 2022).

Recent research has shown that house fly populations have developed significant resistance to various insecticides, including synthetic pyrethroids and neonicotinoids (Koc et al., 2012; Erdogan & Cetin, 2020; Abobakr et al., 2022; Polat, 2022). This rapid increase in resistance poses a significant challenge for effective management of these pests. Therefore, scientists are exploring various insecticides and alternative methods that are effective against house flies. Among these, insect growth regulators (IGRs) are particularly notable. IGRs are classified into four main groups: ecdysone agonists (e.g., Tebufenozide and Methoxyfenozide), classic IGRs (e.g., diflubenzuron, cyromazine, and novaluron), juvenile hormone analogs (JHA) (e.g., methoprene and pyriproxyfen), and anti-juvenile hormone agents (e.g., terpenoid imidazoles and fluoromevalonate). IGRs and JHAs are commonly used in vector control, while ecdysone agonists are mainly applied against agricultural pests, particularly larvae of the Lepidoptera and Coleoptera. However, despite their effectiveness, anti-juvenile hormone agents are rarely used in pest control due to their toxicity to non-target organisms (Tunaz & Uygun, 2004; Oz et al., 2024). IGRs containing JHA and other IGR elements are the most frequently used insecticides in this category.

Juvenile hormones (JHs) significantly influence various physiological and biochemical processes in insects during different life stages. In the larval stage, JHs suppress metamorphosis by delaying development, thus preventing the transition to the pupal and adult stages. In adult insects, JHs are essential for pheromone production, the development of accessory glands, and notably, in females for egg and ovarian maturation (Hu et al., 2019). Chitin, a polymer of N-acetylglucosamine, is an essential component of the cuticle and exoskeleton of insects. Exposure to IGRs, commonly used as larvicides in vector control, disrupts insect development by inhibiting the polymerization of N-acetylglucosamine, a process facilitated by chitin synthase enzymes. These developmental disruptions are typically evident during molting (Göktay & Kışmalı, 1990; Özparlak, 2003; Sankar & Kumar, 2023). The severity of developmental and molting disorders depends on the timing of IGRs exposure as well as the extent and duration of exposure and dosage. Mortality usually occurs during the larval or pupal stage. Late-stage larvae exposed to IGRs may survive to adulthood but often suffer from deformities in body structures, such as wings and legs, leading to reduced survival and mating success (Post et al., 1974; Merzendorfer, 2013; Ser & Çetin, 2016).

One of the integrated pest management strategies used against vector insects is the Sterile Insect Technique (SIT). SIT is an eco-friendly method employed in area-wide pest management to suppress or eliminate detrimental insect populations. This technique involves rearing large quantities of the target pest, sterilizing them, and releasing them into the environment to reduce their reproductive capacity. Sterility in insects can be induced by genetic modification, chemical means, or ionizing radiation (Parker & Metha, 2007). The first study on chemical sterilization was conducted by Goldsmith & Frank (1952), who used aminopterin, a folate antagonist, on *Drosophila melanogaster* Meigen, 1830 (Diptera: Drosophilidae), resulting in infertility by slowing ovarian development in females. Motivated by these findings, scientists and manufacturers have since then tested the chemosterilant effects of over 400 chemicals on various insect species, including IGRs.

In this study, we investigated the sterilizing, oviposition-reducing, and egg-to-adult transition inhibiting effects of two IGRs: diflubenzuron, a chitin synthesis inhibitor, and pyriproxyfen, a juvenile hormone analog, used as a larvicide in house flies, with the goal of assessing their chemosterilant and toxic effects on some Turkish house fly populations.

Materials and Methods

House fly populations

To collect house fly specimens for research, field collections were conducted between June 2020 and August 2021 in five Turkish provinces (Antalya, Bursa, Edirne, Gaziantep, and İzmir) spanning four geographical regions: the Mediterranean, Aegean, Southeastern Anatolia, and Marmara (including Southern Marmara and Marmara-Thrace) (Figures 1-2, Table 1). The five collection sites were selected for their diverse geographic locations as well as their distinct climatic and agricultural characteristics. This ecological variation enabled us to evaluate the efficacy of IGRs across a broad range of vector breeding habitats representative of different regions in Türkiye. In each province, house flies were captured from different areas of a cattle farm using sweep nets. Over 200 house flies were placed in 20x20x20 cm mesh cages and transported to the laboratory. During transport, their survival was ensured by providing moisture with water-soaked cotton balls, nourishment with milk-soaked cotton, and additional energy with sugar.



Figure 1. A map showing the provinces where house flies were collected from the field.



Figure 2. House fly collection using a sweep net on cattle farm.

Table 1. Locations of house fly collections from the field

City	District	Neighborhood	Location
Antalya	Kepez	Karşıyaka (Varsak)	36,99272°N-30,71738°E
Bursa	Nilüfer	Fadıllı	40,16571°N-28,72822°E
Edirne	Merkez	Bosna	41,62572°N-26,56585°E
Gaziantep	Oğuzeli	Çaybaşı	37,03459°N-37,52870°E
İzmir	Çeşme	Germiyan	38,31391°N-26,47018°E

House flies collected from different locations were transported to the Vector Ecology and Research Laboratory in the Biology Department at the Faculty of Science at Akdeniz University. There, they were housed in mesh cages for culturing. To promote healthy development and ensure the sustainability of the cultures, flies were provided with ample food (Wheat bran, milk, and sugar) and water. The laboratory conditions were meticulously controlled, maintaining a temperature of $26\pm 2^\circ\text{C}$, humidity levels of $60\pm 5\%$, and a photoperiod of 12 hours of light followed by 12 hours of darkness.

Chemosterilant activity tests

Pupae from field collections and WHO population were individually placed in Falcon tubes lined with cotton soaked in sugary water to prevent mating and facilitate sex determination upon reaching adulthood. We meticulously monitored the emergence of adult flies from the pupae daily, recording the emergence dates on the Falcon tubes to classify individuals as 1, 3, or 5 days old. Adults were grouped by their days post-emergence, with sex determined for each group. For the experiments, two virgin males and two virgin females from each group were placed into $20\times 20\times 20$ cm mesh cages to evaluate the effects of diflubenzuron and pyriproxyfen at final concentrations of 5% and 10%. The feeding solution was prepared by initially mixing 40% sugar water, into which the appropriate volume of IGR stock solution was added to achieve the desired concentrations. Cotton pads soaked in this IGR-sugar solution were placed in petri dishes inside the treatment cages (Howard & Wall 1995b; 1996b). A separate petri dish containing cotton soaked in milk was also placed in each cage. In the control cages, cotton pads soaked in 40% sugar water (without IGRs) and milk were similarly provided, ensuring that the only variable between groups was the presence of the active ingredients. All feeding materials were freshly prepared and renewed daily to ensure consistent and continuous exposure throughout the experimental period. Each treatment condition (including different age groups and concentrations) was replicated three times, as were the control groups, to ensure statistical robustness. The flies had continuous access to the test solutions, which were freshly prepared and renewed daily throughout the experimental period. This setup aimed to mimic potential field exposure through bait-based delivery methods.

Figure 3. General view of the $20\times 20\times 20$ cm cages used in laboratory experiments.

Table 2. Information on insect growth regulators used in the study (PubChem, 2019)

Name	Mode of action	CAS Number	IUPAC Name
Diflubenzuron	Chitin Synthesis Inhibitor	35367-38-5	<i>N</i> -[(4 chlorophenyl)carbamoyl]-2,6-difluorobenzamide
Pyriproxyfen	Juvenile Hormone Analog	95737-68-1	2-[1- (4-phenoxyphenoxy)propan-2-yloxy]pyridine

Egg counts for both the experimental and control groups were conducted using a digital stereo microscope and analysis software. We measured the dimensions-length and width-of the eggs to compute the egg area index. After counting and measuring, the eggs were placed into culture media composed of milk and bran to observe the transition rates from egg to adult. House flies that reached adulthood were then relocated to mesh cages equipped with cotton soaked in sugar, water, and milk. This setup facilitated the examination of sterility effects in the subsequent generation.

To closely mimic field conditions, large mesh cages measuring 120×120×120 cm were established for population monitoring over a 45-day period (Figure 4). Each cage was populated with 100 virgin male and 100 virgin female *M. domestica*. In the treatment cage (IGR group), flies were provided with a potential oviposition site consisting of a 5×10×20 cm plastic container filled with milk and Wheat bran mixture. In addition, separate plastic cups containing plain water, 40% sugar water, and 40% sugar water supplemented with 10% diflubenzuron or pyriproxyfen were placed inside the cage. In the control cage, the same setup was used, except that the sugar solution did not contain any IGRs. Each day, all containers and feeding materials were checked, and fresh solutions and food were added as needed to maintain surface moisture and ensure continuous exposure. Population densities in each cage were recorded at the end of the 45-day trial period to evaluate the effects of the treatments.



Figure 4. General view of the 120x120x120 cm cages used in field simulation experiments.

Data analysis

The evaluation of the data obtained from the tests was conducted using the following methods (Abbott 1987):

1. For the effect on egg production rates, the data were transformed into an index using the following equation:

$$\text{Egg production decrease rate (\%)} = \left(\frac{N_c - N_t}{N_c} \right) \times 100$$

N_c : Number of eggs in control N_t : Number of eggs in treatment

2. For the effect on emergence from eggs to adults, the data were transformed into an index using the following equation:

$$\text{Emergence decrease rate (\%)} = 100 - \left(\frac{E_c - E_t}{E_c} \times 100 \right)$$

E_c : Emergence rate from eggs of control individuals E_t : Emergence rate from eggs of individuals exposed to insecticide

3. For the effect on egg area index, the data were transformed into an index using the following equation:

$$\text{Egg area index decrease rate (\%)} = 100 - \left(\frac{A_c - A_t}{A_c} \times 100 \right)$$

A_c : Egg area index of control individuals A_t : Egg area index of individuals exposed to insecticide

The data garnered from both the field-collected populations and the WHO population were analyzed through variance analysis (ANOVA), focusing on percentage values and egg area index data to compare these two sets comprehensively. Additionally, the percentage data collected across various doses and different age groups were further examined using the Duncan multiple comparison test, with a significance level set at $p \leq 0.05$. This analysis was conducted using SPSS Statistics Base version 23 for Windows, facilitating a thorough and statistically sound comparison of the effects observed in the different experimental conditions.

The outcomes from the field simulation experiment were categorized according to the population density observed in the test cages after 45 days. The classification for chemosterilant efficacy based on the number of emerged individuals per cage was developed specifically for this study, without reference to any existing publication. It was designed using our own emergence data and population benchmarks established under controlled conditions. According to this classification: a population density of 0-500 individuals indicated very high chemosterilant efficacy; 500-1000 individuals represented high efficacy; 1000-1500 individuals indicated moderate efficacy; 1500-2500 individuals reflected low efficacy; and more than 2500 individuals corresponded to very low efficacy. This customized classification system enabled a clear and quantifiable assessment of the chemosterilant's impact on *M. domestica* population dynamics within the experimental framework.

Results and Discussion

Effects of diflubenzuron and pyriproxyfen on egg yield of house fly populations

Diflubenzuron and pyriproxyfen significantly reduced egg yields across all populations and age groups studied, at various doses. Specifically, a 5% concentration of diflubenzuron resulted in a reduction in egg yield ranging from 37% to 100%. Increasing concentration to 10% led to similar reductions, between 35% and 100%. For pyriproxyfen, a 5% concentration decreased egg yields by 31% to 100%, while 10% concentration resulted in reductions from 50% to 100%. Gaziantep population showed relatively lower inhibition effects compared to other populations. However, no formal resistance tests were performed, and thus, no definitive conclusions on resistance can be made. Further analysis showed that neither the age of the insects nor the dosage significantly affected the reduction in egg yield caused by either diflubenzuron or pyriproxyfen (Table 3).

In studies utilizing a 5% concentration of diflubenzuron, the transition from egg to adult stage showed a significant decrease, ranging from 56.84% to 100% in comparison to the control group across all tested populations. Similarly, trials with a 10% diflubenzuron concentration exhibited statistically significant reductions in adult emergence rates, from 91.96% to 100% compared to control groups across all populations (Table 4).

Trials involving a 5% concentration of pyriproxyfen led to reductions in the emergence from egg to adult stage ranging from 67.08% to 96.52%. In cases where no egg yield occurred, the emergence rates from egg to adult stage were not evaluated. The examination of the 10% concentration of pyriproxyfen on emergence rates revealed significant decreases, ranging from 83.95% to 98.06% compared to control groups across all populations (Table 4).

Table 3. Effects of diflubenzuron and pyriproxyfen on egg production

Insect Age (Days)	Chemical	Populations											
		Antalya		Bursa		Edirne		Gaziantep		İzmir		WHO	
		NEPF±SE	IR (%)	NEPF±SE	IR (%)	NEPF±SE	IR (%)	NEPF±SE	IR (%)	NEPF±SE	IR (%)	NEPF±SE	IR (%)
1	Diflu %5	15.67±6.44 AB a	87	50.33±2.76 C a	59	0.00±0.00 A a	100	26.00±5.44 B a	64	29.67±8.83 B a	71	30.50±4.09 B a	72
	Diflu %10	8.50±6.95 AB a	94	43.50±8.12 C a	64	0.00±0.00 A a	100	31.50±9.20 BC a	57	24.83±10.57 BC a	75	0.00±0.00 A a	100
	Pyri %5	12.83±10.49 AB a	91	61.67±14.26 D a	50	0.00±0.00 A a	100	23.67±6.16 BC a	68	32.83±2.99 C a	68	10.17±4.20 AB a	91
	Pyri %10	5.66±4.63 A a	96	40.16±10.07 B a	67	0.00±0.00 A a	100	36.67±7.22 B a	50	21.33±3.08 AB a	79	0.00±0.00 A a	100
	Control	138.33±6.30 B a		122.33±3.78 B a		108.83±6.78 AB a		73.00±2.96 A a		101.16±4.33 AB a		110.50±2.49 AB a	
3	Diflu %5	15.66±11.17 A a	88	49.66±5.20 B a	62	1.83±1.50 A a	98	49.66±7.99 A a	58	35.33±3.19 B b	59	7.83±6.40 A b	91
	Diflu %10	9.50±5.02 A a	64	27.33±7.67 B a	79	0.00±0.00 A a	100	6.83±5.59 A b	94	25.33±2.36 B a	70	4.66±3.81 A b	95
	Pyri %5	10.50±8.58 AB a	90	24.50±1.44 B b	81	0.00±0.00 A a	100	82.50±4.65 C b	31	23.50±7.18 B a	73	0.00±0.00 A a	100
	Pyri %10	4.00±3.27 A a	96	23.17±9.48 B a	82	0.00±0.00 A a	100	24.33±6.68 B a	80	16.33±7.00 AB a	81	0.00±0.00 A a	100
	Control	110.00±8.30 A a		131.33±3.31 A a		84.5±3.93 A a		119.00±11.96 A a		85.50±6.99 A a		91.16±2.12 A a	
5	Diflu %5	27.50±7.77 AB a	70	34.50±9.04 AB a	60	21.17±9.39 A b	71	28.00±11.47 AB a	56	62.16±3.61 B c	37	8.83±7.22 A b	90
	Diflu %10	22.16±1.83 AB a	100	16.33±6.69 A a	81	8.53±3.61 A b	88	41.17±6.33 B a	35	9.00±7.36 A b	91	4.33±3.54 A b	95
	Pyri %5	26.17±2.33 B a	71	33.67±9.53 BC b	61	10.83±6.73 A a	89	23.17±9.85 B a	63	51.50±6.49 C a	47	0.00±0.00 A a	100
	Pyri %10	23.83±4.37 AB a	73	29.00±5.41 AB a	66	0.00±0.00 A a	100	18.17±7.58 AB a	71	33.50±3.49 B a	66	0.00±0.00 A a	100
	Control	89.83±12.0 A a		86.33±7.08 A b		73.83±4.90 A a		63.17±3.89 A a		98.66±9.20 A a		84.33±5.60 A a	

Abbreviations: NEPF: Number of eggs per female; IR: Egg production inhibition rate compared to control; Diflu: Diflubenzuron; Pyri: Pyriproxyfen; SE: Standard Error.

* If the capital letters in a column are different, there is a statistical difference between the IGRs and doses on the same day ($p \leq 0.05$);

† If the lower-case letters in a column are the different, there is a statistical difference between populations ($p \leq 0.05$).

Table 4. The effect of pyriproxyfen and diflubenzuron on the transition from egg to adult stage

Insect Age (Days)	Chemical	Populations											
		Antalya		Bursa		Edirne		Gaziantep		İzmir		WHO	
		EATR±SE (%)	RR (%)	EATR±SE (%)	RR (%)	EATR±SE (%)	RR (%)	EATR±SE (%)	RR (%)	EATR±SE (%)	RR (%)	EATR±SE (%)	RR (%)
1	Diflu %5	8.42±2.81 A a	90.42	12.41±6.12 A a	85.84	-	-	1.93±0.86 A a	97.10	0.00±0.00 A a	100	4.17±0.81 A a	92.52
	Diflu %10	0.00±0.00 A a	100	2.29±0.12 B a	97.39	-	-	0.77±0.72 A a	99.08	0.00±0.00 A a	100	-	-
	Pyri %5	8.33±2.83 A a	90.52	6.67±0.41 A a	92.39	-	-	2.85±1.20 A a	95.69	4.17±2.8 A a	95.15	8.00±0.00 A	91.03
	Pyri %10	6.67±0.00 BC a	92.41	3.87±1.36 AB a	95.58	-	-	10.50±1.05 C a	83.95	1.67±0.46 A a	98.06	-	-
	Control	87.87±2.25 B a		87.62±3.20 B a		84.29±3.25 B a		66.20±2.55 A a		86.00±5.26 B a		89.23±2.29 B a	
3	Diflu %5	4.00±0.00 A a	95.56	4.62±1.30 A a	94.24	6.67±0.00 A	92.28	25.60±1.56 B b	56.84	4.80±2.86 A a	94.67	8.88±0.50 A a	90.51
	Diflu %10	3.50±1.69 B a	96.11	0.00±0.00 A a	100	-	-	2.50±1.77 AB a	98.90	2.00±0.00 AB a	97.78	4.00±0.00 B a	96.21
	Pyri %5	6.25±2.31 A a	93.06	5.00±0.00 A a	93.97	-	-	4.44±1.44 A a	92.51	14.74±5.05 B a	83.62	-	-
	Pyri %10	0.00±0.00 A a	100	0.00±0.00 A a	100	-	-	4.00±1.89 B a	93.26	5.56±1.41 B a	93.82	-	-
	Control	90.00±1.10 C a		82.86±5.80 B a		86.36±0.39 C a		59.31±3.54 A a		90.00±1.56 C a		87.80±2.20 C a	
5	Diflu %5	7.78±2.32 AB a	91.54	2.86±1.20 A a	96.68	9.1±4.33 B	89.82	0.00±0.00 A a	100	5.00±1.16 A a	94.60	0.00±0.00 A a	100
	Diflu %10	1.66±0.79 A a	98.20	6.86±2.79 A a	92.04	0.00±0.00 A a	100	5.60±1.66 A a	91.96	0.00±0.00 A a	100	0.00±0.00 A a	100
	Pyri %5	3.20±0.60 A a	96.52	3.63±1.13 A a	95.79	11.11±2.48 AB	87.57	22.66±6.73 B b	67.08	8.85±2.95 A ab	90.45	-	-
	Pyri %10	2.86±1.20 A a	96.89	1.82±1.36 A a	97.89	-	-	3.08±0.76 A b	95.33	4.50±4.59 A a	95.14	-	-
	Control	92.00±0.62 B a		86.15±3.41 B a		89.41±0.24 B a		68.87±2.64 A a		92.67±1.01 B a		88.40±0.28 B a	

Abbreviations: EATR: Egg to adult transition rate; RR: Egg to adult transition reduction rate; Diflu: Diflubenzuron; Pyri: Pyriproxyfen; SE: Standard Error.

^x If the capital letters in a column are different, there is a statistical difference between the IGRs and doses on the same day ($p \leq 0.05$);

^y If the lower-case letters in a column are the different, there is a statistical difference between populations ($p \leq 0.05$).

Effects of diflubenzuron and pyriproxyfen on adult emergence rates

The investigation into the egg-to-adult transition involved analyzing emergence rates, focusing on the inhibitory effects of diflubenzuron and pyriproxyfen. Our findings revealed that both substances significantly reduced the rates of emergence in all populations and age groups, with statistically significant reductions ranging from 56.84% to 100% compared to control groups. The analysis further indicated that the age of the insects and the dosage applied did not statistically impact the reduction in emergence rates to the adult stage across the studied populations.

Analysis of emergence rates from eggs of control group individuals revealed rates spanning from 59.31% to 92.67% across all populations. The Gaziantep population showed the lowest emergence rate, while the highest rates were found in both the Antalya and Izmir populations. In contrast, within the experimental groups treated with diflubenzuron and pyriproxyfen, emergence rates ranged markedly lower, from 0% to 25.60%. This substantial difference underscores that diflubenzuron and pyriproxyfen, across all concentrations and populations, averaged an efficacy in reducing the egg-to-adult emergence rate by over 80% (Table 4).

The eggs collected were assessed for their area indices by calculating the product of their lengths and widths. Our research identified statistically significant reductions in egg area index in 49 of the 72 experimental groups, encompassing six populations, two types of IGRs, two concentrations, and three age

groups. Meanwhile, no statistically significant difference in egg area index was noted in nine groups. In 14 of the test groups, egg yield did not occur, preventing the measurement of egg area index. The observed reduction in egg area index varied between 1% and 34%. Notably, this decrease in egg areas was found not to impact the rate of adult emergence.

For the field trial simulation, new custom cages measuring 120x120x120 cm were prepared, into which 100 male and 100 female adult house flies, confirmed as unmated, were released for a duration of one month to observe population development. The cages received consistent supplementation with a nutrient solution and active ingredients. To mimic breeding sites more accurately, milk-soaked wheat bran was layered over the existing nutrient medium. The outcomes of this trial, detailed in Table 5, showed that in cages treated with either diflubenzuron or pyriproxyfen, there was an approximate 90% decrease in population density relative to the control group. This significant reduction corroborates the findings from our laboratory experiments.

Table 5. Number of individuals in cages after diflubenzuron and pyriproxyfen application in field simulation trial

IGR	F ₁	F ₂	F ₃	Chemosterilant effect
Control	>1000	>2000	>5000	
Diflubenzuron %10+sugar water	< 500	100-250	<100	Very high
Diflubenzuron %10	< 250	<100	0	Very high
Pyriproxyfen %10+sugar water	<1000	250-500	<250	Very high
Pyriproxyfen %10	<500	100-250	<100	Very high

In the experiments conducted, eggs collected from various populations were incubated in a nutrient medium to observe their development into adult house flies. Upon emergence, the adults were transferred to mesh cages provided with milk, water, and sugar to assess their egg-laying capabilities. When egg-laying was observed, the new set of eggs was again placed in the nutrient medium to monitor hatching rates.

A noteworthy observation from this study was the performance of eggs from 5-day-old individuals of the İzmir population treated with 5% pyriproxyfen. Out of 50 eggs incubated in the nutrient medium, 5 adults emerged, collectively producing 69 eggs. However, to precisely evaluate their hatching rates, these eggs were initially placed on milk-soaked cotton to observe larval emergence, resulting in none of the 69 eggs hatching. Additionally, in the WHO population, eggs from 1-day-old individuals treated with 5% diflubenzuron transitioned to the larval stage without the need for the nutrient medium but subsequently perished within a day. In the case of the Antalya population, it was recorded that individuals treated with 5% pyriproxyfen at 1 day old and those treated with 10% pyriproxyfen at 3 days old failed to lay eggs upon reaching adult form.

Discussion and Conclusion

Recent research has shown that house flies have evolved resistance to many insecticides that target the nervous system (Khan et al., 2013; Ma et al., 2017; Khan et al., 2017; Khan, 2019; Abbas & Hafez, 2023). This resistance has prompted a shift among scientists and practitioners towards alternative pest control methods, resulting in a resurgence of interest in utilizing IGRs as a viable and effective strategy in recent times. Our literature review has revealed that research on the chemosterilant effects of IGRs on insects has predominantly focused on a single insect population (Howard & Wall 1995a, b, 1996a, b; Knapp & Cilek, 1988; Alam & Motoyoma, 2000; Caimi et al., 2002; Charmillot et al., 2002; Myers & Hull, 2003; Moya et al., 2010; Singh & Kaumar, 2015; Rhyne & Richards, 2020; Hasnain et al., 2023). In contrast, our study is distinctive in its approach, as it investigates house fly populations collected from five different provinces across four unique geographical regions of Türkiye. The inclusion of samples from such diverse regions broadens the geographical scope of this study to encompass five distinct areas. The insights gained from these varied regions are expected to offer valuable contributions to practical pest management.

The administration of diflubenzuron and pyriproxyfen at concentrations of 5% and 10% through food led to a statistically significant reduction in egg production compared to control groups across all studied populations and age categories. Notably, the Edirne, WHO, and Antalya populations exhibited significant declines in egg production, with rates of decrease surpassing those reported in prior research. Our review of the literature reveals that global studies in this domain have predominantly utilized trap-based methods to monitor population density, rather than direct egg counting, to evaluate the impact on egg production. Nonetheless, the outcomes of these international studies employing IGRs align with our findings, reinforcing the efficacy of IGRs in significantly reducing egg production among house fly populations.

Alam & Motoyoma (2000) explored the effects of cyromazine on house flies by incorporating concentrations of 500 and 1000 ppm into their drinking water and concluded that cyromazine did not significantly influence egg production, hatchability, pupal formation, or adult emergence. In contrast, our research demonstrated that diflubenzuron and pyriproxyfen markedly decreased both egg production and egg-to-adult transition rate. A key factor underlying this discrepancy is believed to be the dosages used; whereas Alam and Motoyoma administered cyromazine at 500 ppm (0.05%) and 1000 ppm (0.1%), our study employed considerably higher concentrations of 5% and 10%, respectively. Moreover, in our experiment, the IGRs were dissolved in a 40% sugar-water solution, diverging from Alam and Motoyoma's method of using tap water. This variation in solvent is also presumed to influence the attractiveness of house flies to the treated solutions.

In studies conducted by Myers & Hull (2003) on adult *Platynota idaeusalis* (Walker, 1859) (Lepidoptera: Tortricidae), significant inhibition of egg production and hatchability was observed when 90 ppm tebufenozide and 45 ppm methoxyfenozide were used as contact agents, with no notable difference in the chemosterilant effects between the two substances. This finding was echoed by Nisar et al. (2020), who treated adult *Bactrocera zonata* (Saunders, 1842) (Diptera: Tephritidae) with methoxyfenozide, fenoxycarb, lufenuron, pyriproxyfen, and buprofezin via food, with reductions in egg production by 37.5%, 34.8%, 30.9%, 25.1%, and 22.4%, respectively, and decreased egg hatchability of 65.9%, 67.3%, 67.8%, 72.2%, and 72.9%, respectively. They also observed decreases in sperm count of 29.4%, 25.8%, 22.2%, 17.6%, and 16.1% and in egg densities by 36.2%, 32.2%, 27.8%, 20.8%, and 19.6%. These findings corroborate our results, which also showed significant declines in egg production, egg size, and egg-to-adult transitions. Furthermore, Öz et al. (2024) examined the effects of diflubenzuron at 0.5%, 1%, and 2% concentrations on adult German cockroaches, *Blattella germanica* (L., 1767) (Blattodea: Ectobiidae) via both solid and liquid food, revealing decreased egg production and lower survival rates of nymphs from the treated eggs. This body of research supports the efficacy of IGRs in significantly affecting the reproduction and development of various insect species.

In research conducted by Hasnain et al. (2023) on the peach or melon fruit fly, *Bactrocera cucurbitae* (Coquillett, 1899) (Diptera: Tephritidae), egg production was analyzed using methodologies comparable to those in the present study. Exposure of *B. zonata* to concentrations ranging from 50 to 300 ppm/5 ml of pyriproxyfen, novaluron, lufenuron, buprofezin, and flubendiamide resulted in a 15% to 55% reduction in egg production compared to the control. In contrast, our study recorded inhibition rates ranging from 31% to 100% across various concentrations in four distinct populations. A major factor contributing to this discrepancy is the differing ecological roles of the target pests: *B. zonata* is primarily an agricultural pest, while house flies are considered public health pests. The use of chemical insecticides, such as fenoxycarb and related compounds, is substantially more widespread in agriculture than in public health pest management.

When evaluating the impact of diflubenzuron and pyriproxyfen concentrations on egg production across all studied populations, establishing a clear and statistically significant relationship between increasing concentrations and reduced egg output proved challenging. A review of the existing literature supports our findings, indicating that even a single exposure to insecticide can lead to reduced egg production. For example,

Alam & Motoyama (2000) observed no concentration-dependent effect on egg production in house flies orally exposed to 500 and 1000 ppm of cyromazine. Similarly, Hasnain et al. (2023) reported no direct correlation between increasing concentrations of various IGRs (pyriproxyfen, lufenuron, novaluron, buprofezin, and flubendiamide) and reduced egg production in *B. zonata*; intriguingly, higher concentrations often resulted in more pronounced chemosterilant effects.

Conversely, some studies have indicated that the chemosterilant effects may intensify at higher concentrations. For instance, Charmillot et al. (2002) reported a reduction in egg production in *Cydia pomonella* L. (Lepidoptera: Tortricidae) with prolonged exposure to fenoxycarb and found that even female-only contact with the IGR was sufficient to suppress egg laying. Such discrepancies in concentration-related outcomes may be attributed to the species-specific responses observed in different studies. Given the limited number of investigations on the effects of orally administered IGRs on egg production, our review of the literature suggests that a straightforward correlation between increasing concentrations and inhibition of egg production cannot be universally established. This variability likely depends on both the species involved and the specific active ingredients used.

In a study by Singh & Kaumar (2015), virgin female *Sarcophaga ruficornis* (Fabricius, 1794) (Diptera: Sarcophagidae) were topically treated with pyriproxyfen at concentrations of 50 and 100 µg/5 µl to evaluate its reproductive effects and impact on the F₁ generation. The treatment resulted in increased adult mortality, a significant reduction in fertility, and a 90% decrease in larval transition rates. In the F₁ generation, elevated mortality, reduced pupariation, and an increase in deformed adult emergence were observed, with a positive correlation between concentration and morphological abnormalities. These findings highlight the complexity of the relationship between IGR concentration and biological effects, suggesting that outcomes can vary considerably depending on both the species and the specific insecticide used.

In our investigation of the effects of diflubenzuron and pyriproxyfen on insect egg-laying capacity, we carefully designed the experimental setup by placing Falcon tubes containing cotton soaked in a 40% sugar solution individually for each group to prevent mating prior to their introduction into the test cages. The study included 72 experimental sets, covering six populations, two IGR types, two concentrations, and three age groups. Among these, 13 sets recorded the highest egg production from 5-day-old insects, while 5 sets involving the same age group showed the lowest production. No egg production was observed in 4 sets, and the remaining 2 sets showed no statistically significant difference among insect ages. Delayed exposure (associated with increased insect age) correlated with higher egg yields in 5-day-old individuals. When analyzing egg yield inhibition rates relative to the control group, the highest levels were observed in experiments with insects aged 3, 1, and 5 days, in that order. This pattern likely reflects the biological cycle of house flies, which reach sexual maturity within 24-48 hours and typically begin oviposition 3-4 days after emergence. These results suggest that the potential for reproductive system damage diminishes with delayed exposure. Our findings are consistent with previous studies, including those by Knapp & Cilek (1998), Howard & Wall (1995b), Alam & Motoyama (2000), and Caimi et al. (2002), which collectively support the observed trends.

In this study, egg area indices were calculated by multiplying egg length by their maximum width. The effect of IGRs on reducing the egg area index was assessed by statistically comparing values from experimental groups to those of the control groups. Among the 72 experimental groups established (covering six populations, two IGR types, two concentrations, and three age groups) statistically significant reductions in egg area index were observed in 49 groups. In contrast, 9 groups showed no significant differences, and in 14 groups, no egg production was recorded, making it impossible to calculate the egg area index. The analysis indicated that neither IGR concentration, insect age, nor duration of exposure had a statistically significant effect on the egg area index. Although some trial sets showed egg area indices higher than those of the control group, these increases were not statistically significant.

Furthermore, when examining individuals that transitioned from the egg stage to the adult stage from the measured eggs, it was noted that smaller adults emerged from eggs with a lower egg area index. Nonetheless, the egg area index, whether low or high, did not affect the transition to the adult stage, indicating a similar inhibition rate across transitions to the adult stage. This study's findings contrast with those of other studies indicating a positive correlation between body size and egg production under normal conditions. For instance, Barnard et al. (1995) found that in female house flies at low population densities, a 1 mg increase in average pupal mass led to an estimated 10.6 ± 1.5 additional eggs laid. This discrepancy highlights the complexity of understanding the effects of IGRs on insect development and reproduction and suggests that while IGRs can reduce egg production and modify egg size, these changes may not directly correlate with reproductive capacity or adult size under controlled conditions.

In our study, adult house flies were fed diflubenzuron and pyriproxyfen at concentrations of 5% and 10%, and the emergence of adults from the eggs they laid was monitored by seeding the eggs onto a diet of milk and Wheat bran. The emergence rates were then compared to those of the control groups to assess the transition from egg to adult stage. The results revealed statistically significant reductions in transition rates across all concentrations and age groups by the end of the experiments. When data from all trial sets were analyzed collectively, both IGRs were found to inhibit the transition from egg to adult by an average of over 90%, regardless of concentration or insect age. It was noted that the age of the insects and the duration of their exposure had no discernible impact on the inhibition of the transition. While the inhibition rates seemed to increase with higher concentrations, this trend was not statistically significant. Thus, administering adult house flies with food containing 5% or higher concentrations of diflubenzuron or pyriproxyfen led to a 90% reduction in the transition from egg to adult stage. Upon examining the milk and bran diet on which the eggs were laid, no unemerged pupae were found in the experimental groups, indicating that the chemosterilant effects primarily target the egg and larval stages. The suppression of pupation and adult emergence, especially at higher concentrations of JHA and CSI, suggests a transovarial effect, in which parental exposure to these IGRs adversely affects the developmental stages of their offspring, resulting in a marked disruption of population continuity.

The transovarial effects of novaluron, pyriproxyfen, azadirachtin, and buprofezin on adults of *Stephanitis pyrioides* (Scott, 1874) (Hemiptera: Tingidae) and *Teleonemia scrupulosa* Stål, 1873 (Hemiptera: Tingidae), as explored in studies by Joseph (2019; 2022), respectively, highlight the potential for IGRs to reduce offspring viability in insects significantly. These studies demonstrated that topical application of these substances could effectively decrease nymph emergence in a manner that is not concentration-dependent, indicating that even low concentrations can be as effective as the label concentration in producing transovarial effects.

An interesting aspect of IGR activity involves the behavior of insects such as house flies, which often deposit feces near their oviposition sites. This behavior may result in eggs and early larval stages being exposed to IGRs through fecal matter, potentially inhibiting egg hatching or adult emergence. Supporting this hypothesis, Ivei & Wright (1978) reported negligible levels of diflubenzuron in the eggs of treated *M. domestica* and *Stomoxys calcitrans* (L., 1758) (Diptera: Muscidae). In contrast, Medina et al. (2002) detected diflubenzuron in 20% of feces and 1% of eggs following topical application to *Chrysoperla carnea* Stephens, 1836) (Neuroptera: Chrysopidae). Additionally, Trostanetsky et al. (2015) observed inhibited egg hatching in *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) after adults were treated with novaluron attributing the effect to the presence of the compound in feces. When the eggs were transferred to a novaluron-free environment, hatching rates returned to normal. Our research, which involved seeding eggs onto a sterile milk and bran diet, suggests that the transovarial transfer of IGRs may play a significant role in inhibiting adult emergence rather than direct contamination of eggs or larvae via feces. This hypothesis is supported by Casana-Giner et al. (1999), who investigated the chemosterilant effects of ten IGRs on Mediterranean fruit fly, *Ceratitidis capitata* (Wiedemann, 1824) (Diptera: Tephritidae), demonstrating

that even a 1000 ppm concentration of lufenuron could completely inhibit egg hatching when administered to adult females, and similar sterilizing effects were also observed at varying concentrations and exposure durations across different IGRs and sexes. These findings collectively underscore the complex mechanisms by which IGRs can affect insect reproduction, suggesting that both direct exposure and environmental contamination via fecal matter can contribute to the observed reduction in offspring viability. Furthermore, they highlight the importance of considering both the method of IGR application and the behaviors of target insect species in pest management strategies.

To simulate field conditions, we designed large custom-made cages (120×120×120 cm) and introduced 100 virgin male and 100 virgin female *M. domestica* individuals into each cage. Throughout the month-long experiment, flies were provided with a consistent supply of food, water, and IGRs (diflubenzuron and pyriproxyfen). To mimic natural breeding conditions, a mixture of milk and bran was layered onto the existing diet. Our findings revealed a dramatic reduction (approximately 90%) in population density in treated cages compared to controls. This suppressive effect persisted in subsequent generations, with the F₂ population reduced by 90%, and near elimination observed by the F₃ generation. Although our trials were conducted in controlled settings, these results suggest strong chemosterilant potential in real-world applications as well. Supporting this, Howard and Wall (1996a) reported that traps containing a 50% sucrose, and 10% triflumuron solution achieved significant population suppression of house flies in Indian barns, reducing egg-to-adult emergence to below 1%. However, once traps were removed, fly populations quickly rebounded, highlighting the importance of sustained treatment in field environments. Taken together, our laboratory outcomes and previous field studies suggest that diflubenzuron and pyriproxyfen may be similarly effective in outdoor settings. We believe that further open-field evaluations are warranted to validate the long-term applicability of these IGRs in integrated house fly control programs.

Several studies have explored the chemosterilant potential of various IGRs against agricultural pests, highlighting differences in efficacy based on compound type, dosage, and delivery method. In a recent study on *B. cucurbitae*, Kainat et al. (2025) tested multiple IGRs, including lufenuron, pyriproxyfen, novaluron, buprofezin, and flubendiamide and found that lufenuron exhibited the highest efficacy, reducing fecundity by 68.4% and adult emergence by 70.97% at 300 ppm under laboratory conditions. Its performance was further validated in field trials using bait trap applications that led to substantial reductions in fruit fly damage. Similar success was reported in *C. capitata* control: Navarro-Llopis et al. (2004) achieved up to 80% population reduction in orchards using lufenuron n-based protein bait traps, and Alemany et al. (2008) documented over 63% reduction in female capture rates across a 300-hectare area with lufenuron traps. These findings emphasize not only the broader chemosterilant utility of IGRs but also position lufenuron as particularly effective among them. In line with this growing body of evidence, our study confirms that other IGRs, diflubenzuron and pyriproxyfen, also suppress reproductive parameters in *M. domestica*, supporting their potential role in integrated control strategies for synanthropic pests.

In conclusion, our findings clearly demonstrate the strong chemosterilant potential of diflubenzuron and pyriproxyfen against *M. domestica* under controlled laboratory conditions. Significant reductions were observed in egg production, egg area index, and the transition rate from egg to adult stage, with inhibition rates exceeding 90% across various concentrations, age groups, and populations. The consistency of these results across multiple experimental sets reinforces our confidence in the efficacy of these IGRs. While traditionally used as larvicides in both agricultural and public health settings, IGRs have recently attracted attention for their chemosterilant effects as well. To our knowledge, this is the first study in Türkiye to demonstrate the chemosterilant efficacy of these two IGRs specifically against house flies. Additionally, evidence pointing to transovarial effects and indirect exposure through fecal matter suggests that these compounds can disrupt population continuity beyond direct contact. While our cage system provided a reliable simulation of population dynamics, we acknowledge that the behavioral flexibility and ecological adaptability of house flies may be more pronounced in natural settings. Therefore, we believe that further open-field trials are needed to validate our results, which may pave the way for the broader application of IGRs in integrated house fly management programs.

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