# Choice of optimal biocide combination to control flies (Diptera: Muscidae)

# Mihaela Kavran<sup>1</sup>, Marija F Zgomba<sup>1</sup>, Aleksandra M Ignjatovic-Ćupina<sup>1</sup>, Sanja D Lazić<sup>1</sup>, Dušan V Petrić<sup>1</sup>

<sup>1</sup> University of Novi Sad, Faculty of Agriculture, Department for Plant and Environmental Protection, Novi Sad, Serbia

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# Abstract

**Introduction.** Flies – by feeding on decaying matter, human waste and food – have been implicated in the spread of numerous animal and human diseases. Excessive fly populations are generally associated with livestock units and domestic waste due to decaying organic matter. A large number of flies cause extreme disturbance in the behavior of the host, resulting in skin irritation, lesions, wounds, and secondary infections are likely to appear.

**Objective.** The aim of this study was to evaluate the effects of combined applications of larvicide (cyromazine) and adulticides (acetamiprid in formulation with pheromone and thiamethoxam) on the suppression of fly populations.

**Materials and methods.** The study was conducted on a pig farm. The piglet farms are one of the most favorable places for fly breeding. Three units were used for biocide applications and a fourth unit as the control where biocides were not applied. The monitoring of pre- and post-treatment of adult fly populations was carried out by glued cardboards. The cards were hung on metal rods above piglet's cage. This monitoring method served as a parameter for the estimation of biological effectiveness.

**Results.** The highest degree of fly control (88.4% mortality 8 days after treatment) was achieved when a combination of cyromazine and thiamethoxam was used. A biocide based on sex pheromone (Z)-9-tricosene + acetamiprid was the most effective on flies 3 days after biocide application, with a mortality rate of 69.1 %. Thiamethoxam achieved the highest reduction of flies 6 days after treatment, with 78.19% obtained mortality.

**Conclusion.** Biological efficacy of the applied biocides in combination ciromazine + thiamethoxam and thiamethoxam alone was justified.

# Keywords

Acetamiprid, cyromazine, fly control, thiamethoxam, (Z)-9-tricosene

# INTRODUCTION

The development of agriculture in a country can be evaluated by the percentage of livestock in total agricultural production, more developed agriculture having a higher share of livestock production. High fly populations may cause increased stress to the animals and farm workers, and create difficulties with neighbours and surrounding businesses. Flies could be responsible for significantly decreased meat and milk production on farms. Using cattle inventories and average prices for 2005 – 2009, and median monthly infestation levels, national losses are estimated to be \$360 million for dairy cattle, \$358 million for cow-calf herds, \$1.268 million for pastured cattle, and \$226 million for cattle on feed, for a total impact to US cattle industries of \$2.211 million per year [1].

A large number of flies cause extreme disturbance in the behaviour of the host, resulting in skin irritation, lesions, wounds, and secondary infections are likely to appear.

Flies play an important role in the transmission of pathogens, such as viruses, fungi and parasites in many regions of the world [2]. Adult flies harbour more than 100 pathogen species associated with more than 65 diseases of humans and animals [3, 4, 5]. The majority of these are *Shigella sp., Vibrio cholerae, Escherichia coli, Staphylococcus* 

Address for correspondence: Mihaela Kavran, University of Novi Sad, Faculty of Agriculture, Department for Plant and Environmental Protection, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia E-mail: mihaeladzombic@hotmail.com

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*aureus, Salmonela sp.* [6, 7, 8]. The porcine reproductive and respiratory syndrome (PRRS) virus, a member of the Arterivirus group, is an economically significant pathogen leading to a decline in pig reproduction and increased perinatal mortality of piglets. Moreover, the virus causes increased susceptibility to secondary infections of the respiratory and reproductive systems [9].

*Musca domestica*, a widely present species in farms of the region, can cause serious sanitary problems because of its high reproductive potential, feeding habits and abilities to disperse. Organic waste from intensive animal production provides excellent habitats for the growth and development of these insects [10].

The most effective fly control measures include both suppression of larvae (using larvicides) and adult forms (using adulticides). Fly control, which consists only of suppression of the adult form with conventional biocides, has only a shortterm effect [11, 12]. With the exception of chemical treatment, which is still the most important component of a strategy for controlling flies on farms, an essential preliminary step in fly population reduction is maintaining a high level of hygiene.

Organophosphates, carbamates, pyrethroids and Insect Growth Regulators have been used to control house flies [13, 14, 15], but improper use of biocides combined with the housefly's short life cycle, often less than seven days [16], and high biological potential, create conditions for the development of resistance to biocides. Additionally, resistance development in flies can be favoured by the frequent use of biocides with the same mode of action. Mihaela Kavran, Marija F Zgomba, Aleksandra M Ignjatovic-Ćupina, Sanja D Lazić, Dušan V Petrić. Choice of optimal biocide combination to control flies (Diptera: Muscidae)...

Most studies are based on the topical application of insecticides to the adults of the house fly [17], but only 15% of the total number of all fly forms in farms are adults [18]. Therefore, the use of adulticides, as the only way of fly control, is considered insufficient. This is one of the reasons why it is very important that the strategy of the fly control should include the use of larvicides.

**Objective.** The aim of this study was to evaluate the biocidal effects of combined treatment (lavicide and aduticide) and adulticides alone, and to compare the longevity of their impact (persistence) on fly abundance on a piglet farm. Furthermore, an effort was made to obtain a reduction in the fly population density under the nuisance threshold [19].

### MATERIALS AND METHODS

This study was conducted in a pig farm situated in Kamendin (45° 26' 0" N; 19° 49' 0" E), in the northern part of Serbia.

The pig farm where the study was conducted had several units of similar construction, of which four were used for study. The breeding units were of the same size, with the same number of pigs, and identical number and arrangements of channels for slurry and manure. Three units were used for biocide applications and a fourth unit as control, where biocides were not applied.

Conditions on farms where continuous process of cleaning and removing organic matter is not carried out on a regular basis provide optimal conditions for fly growth, and consequently, increased fly population. In the sites chosen for this study, during the entire process of biocide evaluation, there was overflow or leak of food on the floor. Such a situation, with the surfaces rich in organic matter, is conducive for egglaying and further larvae development. A lot of eggs, larvae and pupae were recorded in the treatment site.

If it is not possible to keep optimal hygiene on a farm and biocide application is inadequate/incomplete, a high level of fly control cannot be expected. The main obstacle to effective fly control could be the construction of the farm.

At the beginning of the biocide evaluation, the 35-day-old piglets occupied every unit. They remained in breeding units following 45 days. Thereafter, they were transferred into units for older pigs. When the piglets were removed, farm workers cleaned the whole construction.

**Monitoring method.** This consisted of glued cardboards  $(20 \times 20 \text{ cm})$ . The black-white side of a cardboard was stuck with a glue that remains effective for sufficient time to catch the flies that landed (stuck to the cardboard) during the entire monitoring time. Adults were counted on the glued side exclusively.

There were four glued cardboards in every piglet breeding unit. The cardboards were hung on the metal rods, 1 m above piglet's cage. All cardboards were replaced with the new ones at weekly intervals, and the used ones were brought to the laboratory for adult identification.

Monitoring started on 27 July 1012 and continued until the end of this experiment on 13 September 2012). This monitoring schedule gave an insight into the density of the pre- and posttreatments adult fly population. During the study period, the sampling was carried out eleven times. According to a literature survey of the region, the *M. domestica* nuisance threshold in pig farms has not been determined so far. The intention of the presented study was to have approximately 50% suppression of the initial fly population as a parameter for further biocide efficacy.

**Biocide treatments.** Larvicide treatments were performed by cyromazine (Neporex – Novartis, Switzerland) in slurry channels placed below the piglets' cages, while sex pheromone (*Z*)-9-tricosene in formulation with acetamiprid (Interflytox Köder – Inter-Harz GmbH, Germany) and thiamethoxam (Agita 10 WG – Novartis, Switzerland) were used for adult control. Treatments against adult flies were applied on surfaces usually frequented by flies, such as resting places around the windows, food boxes, containers and horizontal metal rods. The surfaces treated with particular biocide were of the same size in each unit.

*Treatment 1.* 400 g of Neporex dissolved in 5 l water was applied to the surface of manure in the slurry channels of the first breeding unit After 8 days, cyromazine effectiveness was recorded and application of the same product in combination with the treatment of adult flies with thiamethoxam was performed. The application of the prepared solution (400 g Agita 10 WG + 160 g sugar dissolved in 4 l water) was carried out on the most attractive surfaces for flies to land on (window sills, external sides of food boxes and the metal rods) – total surface area: 21.45 m<sup>2</sup>.

*Treatment 2.* In the second breeding unit, sex pheromone (*Z*)-9-tricosene + acetamiprid was applied on window sills, external sides of food boxes and the metal rods (400 g Interflytox Köder dissolved in 4 l water) – total surface of 21.45 m<sup>2</sup>.

*Treatment 3.* In the third breeding unit thiamethoxam was used (400 g Agita 10 WG + 160 g sugar dissolved in 4 l water) at the same places and on the same total surface of 21.45 m<sup>2</sup>.

*Statistical analysis.* Data collected during the study were analyzed with statistical programme Statistica 10. The degree of efficacy was compared by the least significant difference between treatments for the 95% confidence interval (0.05> p) and Duncan's multiple interval test (ANOVA). Effectiveness of biocide treatments were presented by the number of adult flies. Effectiveness was calculated by Henderson-Tilton's formula [20].

# RESULTS

Identification of the collected material showed that all specimens belonged to only one species – *M. domestica*.

Results obtained by the glued cardboard monitoring method demonstrated that the pre-treatment fly population in all breeding units were of the similar size (Tab. 1).

The fly population significantly decreased 2 days after biocides treatment in three breeding units, compared with control site, but did not differ between units (Fig 1)<sup>1</sup>. The situation was unchanged up to 8 days after biocides

<sup>1.</sup> Values followed by the same letter are not significantly different from each other at P = 0.05 (Duncan's multiple interval test).

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applications when the effects of (Z)-9- tricosene + acetamiprid become lower. The fly population was significantly reduced between 9-13 days after treatment in all breeding units, including the control unit.

Thiamethoxam and the combination based on cyromazine and thiamethoxam produced good results until 13 days after the treatment, after which the number of flies exceeded nuisance thresholds. Efficacy of biocide based on (Z)-9tricosene + acetamiprid was not acceptable 8 days after treatment. Fly density exceeded identified thresholds.

The combination of cyromazine and thiamethoxam kept the fly population at a low level longer than other treatments (Fig. 1). Although the number of flies recorded after 27 days was higher in comparison to previous evaluation terms, in both treated and untreated units, a statistically significant difference between all treatments and control was still evident.

The biocide based on sex pheromone (Z)-9-tricosene + acetamiprid was the most effective on flies 3 days after application, with the mortality rate of 69.1 % (Tab. 1).

Thiamethoxam achieved the highest reduction (78.19%) of flies 6 days after application, while the combination of cyromazine and thiamethoxam gave the best results 8 days after treatment, with a mortality rate of 88.4%.

Nine days after biocide application combination of larvicide (cyromazine) and adulticide (thiamethoxam) showed 64.18% mortality, which was the highest recorded at this evaluation time (Tab. 1). Both treatments with adulticide alone caused lower reduction of initial fly population.

#### DISCUSSION

The impact of (Z)-9-tricosene on the *M. domestica* in outdoor situations was studied by Hanley et al. [21]. The authors investigated the efficacy of the toxic targets that were prepared with a sugar/insecticide (Z)-9-tricosene mix. They concluded that the combination was not sufficiently attractive/detrimental to house flies to provide an effective and economic lure in outdoor situations. In the presented study, the biocide based on (Z)-9-tricosene + acetamiprid also did not produce the expected results. Suppression of the fly population was kept on low level for about one week, after which the fly population density considerably increased a week later.

Ibragimkhalilova and Eremina [22] confirmed the high toxicity of neonicotinoids on house flies. When some neonicotinoids used as baits with sugar it was found that thiamethoxam was more active than acetamiprid and imidacloprid [23].

Cyromazine has been studied since 1975 as a good potential for controlling manure-breeding flies [24]. Cyromazine

interferes in the normal development of fly larvae. Products based on cyromazine showed efficacy in fly control and demonstrated in different species of flies: *M. domestica* [25], *Musca autumnalis* [25], *Stomoxis calcitrans* [26], *Fannia femoralis* [27].

In the present study combination of thiamethoxam and cyromazine showed higher degree of effectiveness than thiamethoxam alone and biocide based on (Z)- 9- tricosene + acetamiprid.

Scientists thought that flies would not be able to create a resistance to cyromazine, but resistance to this IGR has been registered in the United Kingdom [28]. The resistance level was very low, but considering their biological potential and ability of flies to create fast resistance, cyromazine treatments should be carefully monitored and controlled.

Sheppard et al. [29] believe that the application technique could affect the rapidity of resistance creation. They stressed the importance of the technique for applying cyromazine, emphasizing that spray treatment, as opposed to administering cyromazine via the feed, results in a slower development of resistance. Moderate to high resistance could be developed using cyromazine through the feed [30].

Sánchez Arroyo [31] and Burgess [19] gave nuisance thresholds for flies in farms by different monitoring methods that were dissimilar to the glued cardboards used in presented study, and therefore cannot be compared.

Biocide effects, according to the selected value below 50% of initial population, were recorded in the sequence of evaluations that could demonstrate both the efficacy and persistence of the biocide used. Treatment with (Z)-9-tricosene + acetamiprid produced short-term effects. Only the third day evaluation of the treatment offered an acceptable suppression of the fly population density. Combination of cyromazine and thiamethoxam decreased fly population density under the threshold level gradually until the day 13 after the treatment. Thiamethoxam alone gave suppression higher than 50% on the second day after treatment, but had a shorter duration than the previous combination – until day 8, post-treatment application. The results of the present study reveal that all treatments, to some degree, can induce mortality of house flies.

### CONCLUSION

In conclusion, according to the above-mentioned results, a combination of cyromazine and thiamethoxam is preferred to sex pheromone (Z)-9-tricosene + acetamaprid and thiamethoxam. Sex pheromone (Z)-9-tricosene + acetamiprid should be used in combination with some IGR or other substances with larvicidal effects, or as a part of integrated pest management in fly control. The application of

Table 1. Fly mortality after biocide application evaluated by the glued cardboard monitoring method

Biocides	PT	Days after treatment															
		2		3		6		8		9		13		20		27	
	N	Ν	%	Ν	%	Ν	%	Ν	%	Ν	%	Ν	%	Ν	%	Ν	%
(z)-9-tricosene + acetamiprid	80	50	45.19	36	69.10	77	33.90	140	10.24	68	0	92	0	269	0	276	0
thiamethoxam	85	38	60.80	26	76.75	27	78.19	53	68.27	51	23.15	153	0	162	0	343	0
cyromazine + thiamethoxam	94	72	34.60	29	77.17	30	78.66	22	88.40	27	64.18	59	45.99	282	0	350	0
	PT – pre	e-treati	nent peri	od; N -	- total nu	mber o	of flies on	cardbo	oard; % –	perce	ntage of I	nortali	ty				

adulticides, as the only way of reducing the fly population, is not compatible with sound fly control strategy. The findings of this study demonstrate that the biological efficacy of the applied biocides in combination ciromazine + thiamethoxam and thiamethoxam alone, was justified.

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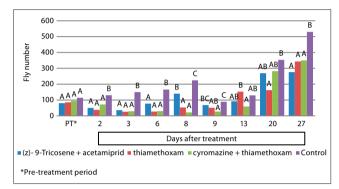


Figure 1. Suppression of fly population density with different biocides in pig breeding units

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