eISSN 2236-5257
10.46551/ruc.v24n1a3

EFFICACY ASSESSMENT OF LARVICIDES IN THE CONTROL OF FLIES IN A LAYING INDUSTRIAL AVIARY

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ABSTRACT

The eggs production through the confinement of chickens generates some inconveniences, among which the accumulation of manure, rich in organic matter that provides an environment conducive to the development and proliferation of *Musca domestica*. The objective of this work was to evaluate in a controlled environment the larvicidal efficacy in suppressing *Musca domestica* larvae. Material and methods used were the distribution of plastic trays exposed to oviposition in the farm sheds' vicinity, applying the treatments in a controlled environment.

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Recebido em	Aceito em	Publicado em
25/11/2021	28/03/2022	08/04/2022

Results shown that the isolated and intercropped treatments were effective in controlling the larvae. Concluding that the evaluation of the larvicidal efficacy of the Ciromanzine 50% principles; Triflumuron 48%; Ciromanzine 10%; and dolomitic limestone were effective in controlling larvae development in a controlled environment.

Key words: Cyromazine; Triflumuron; Dolomitic Limestone.

INTRODUCTION

Breeding laying hens in confinement is an important activity that drives the economy. In grange, poultry are kept in cages and there is a constant accumulation of feces. The availability of substrate rich in organic matter is favorable to the development of synanthropic Diptera, among them *Musca domestica*, considered the main pest of poultry facilities[1,2,3]. Exposure of poultries manure to humidity and high temperatures provides a suitable environment for *M. domestica*[4]. The short biological cycle of *M. domestica* allows the rapid colonization of the environment and the proliferations cause discomfort and stress for workers and poultry, and may reduce the value of poultry products[3]. Flies cause extreme changes in host behavior, promoting skin irritation, injury, wounds and susceptibility to secondary infections[5]. They are also known as vectors of over 100 pathogens such as protozoa, bacteria, helminths and viruses[6,7].

Controlling the proliferation of flies in grange is of paramount importance for the activity and the environment. Studies are performed to evaluate the used methodologies for the control and, consequently, the mitigation of negative effects on the environment[8,9]. The use of insecticide in the control of *M. domestica* is a commonly used alternative, the most effective measures include the suppression of larvae, with larvicides use and adulticides use for control of adult's population. The conventional control with the use of biocides for adult individuals has a short term of effect[10,11].

Globally, *M. domestica* has developed resistance to various classes of insecticides and some factors have contributed to its resistance capacity, including adaptability to environmental conditions, short biological cycle, cross-resistance potential and overuse of insecticides[12]. With the exception of chemical treatment, which is still the most important strategy control

component, the preliminary action for population reduction is to maintain a high level of hygiene[5].

The objective of the present study was to evaluate in a controlled environment the larvicidal efficacy of the principles, Cyromazine 50%; Triflumuron 48%; Cyromazine 10%; and dolomitic limestone with isolated and intercropped treatments, in sterilized manure samples, exposed to oviposition near the laying industrial aviary sheds in the Montes Claros region, Minas Gerais, Brazil.

MATERIALS AND METHODS

The research was conducted at the premises of the poultry laying enterprise in the municipality of Montes Claros, with location (16º53'48" south latitude and 43º56'58" west latitude), and on the premises of the Laboratory of Bioprospecting and Genetic Resources, and the Laboratory of Zoology, at Professor Darcy Ribeiro University Campus, at the State University of Montes Claros, Unimontes.

Manure collection and sterilization test

Manure samples were collected in sheds with high index of fly proliferation, in the Montes Claros laying grange, being taken to the Unimontes Bioprospecting and Genetic Resources Laboratory, and subjected to the sterilization testing by wet steam autoclaving per period of twenty minutes with a temperature of 121ºC. In the sequence, the samples were distributed in two plastic trays with dimensions of 30 cm x 20 cm x 7 cm deep, representing a surface area of 0.06 m² per tray.

Manure sterilization consisted of obtaining an initially sterile substrate, free from eggs and larvae, and with new conditions to test the larvicidal efficacy of the principles and dolomitic limestone in the suppression of *M. domestica* larvae.

The trays were placed in a room with a suitable environment for the development of *M. domestica* larvae, being covered with mesh to ensure isolation between the substrates and the environment, allowing the observation regarding the emergence of larvae and of adult flies. The effectiveness of manure sterilization was confirmed after 10 days, from daily and uninterrupted observations, with no emergence of larvae and emergent individuals.

Bioassay

New collections were performed in grange sheds and subjected to the sterilization conditions described above. The autoclaved substrate was humidified by mean of low-pressure sprays to achieve pasty consistency, similar to naturally found manure, being transferred to thirty-three plastic trays with the dimensions described above, totaling a surface area of 1.98 m².

Then the trays were randomly distributed and exposed to oviposition for a period of 24h, near the nuclei that presented the highest *M. domestica* infestation, with a total area of 10,38[illegible] m². After 24 hours of exposure, the trays were collected and taken to the room under conditions free of external contamination.

Treatments

After identification, the following treatments were applied separately and in consortium: T1 control treatment (without product use). Isolated treatments: T2 Cyromazine 50%; T3 Triflumuron 48%; T4 Cyromazine 10%; T5 Dolomitic limestone. Intercropped treatments: T6 Cyromazine 50% + Triflumuron 48%; T7 Cyromazine 50% + Cyromazine 10%; T8 Cyromazine 50% + Dolomitic limestone; T9 Triflumuron 48% + Cyromazine 10%; T10 Triflumuron 48% + Dolomitic limestone; T11 Cyromazine 10% + Dolomitic limestone, with three replicates per treatment.

For treatment's preparation, the manufacturers' guidelines were followed with experiment scale adjustments. The dilution of the principles for isolated and intercropped applications observed the following order: Cyromazine 50% = 5g / liter of water; Triflumuron 48% = 10 ml / liter of water; Cyromazine 10% = 1g / liter of water. In the isolated treatments were administered 12 ml of the dilution of the respective principle per tray, in the intercropped treatments were administered 6 ml of the dilution of each principle respectively per tray, using low-pressure sprays. Dolomitic limestone was applied in a thin layer of 60 g over the manure in the isolated treatment and 30 g in the intercropped treatment respectively (Table 1). After applying the treatments, the trays were kept in isolation from the internal and external environment of the room.

Table 1. Control treatments, isolated, intercropped and their respective dilution and application.

Treat.	**Control/Isolated/Intercropped**	**Dilution**	**Application**

T1	[1]Control	*0	**0
T2	[2]Cyromazine 50%	5g / liter	12 ml
T3	[2]Triflumuron 48%	10 ml / liter	12 ml
T4	[2]Cyromazine 10%	1g / liter	12 ml
T5	[2]Dolomitic Limestone	*0	60g
T6	[3]Cyromazine 50% + Triflumuron 48%	[1]0	6 ml + 6 ml
T7	[3]Cyromazine 50% + Cyromazine 10%	[1]0	6 ml + 6 ml
T8	[3]Cyromazine 50% + Dolomitic Limestone	[1]0	6 ml + 30g
T9	[3]Triflumuron 48% + Cyromazine 10%	[1]0	6 ml + 6 ml
T10	[3]Triflumuron 48% + Dolomitic Limestone	[1]0	6 ml + 30g
T11	[3]Cyromazine 10%+ Dolomitic Limestone	[1]0	6 ml + 30g

[1] = Control treatment; [2] = Isolated treatment; [3] = Intercropped treatment; *0 = Undiluted; **0 = without application; [1]0 = Dilution obtained from isolated treatments.

Substrate Observation and Screening

Over the next 10 days, it was collected daily at the same time, after mixing and homogenization of the substrate, randomly, one sample in each of the thirty-three trays. The samples were conducted to Unimontes zoology laboratory for substrates' observation and screening.

The samples were analyzed in a stereoscopic magnifying glass, being quantified the number of eggs and larvae of 1st, 2nd and 3rd stages. Seven days after the first application of the treatments, it was performed the second application, according to the time interval usually practiced in the larval control actions of the enterprise itself, following the same procedures of the first application. Once the screening and observation of the substrates were finished, the daily monitoring was carried out with the objective of monitoring the emergence of pupae and emerging individuals trapped in the trays through the screens.

Statistical analysis

It was used the completely randomized design in triplicate. After obtaining the results, as the data were obtained by counting, it was used the square root transformation. After verifying the

statistical assumptions, the data were submitted to analysis of variance. If there being significance of effects of the treatments by the F test, the Scott-Knott test means were grouped at a 5% significance level. For data analysis was used the R Core Team[13] statistical package (Development Core Team, 2013).

RESULTS

294 eggs were quantified; 4,717 1st stage larvae; 3,325 2nd stage larvae; 2,073 3rd stage larvae; and 377 flies (Table 2).

Tabela 2. Counting eggs, 1st, 2nd and 3rd stage larvae and flies with different treatments.

Treat.	Eggs	L1	L2	L3	Flies
T1	30	985	612	515	149
T2	55	576	460	228	17
T3	28	569	363	152	31
T4	77	801	547	369	16
T5	18	345	273	136	14
T6	54	585	430	144	37
T7	5	278	178	220	40
T8	3	223	135	98	15
T9	18	174	151	99	35
T10	3	83	67	41	11
T11	3	98	109	71	12
Total	**294**	**4.717**	**3.325**	**2.073**	**377**

T1 Control; T2 Cyromazine 50%; T3 Triflumuron 48%; T4 Cyromazine 10%; T5 Dolomitic Limestone; T6 Cyromazine 50% + Triflumuron 48%; T7 Cyromazine 50% + Cyromazine 10%; T8 Cyromazine 50% + Dolomitic Limestone; T9 Triflumuron 48% + Cyromazine 10%; T10 Triflumuron 48% + Dolomitic Limestone; T11 Cyromazine 10%+ Dolomitic Limestone.

Estimates of mean squares for the number of eggs, 1st, 2nd and 3rd stage larvae, and flies, evaluated according to the isolated and intercropped treatments, indicated the absence of significant effect of the "Treatment" variation source at the 5% level of probability ($p > 0.05$)

for the number of eggs and presence of significance at the level of 1% ($p \leq 0.01$) for the number of 1st, 2nd and 3rd stage larvae and flies in the analysis of variance respectively (Table 3).

Table 3. Summary of the mean squares for the number of eggs, 1st, 2nd and 3rd stage larvae and flies.

FV	GL	Eggs	L1	L2	L3	Flies
Treatments	10	4,27 NS	49,62 **	31,69 **	23,18 **	5,99 **
Residue	22	2,26	10,74	5,86	1,68	0,66
CV (%)	-	56,37	30,08	25,77	17,39	25,42

* Estimates of the mean square followed by NS and ** indicate the absence of significant effect of the "Treatment" variation source at the 5% probability level ($p > 0.05$) and presence significance at the 1% level ($p \leq 0, 01$) by the F test, respectively.

In the multiple comparative analysis of the means by the Scott-Knott test, the intercropped treatments: T10 Triflumuron 48% + Dolomitic limestone; T11 Cyromazine 10% + Dolomitic limestone presented statistically significant differences in relation to the other treatments regarding the average number of 1st, 2nd and 3rd stage larvae and flies. The average number of flies found in the control treatment was up to 10 times higher compared to other treatments (Table 4).

Table 4. Multiple comparative analysis of means of the number of eggs, 1st stage larvae, 2nd stage larvae, 3rd stage larvae and flies from different treatments.

Treat.	Eggs	L1	L2	L3	Flies
T1	10,00 a	328,33 a	204,00 a	171,67 a	49,67 a
T2	18,33 a	192,00 a	153,33 a	76,00 b	5,67 b
T3	9,33 a	189,67 a	121,00 a	50,67 c	10,33 b
T4	25,67 a	233,67 a	182,33 a	123,00 a	5,33 b
T5	6,00 a	115,00 b	91,00 a	45,33 c	4,67 b
T6	18,00 a	195,00 a	143,33 a	48,00 c	12,33 b
T7	1,67 a	92,67 b	59,33 b	73,33 b	13,33 b
T8	1,00 a	74,33 b	45,00 b	32,67 c	5,00 b
T9	6,00 a	58,00 b	50,33 b	33,00 c	11,67 b

T10	1,00 a	27,67 b	22,33 b	13,67 d	3,67 b
T11	1,00 a	32,67 b	36,33 b	23,67 d	4,00 b

* Means followed by equal letters do not differ statistically at the 5% level by the Scott-Knott test.

The sterilized manure provided an attractive and effective environment regarding the oviposition in the nuclei with the highest incidence of flies, presenting expressive numbers of eggs, larvae and flies. The collection, screening and observation of samples that were previously expected to close in 15 days, extended for another 14 days, totaling an interval of 29 days. It was estimated that pupae would appear between 7 and 10 days early in the experiment, however, it occurred only 16 days after initiation.

DISCUSSION

In the study of cyromazine resistance in populations of *M. domestica* collected in grange of five Brazilian cities: Petrópolis - RJ, Promissão SP, Ibiuna - SP, Monte Mor -SP and Montes Claros - MG, indicated the presence of Cyromazine resistance in Petrópolis, Montes Claros and Promissão populations, the Ibiúna and Monte Mor populations showed sensitivity[14].

In this work the search for alternatives to fly control, with application of the principles Cyromazine 50%; Triflumuron 48%; Cyromazine 10%; and dolomitic limestone in an isolate and intercropped way presented satisfactory response in controlled environment, with efficiency in suppressing *M. domestica* larvae and emerging adult individuals, and population reduction. These results are similar to the comparative study of biological, chemical and cultural control strategies about *M. domestica* resistant to Cyromazine in a poultry house in La Matanza, Buenos Aires - Argentina, where the combined use of limestone (cultural control) and Cyromazine 50 % reduced faster the fly population in relation to the combination of Cyromazine 1% and limestone[15]. In our study, the combination of Cyromazine 50% and dolomitic limestone presented reduction in the number of flies; however, the combination of Cyromazine 10% and dolomitic limestone presented a more significant reduction in the number of flies.

In the ideal combination study of biocides to control flies conducted on a pig farm in Kamendin, northern Serbia, the application of the combined principles Cyromazine and

thiamethoxam and thiamethoxam alone presented biological efficacy, thiamethoxam presented a reduction of 78.19% of the flies 6 days after application and the combination of Cyromazine and thiamethoxam presented better results 8 days after treatment, with a mortality rate of 88.4%, presenting a significant reduction in the fly population between 9 and 13 days after treatment[5]. In our work the treatments with isolated and intercropped application of the principles Cyromazine 50%; Triflumuron 48%; Cyromazine 10% has been shown to be effective in suppressing larvae and emerging adult individuals, with the consequent population reduction of flies at an interval of 29 days after treatment, presenting significant results. It has been shown that the intercropped use of the treatments: T10 Triflumuron 48% + dolomitic Limestone; T11 Cyromazine 10% + dolomitic limestone presented statistically significant results compared to other treatments.

CONCLUSION

The evaluation of the larvicidal effect of the principles and dolomitic limestone in a controlled environment presented efficiency in suppressing *M. domestica* individuals.

ACKNOWLEDGEMENTS

The authors thank the UNIMONTES Postgraduate Program in Biotechnology (PPGB), the Minas Gerais State Research Support Foundation (FAPEMIG) and Somai Nordeste S / A for their logistical and financial support.

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