ROCIO YANET FARRO BARBARÁN

INSECTICIDE RESISTANCE AND ITS EFFECTS ON THE BIOLOGICAL AND REPRODUCTIVE OUTPUTS OF TWO FIELD COLLECTED POPULATIONS OF THE HOUSE FLIES *Musca domestica* L. (DIPTERA: MUSCIDAE)

LAVRAS – MG
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Dissertation presented to the Universidade Federal de Lavras, as part of the requirements of the postgraduate program in Entomology, to obtain title of Master.

Prof. Dr. Khalid Haddi

Advisor

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RESISTÊNCIA A INSETICIDAS E SEUS EFEITOS NOS PRODUTOS BIOLÓGICOS E REPRODUTIVOS DE DUAS POPULAÇÕES COLETADAS EM CAMPO DA MOSCAS DOMÉSTICAS *Musca domestica* L. (DIPTERA: MUSCIDAE)

Dissertation presented to the Federal University of Lavras, as part of the requirements of the postgraduate program in Entomology, to obtain title of Master.

APPROVED on May 27, 2022

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LAVRAS-MG
2022

DEDICATE

To my parents, Jorge and Vilma, for their love, example, and encouragement throughout life;
My dear brother and sisters, Jorge, Verónica, and Lourdes, always companions;
My nephews and nieces for whom I hope to be a positive role models.
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To God, for giving me the opportunity each day to get up strong to contribute through my work to science and society, with love, service, and gratitude.

OFFER

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GENERAL ABSTRACT

The housefly (*Musca domestica* L., Diptera: Muscidae) is associated with economic losses by contaminating poultry products. Occurring cases of control failure and selection of resistant populations by synthetic insecticides misuse. Requiring studies of the physiological mechanisms of this resistance to determine the appropriate solutions to return the balance to that population, hence, the main objective of this work was to evaluate the effects of insecticide exposure in two populations of *M. domestica* to ten synthetic insecticides in their biological and reproductive parameters, to improve the integrated pest management for those populations. For this purpose, this dissertation was structured into two chapters. CHAPTER I consists of the comparative assessment of the susceptibility to insecticides by dose-response curves between a population of adult flies, one with no history of insecticides (POPUFLA) and another with a history of insecticide application (POPNEP), as well as analyzing the mortality of third-stage-larvae and pupae, at the maximum field recommended dose (MFRD). The results showed that the resistance ratios (LC_{50} POPNEP/ LC_{50} POPUFLA) were 20.5; 13.1; 12.4 and 12.2 folds for deltamethrin, thiamethoxam, lambda-cyhalothrin + thiamethoxam and lambda-cyhalothrin indicating potential insecticides' resistance occurrence in POPNEP. Chlorfenapyr, imidacloprid, cyantraniliprole, chlorpyrifos, and spinetoram showed control effectiveness for the two populations. Larval and pupal mortalities ranged from 50 to 100% in the two populations for all the insecticides including azadirachtin. In CHAPTER II, an age-stage, two-sex, life table was used to evaluate insecticide resistance's potential effects on flies' biological parameters. Significant statistical differences were found for the egg, and third larval stage durations (H= 47.86; *df* =1; *P* <0.001), which prolonged the total development of POPNEP (15.71 ± 0.38 days) concerning POPUFLA (15.69 ± 0.25 days). Also, at the POPNEP females presented a higher intrinsic rate of increase (r_m=0.197 females/ female/day) but, a longer mean generation time (T=33.21 days) compared to POPUFLA (r_m=0192; T= 31.36). The overall findings suggest that the POPNEP is an insecticide-selected population that developed a fitness cost in terms of long development times but also has a reproductive advantage over the susceptible population POPUFLA. Both the encountered developmental cost and reproductive advantage linked to insecticide resistance should further be studied and verified under field conditions.

Keywords: *Musca domestica*. Integrated pest management. Toxicology. Fertility life table.

RESUMO GERAL

A mosca doméstica (*Musca domestica* L., Diptera: Muscidae) está associada a perdas econômicas por contaminar produtos avícolas. Ocorrendo casos de falha de controle e seleção de populações resistentes por uso indevido de inseticidas sintéticos. Requerendo estudos dos mecanismos fisiológicos dessa resistência para determinar as soluções adequadas para devolver o equilíbrio a essas populaçãoes, portanto, o objetivo principal deste trabalho foi avaliar os efeitos da exposição de inseticidas em duas populações de *M. domestica* a dez inseticidas sintéticos em seus parâmetros biológicos e reprodutivos, para melhorar o manejo integrado de pragas para essas populações. Para isso, a presente dissertação foi estruturada em dois capítulos. O CAPÍTULO I consiste na avaliação comparativa da suscetibilidade a inseticidas por curvas dose-resposta entre duas populaçãoes de moscas adultas, uma sem histórico de inseticidas (POPUFLA) e outra com histórico de aplicação de inseticidas (POPNEP), além de analisar a mortalidade de larvas de terceiro estágio e pupas, na máxima dose recomendada em campo (MDRC). Os resultados mostraram que as razões de resistência (LC_{50} POPNEP/LC_{50} POPUFLA) foram de 20,5; 13,1; 12,4 e 12,2 vezes para deltametrina, tiametoxam, lambda-cialotrina + tiametoxam e lambda-cialotrina indicando potencial ocorrência de resistência a inseticidas em POPNEP. Clorfenapir, imidaclopride, ciantraniliprole, clorpirifós e espinetoram mostraram eficácia de controle para as duas populações. As mortalidades de larvas e pupas variaram de 50 a 100% nas duas populações para todos os inseticidas, incluindo azadiractina. No CAPÍTULO II, uma tabela de vida de dois sexos, por estágio de idade, foi usada para avaliar os efeitos potenciais da resistência a inseticidas sobre os parâmetros biológicos das moscas. Diferenças estatísticas significativas foram encontradas para a duração do ovo e do terceiro estágio larval (H= 47,86; *df* =1; *P* <0,001), o que prolongou o desenvolvimento total da POPNEP (15,71 ± 0,38 dias) em relação à POPUFLA (15,69 ± 0,25 dias). Além disso, no POPNEP as fêmeas apresentaram maior taxa intrínseca de crescimento (r_m=0,197 fêmeas/fêmea/dia), porém, maior tempo médio de geração (T=33,21 dias) em relação ao POPUFLA (r_m=0192; T= 31,36). Os resultados gerais sugerem que a POPNEP é uma população selecionada por inseticida que desenvolveu um custo de aptidão em termos de longos tempos de desenvolvimento, mas também tem uma vantagem reprodutiva sobre a população suscetível POPUFLA. Tanto o custo de desenvolvimento encontrado quanto a vantagem reprodutiva ligada à resistência a inseticidas devem ser mais estudados e verificados em condições de campo.

Palavras-chave: *Musca domestica*. Manejo integrado de pragas. Toxicologia. Tabela de vida e fertilidade.

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1 GENERAL INTRODUCTION

The order Diptera is one of the most diverse of insects. These insects are not only diverse in species but also in richness, ecological habits and also have an economic importance; being the *Musca domestica* Linnaeus (1758) the most recognized dipteran worldwide (RESH; CARDÉ, 2009).

Flies have medical and veterinary interest, are cosmopolitan, synanthropic (adapted to the human environment), inhabiting both urban and rural houses (BENNETT, 2006; KEIDING, 1986), being an important incidental vector of several pathogenic organisms such as viruses, bacteria, rickettsia, protozoa and helminths (PALACIOS et al., 2009). They are also associated with economically important diseases of animals. Therefore, they are of considerable importance from a hygienic and epidemiological point of view (ZAHRADNÍK; CHVALA, 1990). In animal production such as poultry farming, flies cause continuous irritation by feeding on the animal's secretions from the eyes, nose and wounds. Such discomfort affects the feeding process of the bred animals, significantly reducing the production and consequently leading to economic losses to the producer (MONTILLA-CORONADO, 2017).

Since 2011, Brazil occupies the seventh position among the largest chicken egg producers in the world. In 2021, egg production reached around 54.5 billion units, which represents an increase of 1.8% higher than that registered in the previous year (ASSOCIAÇÃO BRASILEIRA DE PROTEÍNA ANIMAL, 2018). Flies can be a nuisance not only for animal producing units but also for the workers and the neighborhood around the farms. When the number of insects exceeds acceptable levels, measures must be taken to control the population, avoiding nuisances and lawsuits (PASCALE; KUNZ, 2011). Recently, the small city of Nepomuceno was a local example of this situation, where flies have become an uncontrollable pest, not only for the animal producing units, becoming increasingly resistant to control strategies, but also for the community. This has led to legal, social and economic problems (EPTV 2, 2019). In the light of such situation, the present research tried to understand the underlying reason of such flies' infestations aiming to help with the integrated management of this pest at local and regional levels.

2 OBJECTIVES

2.1 General objective

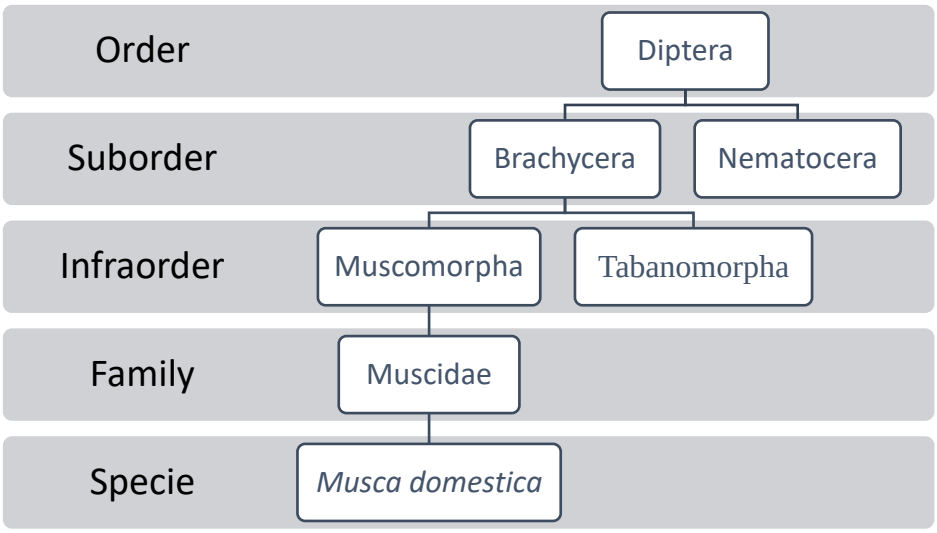
The general objective of the present research was (i) to assess the insecticides resistance status of two field colected populations of *Musca domestica* and (ii) to investigate the effects on the flies biologial and reproductive traits of any potential resistance with a general perspective of (iii) improving the integrated management strategies of this pest.

3 BIBLIOGRAPHICAL REVIEW

3.1 Systematic and Taxonomy of *Musca domestica*

The Diptera order is known to have as a common characteristic a pair of functional membranous wings and a pair of modified wings called halteres that are club-shaped and that serve to stabilize and generate rapid movements during flight while a few of them are wingless, being the most significant representatives the mosquitoes, fruit flies, midges, black flies, and house flies (CEDRIC GUILLOTT, 2005; RESH; CARDÉ, 2009). It is estimated that 10% of the described diversity of the world belongs to this taxon (PAPE; EVENHUIS, 2021) . As shown in Figure 1, the Diptera order is divided into two suborders: Brachycera and Nematocera. Two medical and veterinary important infraorders emerge from Brachycera, the Muscomorpha and the Tabanomorpha. Finaly inside the Muscomorpha is inserted the Muscidae super family, to which *Musca domestica* belongs, the object of this study (BRITTO et al., 2008; TRIPLEHORN; JOHNSON; BORROR, 2005).

Figure 1 – Taxonomic hierarchy of the order diptera with the clades of interest for this research.



3.2 General characteristics of *Musca domestica*

Musca domestica is the most recognized dipteran worldwide (DE CARVALHO et al., 2005) with medical and veterinary interest. This fly is cosmopolitan, highly synanthropic, being an important mechanical vector of more than 100 associated pathogenic organisms (SANCHEZ-ARROYO, 2011) and implicated in their transmission to humans and livestock(PALACIOS et al., 2009) (MATHISON; PITT, 2014) (MALIK; SINGH; SATYA, 2007).

3.3 Life history

The housefly is a holometabolous insect; that goes through a complete life cycle of, egg, larva, pupa and adult; and where all the stages differ in their feeding and behavioral habits. The complete cycle from egg to adult lasts an average of ten to fourteen days; that is to say approximately 15,5 hours in the egg stage, 5 to 6 days in the larval stage (LI, LII and LIII) and 6 to 7 days in the pupal stage and also considering a period of maturation of the eggs inside the female (period of pre -oviposition) of about 3 days, all this about a temperature of 25 °C and a RH close to 90% (KEIDING, 1986). High temperatures favor the increase of populations, accelerate their biological cycle and give rise to a greater number of generations per year (SANCHEZ-ARROYO, 2011; SCHLAPBACH, 2007).

It is estimated that at any given time, only 15% of an existing fly population are adult flies, with eggs, larvae and pupae accounting for the remaining 85%. In this way, the detection must be oriented to all the biological stages and not only to the adult ones (as it is erroneously and commonly oriented) for a more complete management of the pest (SALAS; LARRAÍN, 2012) especially inside animal farms.

3.3.1 The egg

The egg may occur singly, arranged in palisade or in disarranged groups (MULLEN; DURDEN, 2019) (Figure 2a). The egg is generally banana shaped, creamy in color, 1 to 1,2 mm long, and concave dorsally that remains in that stage for about 15 hours at 25°C on average (KEIDING, 1986). The period of maturation of the eggs inside the female (period of pre-oviposition) is about 3 days, the females seek organic matter in decomposition, fermented like poultry manure with a certain degree of humidity (maximum 90% RH) for oviposition (KEIDING, 1986). Volatile hormones released at the time of oviposition serve as an attractant for other females to oviposit in the same place, so it is common to find large numbers of eggs of the same age, in the same oviposition spot (TANG et al., 2016). It has been reported that a female can lay up to 120 eggs per clutch in a total of 5 to 6 clutches in her lifetime, generating a large number of offspring, especially in tropical climate zones (BRITTO et al., 2008) .

3.3.2 The larvae

Once the egg hatches, the larvae (also known as maggot) emerge and go through three stages (L1, L2 and L3) separated by a molt (Figure 2b, c, d), which usually lasts 5 to 6 days at 25°C (KEIDING, 1986). The translucent neonate larva (L1) immediately penetrates the decomposed organic (ORGANIZACION PANAMERICANA DE LA SALUD, 1962). The instar L1 grows from 1 to 3 mm, the instar L2 from 3 to 5mm and the instar L3 from 5 to 13mm. The larvae body is soft, prone to desiccation, tapered, with a head greatly reduced at the pointed end. The mouth has strong mandibles that serve not only for feeding but also for locomotion by tearing and loosening the substrate (MULLEN; DURDEN, 2019). Larvae strongly avoid light and require warm temperatures (15° - 40°C) and humidity (maximum 90% RH) that is why they like to cluster in high numbers (hundreds or thousands) (KEIDING, 1986). Another option to

regulate its temperature is to move inside the poultry manure to avoid heat loss but at the same time keep itself in a point where oxygen is still flowing (ORGANIZACION PANAMERICANA DE LA SALUD, 1962). Larvae actively feed on solubilized substances and bacteria, as the larva grows; it gradually adopts a creamy white or yellowish color. Before completing the third larval stage, they stop feeding and seek for a place providing adequate temperature (15°- 20°C) and humidity conditions, where they bury themselves in the substrate and pupate (BRITTO et al., 2008).

3.3.3 The pupae

When the larva is ready to pupate, it contracts its tegument towards the cephalic part, to form a barrel-shaped sheath with rounded ends, called a puparium (Figure 2e). The puparium measures 0.63 cm on average, initially with the same color and consistency of the larva that changes to a dark brown color and hard consistency after sclerotization. This process takes 24 hours at maximum (KEIDING, 1986; ORGANIZACION PANAMERICANA DE LA SALUD, 1962). The true pupa inside the puparium is immobile and does not feed for about 6 to 7 days at 25°C and between 75 to 90% RH (KEIDING, 1986). It uses its prothoracic and posterior spiracles to breath keeping them embedded in the wall of the puparium which differentiates houseflies from others flies (MULLEN; DURDEN, 2019).

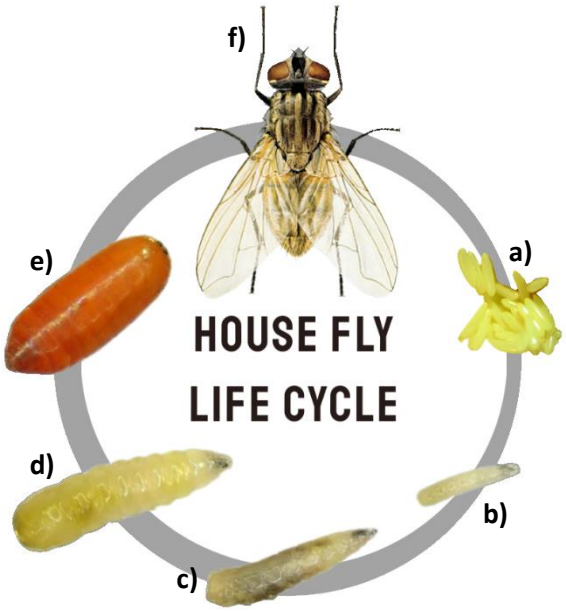
3.3.4 The adult

When the adult housefly is fully formed and ready to emerge from the puparium, it uses the swelling and shrinking of the ptilinal sac located in the head, to break and push out the front end of the puparium (BRITTO et al., 2008). The house fly that emerges is soft, pale, has folded wings and immediately seeks a refuge, preferably a high place where it can hang upside down, and spread its wings, until its body dries up and finishes sclerotizing (KEIDING, 1986).

Adult house flies are easily recognized for having 6 to 7 mm long, with the female usually larger than the male, and both wings are longer than the abdomen with the pronounced upward bend in the fourth longitudinal wing vein (GEDEN et al., 2021; SANCHEZ-ARROYO, 2011) (Figure 2f). The thorax is gray-yellow to dark gray presenting four dark longitudinal and narrow stripes in the mesonotum region and the

abdomen is yellowish with a median dark stripe (BENNETT, 2006) and irregular dark markings on the sides (SANCHEZ-ARROYO, 2011). Houseflies have licking-type mouthparts (TRIPLEHORN; JOHNSON; BORROR, 2005) . The head present a pair of compound eyes and three ocelli (BENNETT, 2006; GEDEN et al., 2021; SANCHEZ-ARROYO, 2011). The sexes can be easily separated by noting the space between the composed eyes. In males, the composed eyes are nearly meeting at the dorsal mid line and so called holoptic while in females they are more widely separated from dorsal mid line and called dichoptic. Adult flies need to feed at least two times a day, and water is important as they cannot survive without it for more than 48 hours (WORLD AND HEALTH ORGANIZATION, 1997).

Figure 2 – House fly life cycle



House fly stages:
a) Eggs. b) Larva I. c) Larva II.
d) Larva III. e) Pupa and
f) Adult.

3.3.5 Reproduction

Mating can occur at any time after the adult fly is fully active, that is, approximately 15 hours after emergence (ORGANIZACION PANAMERICANA DE LA SALUD, 1962). Housefly females generally mate only once, store the male's sperm in their spermatheca and gradually release it to fertilize the eggs for up to three weeks (KEIDING, 1986). Houseflies under controlled laboratory conditions and attending to their nutritional requirements (specially proteins), are ready to oviposit after 3 to 5 days

after copulation and each female can produce up to 150 eggs per gonotrophic cycle (GEDEN et al., 2021).

3.4 Economic importance of poultry farming in Brazil

According to Lopes (2010), the Brazilian poultry industry is currently experiencing the Super Industrial Period where scientists and technicians together develop their own technology in relation to the different climatic and topographical conditions of each region (LOPES, 2010).

Decades of efforts have succeeded in a booming activity According to the Brazilian Association of Animal Protein (ABPA, 2018). In 2017, the production of chicken meat was 13.5 million tons, making Brazil the biggest producer behind only the United States and coming to be the biggest world exporter of chicken. Domestic consumption in 2017 reached 66.9% while the percentage of exports was 33.1%, which would be 4.3 million tons exported to more than 150 countries. The consumption per person in 2017 was around 42.07 kg/year (ASSOCIAÇÃO BRASILEIRA DE PROTEÍNA ANIMAL, 2018). Poultry farming accounts for approximately 1.5% of the Brazilian Gross Domestic Product (GDP) and for the generation of almost 5 million direct (poultry) and indirect jobs (companies belonging to the production chain), and more than R$ 6 billion in taxes (ASSOCIAÇÃO BRASILEIRA DE PROTEÍNA ANIMAL, 2018), granting it a social importance for the maintenance of many Brazilian families. The state of Minas Gerais is the second largest producer of eggs in the country, with the main producers being the municipalities of Itanhandú, Montes Claros, Uberlandia, Sao José da Lapa, Pasa Quatro, Nepomuceno and divinópolis, which account for 51% of the state production (SANTOS FILHO et al., 2011).

3.5 Health problems related to houseflies in bird breeding

Inevitably, the development of the poultry industries generates environmental impact. The waste generated by the large poultry farms when poorly disposed of may become an attractive spot to houseflies (GEDEN et al., 2021). In Brazil, wire cages used for egg-laying hens are typically suspended above the ground), with bird feces and spilled feed accumulating in piles beneath them resulting in high fly densities (GEDEN et al., 2021), compared to other housing systems (HY-LINE INTERNATIONAL, 2017).

Uncontrolled management of poultry manure is a highly desirable medium for fly populations (HY-LINE INTERNATIONAL, 2017). Additionally, Flies develop in large numbers, causing discomfort, stress, and decreased production (SANCHEZ-ARROYO, 2011)and the impact of flies' infestations in the poultry industry would undeniably incur a significant reduction in the production of poultry meat and eggs, causing significant economic losses (SCHLAPBACH, 2007)

Apart from this, the flies are important vector of various disease. When developing in decomposing material or manure loaded with microorganisms, the flies' bodies, covered by bristles, are impregnated with these microorganisms both externally (when moving) and internally (when feeding) (WORLD AND HEALTH ORGANIZATION, 1997), and so can mechanically transport them to humans. (NEVES, 1992). More than 100 pathogens are associated with the houseflies (MALIK; SINGH; SATYA, 2007). The most common diseases transmitted by flies include avian influenza, Newcastle disease (HY-LINE INTERNATIONAL, 2017), enteric infections such as diarrhea, dysentery, typhoid, cholera (WORLD AND HEALTH ORGANIZATION, 1997), Coccidiosis, helminthic infections (FREITAS DA SILVA CAMPOS et al., 2018), eye infections such as trachoma and epidemic conjunctivitis, certain skin infections such as yaws, cutaneous diphtheria and some mycoses (GREENBERG, 2019). The diseases mentioned can affect both birds and humans, with the exception of coccidiosis, which only affects immunosuppressed people (SILVA-DÍAZ et al., 2016)

Another type of nuisance caused by flies are the specks of dirt (light and dark punctuated secretions) that they leave on their resting surface in the poultry facilities (i.e., windows, railings, cables, etc.,) both inside and outside and that gradually deteriorate the structures (KEIDING, 1986). In addition to the fact that the simple presence of these insects generates preconceptions, being synonymous with unhealthy and unhygienic conditions (WORLD AND HEALTH ORGANIZATION, 1997).

Houseflies' infestations are a problem that require monitoring and control being vital to chicken and human health (SANCHEZ-ARROYO, 2011). Understanding of the life cycle of flies and their interaction with their environment is important to study in order to develop suitable strategies to reduce their impact (HY-LINE INTERNATIONAL, 2017).

3.5.1 Spread of house flies and resulting social and legal problems

Because the flies produce many individuals in a relatively small amount of decaying organic matter (SANCHEZ-ARROYO, 2011), flies progenies reach a high density, which forces new born individuals to disperse from the place of breading to other spaces (POTENZA; TUCCI, 2014). They are attracted to the places that emit sweet, fermentation or putrefying scents that can be encountered in some food factories, farms, stables and urban areas (CHAKRABARTI; KAMBHAMPATI; ZUREK, 2010). According to Potenza 2014, the housefly has a flight range of up to 20 km, traveling at 6 to 8 km per hour. But the flies have a preference for staying up to 1,6 km from the initial breeding point, and when not the ideal conditions, they disperse to more distant places (POTENZA; TUCCI, 2014). The scenario in and around the aviaries is not very different, although breeding substrates are generally available in high amounts. Therefore, up to 60%flies remain in the aviaries and nearby areas, and up to 27% move to more distant areas where they can find adequate breeding conditions (POTENZA; TUCCI, 2014).

Poor monitoring, lack of implementation or execution of an integrated pest management in some laying poultry houses has resulted in many public complaints in the state of Minas Gerais about out-of-control fly populations. Reports such as the one published by the Globo network and Lavras TV entitled " MP apura irregularidades em aviário por infestação de moscas em, MG " dated February 6, 2019 (EPTV 2, 2019; LAVRAS TV, 2019). Establishments that do not comply with sanitary regulations may be subject to sanctions and temporary or permanent closures, which affects the local economies, incurring in social problems such as mentioned in the journalistic report of the Globo Network of August 14, 2019 entitle “Funcionários de aviário interditado após infestação de moscas fazem manifestação” (EPTV 1, 2019).

3.6 Housefly control

The control of flies begins with prevention, that is, sanitation and cleaning, eliminating possible breeding sites. The floors must be made of concrete, have drains, and should be rinsed daily to avoid attractive odors. The manure must be cleaned, buried or composted properly (RATNASARI, 2013). Parallel to prevention, fly monitoring is essential to determine when and how to act.

3.6.1 Fly activity level monitoring

Monitoring house-flies activity on animal facilities is necessary in order to know and maintain the flies' populations below a level (action threshold) and being able to react quickly according to the integrated pest management strategies (GERRY, 2020). Fly control should begin when an increase in populations is detected based on the action threshold followed continue monitoring to confirm the effectiveness of the treatments (FOOD AND DRUG ADMINISTRATION, 2011). In Brazil, the egg production system is predominantly intensive, in conventional vertical cages, in pyramidal (64%) or in vertical (36%) forms (SILVA, 2019). Three types of houseflies monitoring techniques in poultry facilities are recommended in Brazil Gerry (2020). They include the Fly Ribbons, Spot Cards and Baited Jug Traps (GERRY, 2020).

The Fly Ribbons are hanging tapes with an adhesive substance on their surface that is suspended from one end in the beams of the aviaries to take advantage of the ethology of the flies to perch on hanging things. They should be placed in protected places, where a preference for fly resting has previously been observed, for 2 to 7 days (observing the day that it can reach its maximum capacity for retaining flies), taking into account that dust and direct sunlight alter the capture capacity of the tape when they are placed for longer. A minimum of four or nine tapes (per narrow house or high house, respectively) are needed to detect a doubling of flies' activity (GERRY, 2020). The number of flies per tape per day should be recorded. Control thresholds range from 14 to 43 flies per ribbon.

The spot cards are 3 x 5 inches white pieces of cardboard that are attached to the places where stains are observed due to fly activity, that is, dark and light points typical of flies' excretions. This technique does not differentiate between fly species and does not require trained personnel, but it does require an effort to count, in addition to the fact that a single fly can leave more than one stain on a single cardboard, so it only serves to measure the level of activity and not the number of individuals. Action threshold is 50 to 100 points per card per week and is recommended to use 6 to 7 cards per poultry house a week (GERRY, 2020). For point counting (when they are numerous) Gerry (2020) recommends the Flyspoter software, available at https://www.veterinaryentomology.org/flyspotter-house-fly-monitoring, however, there are currently mobile applications that can do the same job with a single photograph of the card, for example CountThings app available in

Play Store for free. Cards are easily dated and filed to keep track of adult activity (HY-LINE INTERNATIONAL, 2017).

The baited jug Traps are homemade devices constructed of a plastic milk jug with four two-inch side holes, hung from rafters with wire at the level of the highest cages or above the accumulated feces, and contained within the insecticide bait for domestic flies, placing 5 to 6 traps in the longitudinal axis of the poultry shed. The flies die inside and should be counted every week, the threshold level being 300 to 350 per week. However, its long-term use is not recommended due to the possibility that the flies generate resistance to the insecticide and that monitoring will become useless (WATSON; WALDRON; RUTZ, 1994).

3.6.2 Chemical control and problems of insecticide use

When flies infestation reach the action threshold, chemical control becomes imperative and should be carried out within Integrated Pest Management (IPM) (BONILLA et al., 2000). Producers must decide which chemicals to be used and continue monitoring to determine the progress of control, keeping a detailed history of the insecticides used, their formulations and proportions, and including the name of the applicator personnel and the date of application (BELL; WEABER, 2001; RATNASARI, 2013). The rotation between different classes of insecticides can help delay or minimize the development of resistance (RATNASARI, 2013).

In poultry farms, flies' control is relying on insecticides application as principal approach. The insecticides used to control flies can be classified as adulticides and larvicides. Adulticidal products; belonging to organophosphates, pyrethroids, and neonicotinoids; are used to eliminate flies when the infestation is above the tolerable infestation level. For chemical control, two types of formulations can be used, one being liquid and the other in the form of baits. Larvicides like Cyromazine, the active ingredient of Larvadex® and Neporex®, are generally selective to natural enemies and should be preferentially used. Larvadex® is a fly larvae growth regulator, for oral administration to birds, through the feed.

It is well known that many end-users tend to take 'quick' measures to their pest problems, using inappropriate insecticides for the type of pest, in the wrong dose or for long periods of time evidencing poor knowledge about the risks associated with the use

of pesticides, even farmers who are aware of the pesticides harmful effects are unable to put that knowledge into practice (DAMALAS; ELEFTHEROHORINOS, 2011).

From the ecological point of view, it is usual for insecticides and their metabolites to spread beyond the limits of the applied field, since they are easily carried by wind and water, with the aggravating circumstance of being products of great biological activity, contaminating the areas where they reach (CISNEROS, 2019).

The inappropriate use of insecticides in aviaries can cause contamination both in birds and in the eggs, they lay, making them unsuitable for consumption (POTENZA; TUCCI, 2014). For example: larvicides available in Brazil (Ciromazin 1%® and Difly®), which are placed in the feed of the birds, must be removed from the diet of the birds in advance, according to the information sheet of each product, due the birds must not be slaughtered before this period, as for laying birds, the use is not recommended (REZENDE et al., 2019).

Insecticides, in addition to being toxic to pests, are also toxic to animals, including humans, which is why all biosafety measures must be taken both in the manufacture and during the application of these products (CISNEROS, 2019).

3.6.2.1 Repercussions on non-target-fauna

Insecticides can have an impact, directly or through their metabolites (TUDI et al., 2021), on the non-target insect which include insect pollinators such as bumblebees, honey bees, syrphid flies, as well as insect predators and parasitoids (RIYAZ; AHMAD SHAH; SIVASANKARAN, 2021).

The housefly is a persistent insect and most insecticides that intended to control it are toxic to beneficial insects(RATNASARI, 2013). In aviaries most of these beneficial insects are beetles and mites, that are often associated with accumulations of bird manure and in turn are more susceptible than the housefly to these compounds (KAUFMAN; RUTZ, 2018).

The elimination of natural enemies can lead firstly to the rapid resurgence of the pest problem (the one that motivated the application in the first place), and secondly, to the appearance of new pests (CISNEROS, 2019) needing increased amounts of pesticide to be use, and higher control costs (KAUFMAN; RUTZ, 2018).

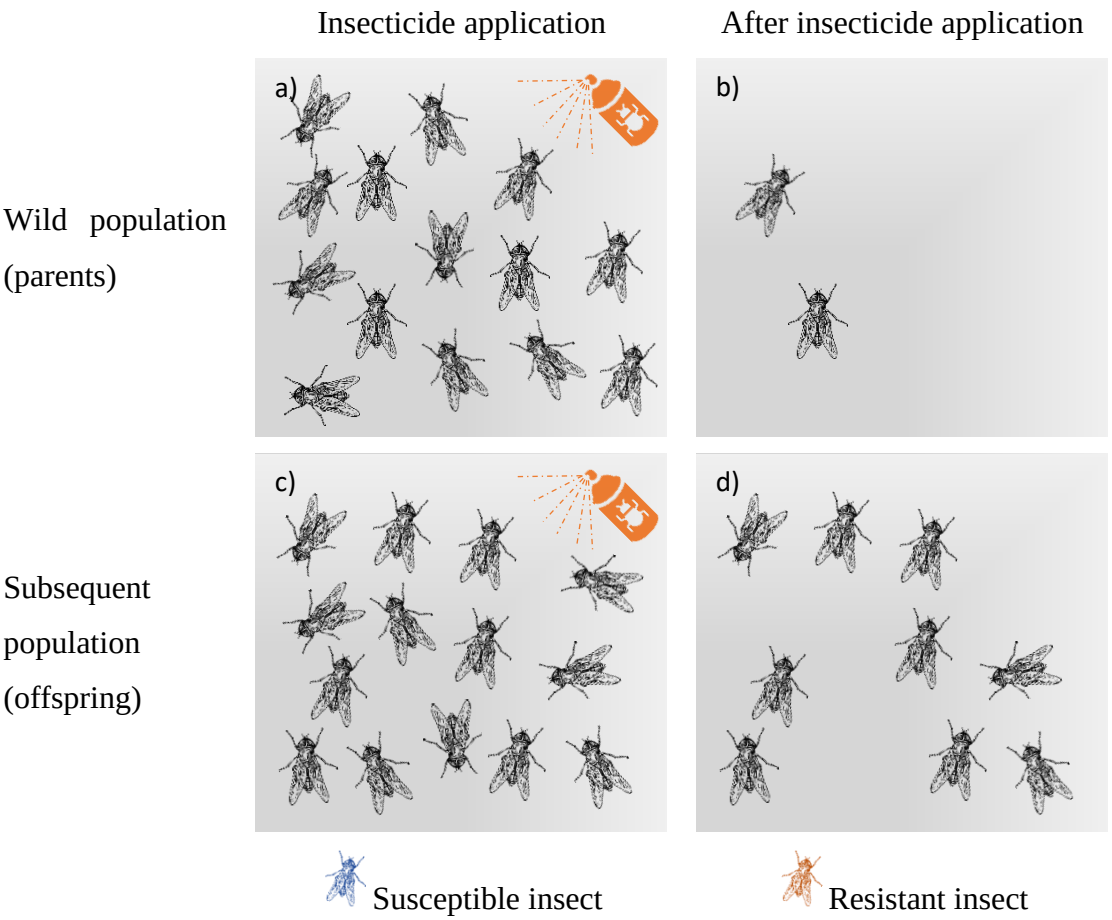
3.6.2.2 Emergence of resistance to synthetic insecticides

The over use of insecticides can lead to setbacks in insect pest control due to the phenomenon of insecticide resistance (CISNEROS, 2019).

In the first place, it is necessary to define the differences between tolerance and resistance to insecticides. Tolerance is the natural absence of susceptibility of an insect population to an insecticide or active compound (CISNEROS, 2019). Resistance is the hereditary capacity of an individual or a population to physiologically and metabolically overcome the toxic action of an insecticide and thus survive and function normally (PONCE et al., 2006).

The basis of resistance has a genetic origin, more specifically in genetic variations. Sometimes these changes, being at the germinal level, can be inherited to the offspring (RODRÍGUEZ, 2005). In wild insect population, most individuals are likely to succumb to exposure to a lethal dose of an insecticide. However, some individuals, which have the ability to efficiently metabolize and/or excrete the insecticide may survive, continue with their biological cycle, reproduce and pass on this characteristic to their offspring, increasing the percentage of resistant survivors within the population (Figure 3). Said offspring, having resistance, will be more difficult to control, requiring higher doses of insecticide or a new one with another mechanism of action (PONCE et al., 2006). That is to say that the insecticide exerts a selection pressure, eliminating susceptible individuals and resistant ones taking their place in ecosystems (CISNEROS, 2019).

Figure 3 - Selection by insecticides of resistant biotypes of pests



In nature there are a few individuals with characteristics that allow them to survive to toxic substances (orange insect). a) Populations exposed to insecticides decrease due to the death of susceptible individuals (blue insect) b) while resistant individuals survive, c) in the next generation resistant individuals reproduce in greater numbers and the population when exposed to the insecticide or the same chemical group, killing the susceptible individuals, d) leaving alive the resistant ones that are now in greater numbers and the population becomes more difficult to eliminate.
Source: The author (2022).

There are three main types of resistance:

-Cross-resistance: occurs when a population that has been "selected" for its resistance to an insecticide becomes also resistant to different insecticide has a similar or the same mode of for having the same target site (CISNEROS, 2019). Such is the case of DDT and pyrethroids whose action is limited by the kdr gene (knockdown resistance), which interferes with the sodium channels in nerve cells (FAO, 2012).

-Multiple resistance: occurs in a population that has acquired resistance to various types of insecticides, both to which it has been exposed and to which it has not been exposed, that is, the population has several resistance mechanisms simultaneously (PONCE et al., 2006).

-Negative cross resistance: occurs when a population resistant to an insecticide is subjected to a new insecticide with a different mode of action, the population of insects returns to a susceptibility close to the original (PONCE et al., 2006). For example, in *Culex quinquefasciatus* mosquito larvae, it has been observed that organophosphates such as Temephos increase susceptibility to pyrethroid insecticides, such as permethrin, and vice versa (CISNEROS, 2019). This phenomenon would be quite useful for resistance management in the field, but unfortunately it is not very frequent (BIELZA, 2005).

In general, resistance genes in organisms that have never been exposed to insecticides are scarce, this is because to achieve this resistance the organisms compromise some other genetic or physiological characteristics, for example, a reduction in fertility, this is called the Fitness Cost (FAO, 2012). Resistance can be reversible in cases of prolonged absence of the insecticide, since the resistant organisms are at a disadvantage, the susceptible ones will reproduce more quickly, repositioning themselves in their ecological niche, reversing the state of susceptibility to the population (FAO, 2012).

REFERENCES

ASSOCIAÇÃO BRASILEIRA DE PROTEÍNA ANIMAL. **RELATÓRIO ANUAL 2018**. São Paulo: ABPA, 2018.

BELL; WEABER. **Commercial Chicken Meet**. 5. ed. Midtown Manhattan, New York City: Springer, 2001. v. 1

BENNETT, S. M. ***Musca domestica* (The Common Housefly)**. Disponível em: <http://www.the-piedpiper.co.uk/th6a.htm>. Acesso em: 15 fev. 2022.

BIELZA, P. La resistencia a insecticidas: de los mecanismos a las estrategias de manejo. **Phytoma**, v. 173, n. 16, p. 1–10, nov. 2005.

BONILLA, J. et al. REDUCCIÓN DEL ESCURRIMIENTO DE PLAGUICIDAS AL MAR CARIBE. p. 1–163, 24 nov. 2000.

BRITTO, L. G. et al. Manual de identificação, importância e manutenção de colônias estoque de dípteras de interesse veterinário em laboratório. **Embrapa Documento 125**, p. 30, 2008.

CEDRIC GUILLOTT. **Entomology**. 3. ed. Netherlands: Springer, 2005. v. 1

CHAKRABARTI, S.; KAMBHAMPATI, S.; ZUREK, L. Assessment of house fly dispersal between rural and urban habitats in Kansas, USA. **Journal of the Kansas Entomological**, v. 83, n. 2, p. 172–188, 2010.

CISNEROS, F. **Control químico de las plagas agrícolas**. 1. ed. Lima, Perú: Sociedad Entomológical del Perú, 2019. v. 1

DAMALAS, C. A.; ELEFTHEROHORINOS, I. G. Pesticide exposure, safety issues, and risk assessment indicators. **International Journal of Environmental Research and Public Health**, v. 8, n. 5, p. 1402–1419, 6 maio 2011.

DE CARVALHO, C. J. B. et al. A catalogue of the *Muscidae* (Diptera) of the Neotropical Region. **Zootaxa**, v. 1, p. 1–282, 16 fev. 2005.

EPTV 1. **Funcionários de aviário interditado após infestação de moscas fazem manifestação**. Disponível em: <https://g1.globo.com/mg/sul-de-minas/noticia/2019/08/14/funcionarios-de-aviario-interditado-apos-infestacao-de-moscas-fazem-manifestacao.ghtml>. Acesso em: 23 fev. 2022.

EPTV 2. **MP apura irregularidades em aviário por infestação de moscas em Nepomuceno, MG**. Disponível em: <https://g1.globo.com/mg/sul-de-minas/noticia/2019/02/06/mp-apura-irregularidades-em-aviario-por-infestacao-de-moscas-em-nepomuceno-mg.ghtml>. Acesso em: 22 fev. 2022.

FAO. **Directrices sobre la prevención y manejo de la resistencia a los plaguicidas**. Rome. FAO, set. 2012.

FOOD AND DRUG ADMINISTRATION. Guidance for Industry Prevention of Salmonella Enteritidis in Shell Eggs During Production, Storage, and Transportation. p. 1–27, 9 dez. 2011.

FREITAS DA SILVA CAMPOS, M. DE F. et al. Identificação parasitológica da cama de frango reutilizada em uma granja avícola. **Revista Brasileira de Ciência Veterinária**, v. 25, n. 1, p. 27–30, 2018.

GEDEN, C. J. et al. House Fly (Diptera: Muscidae): Biology, Pest Status, Current Management Prospects, and Research Needs. **Journal of Integrated Pest Management**, 2021.

GERRY, A. C. Monitoring house fly (Diptera: Muscidae) activity on animal facilities. **Journal of Insect Science**, v. 20, n. 6, 1 nov. 2020.

GREENBERG, B. Chapter 5: Flies and Animal Diseases. Em: GREENBERG, B. (Ed.). **Flies and diseases**. II ed. New Jersey: Princeton University Press, 2019. v. IIp. 268–343.

HY-LINE INTERNATIONAL. FLY MANAGEMENT: SURVEILLANCE AND CONTROL. p. 1–8, 2017.

KAUFMAN, P.; RUTZ, D. Pest Management Recommendations for Poultry. **Penn State Extension**, p. 1–24, 24 jan. 2018.

KEIDING, J. The Houseflies Biology and control. **Insects Biology**, p. 302–323, 1986.

LAVRAS TV. **MP apura irregularidades em aviário por infestação de moscas em Nepomuceno**. Disponível em: <https://lavras.tv/site/2019/02/07/mp-apura-irregularidades-em-aviario-por-infestacao-de-moscas-em-nepomuceno/>. Acesso em: 23 fev. 2022.

LOPES, K. R. F. **Avicultura: da pré-história à produção industrial**. 1. ed. Mossoró: Universidade Federal Rural do Semi-árido, 2010.

MALIK, A.; SINGH, N.; SATYA, S. House fly (*Musca domestica*): A review of control strategies for a challenging pest. **Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes**, v. 42, n. 4, p. 453–469, 2007.

MATHISON, B. A.; PITT, B. S. Laboratory Identification of Arthropod Ectoparasites. **Clinical Microbiology Reviews**, v. 27, n. 1, p. 48–67, 12 jan. 2014.

MONTILLA-CORONADO, R. Pupal parasitoids of flies (Diptera) associated to the bovine cattle manure, in Aragua, Venezuela. **Journal of the Selva Andina Biosphere**, v. 5, n. 2, p. 124–132, 2 nov. 2017.

MULLEN, G. R.; DURDEN, L. A. **Medical and Veterinary Entomology**. 3. ed. San Diego: Elsevier, Academic Press, Book Aid International, 2019. v. 1

NEVES, D. P. Parasitologia humana. **Revista do Instituto de Medicina Tropical de São Paulo**, v. 34, n. 4, p. 288–288, ago. 1992.

ORGANIZACION PANAMERICANA DE LA SALUD. Moscas de importancia para la salud pública y su control. Publicaciones Científicas. p. 1–48, 15 maio 1962.

PALACIOS, S. M. et al. Efficacy of essential oils from edible plants as insecticides against the house fly, *Musca domestica* L. **Molecules**, v. 14, n. 5, p. 1938–1947, maio 2009.

PAPE, T.; EVENHUIS, N. L. **Dipterorum**. Disponível em: <http://www.diptera.org/Summary>. Acesso em: 4 fev. 2022.

PASCALE, J.; KUNZ, A. **Manejo Ambiental na Avicultura**. 1. ed. Concórdia, SC: Embrapa Suínos e Aves, 2011. v. 149

PONCE, G. et al. MODO DE ACCIÓN DE LOS INSECTICIDAS. **Revista de Salud Pública y Nutrición**, v. 7, n. 4, p. 1–14, 2006.

POTENZA, M. R.; TUCCI, E. C. **Controle de pragas na avicultura**. (SBSA, Ed.) XV Simpósio Brasil sul de avicultura. **Anais**. Chapecó: SBSA e VI Poultry Fair, 8 abr. 2014.

RATNASARI, D. K. Eliminate Flies from Manure. **ReseachGate**, p. 1–11, 23 mar. 2013.

RESH, V. H.; CARDÉ, R. T. **Encyclopedia of insects**. 2. ed. AMSTERDAM, BOSTON, LONDON, NEW YORK, OXFORD, PARIS SAN DIEGO, SAN FRANCISCO, SINGAPORE, SYDNEY, TOKYO: Academic Press, 2009. v. 11

REZENDE, L. DO C. et al. Synanthropic diptera affecting layer poultry farms: a review. **Arquivos do Instituto Biológico**, v. 86, 2019.

RIYAZ, M.; AHMAD SHAH, R.; SIVASANKARAN, K. Pesticide residues: Impacts on fauna and the environment. Em: BIODEGRADATION TECHNOLOGY OF ORGANIC AND INORGANIC POLLUTANTS (Ed.). **Biodegradation**. 1. ed. London: IntechOpen, 2021. v. 1p. 1–21.

RODRÍGUEZ, J. C. **RESISTENCIA A INSECTICIDAS: DE LA TEORÍA A LA PRÁCTICA**. Montecillo - Estado de México: Colegio de Postgraduados, Instituto de Fitosanidad, 2005.

SALAS, C.; LARRAÍN, P. Identificación y control integrado de moscas con importancia médica y veterinaria presentes en la región de Arica y Parinacota, Chile. **249**, Boletín INIA - Instituto de Investigaciones Agropecuarias. p. 1–76, 1 fev. 2012.

SANCHEZ-ARROYO, H. House fly, *Musca domestica* Linnaeus. **Featured Creatures**, p. 1–6, mar. 2011.

SANTOS FILHO, J. I. DOS et al. Os 35 anos que mudaram a avicultura brasileira. Em: **Sonho, desafio e tecnologia**. 1. ed. Concórdia: Embrapa, 2011. v. 1p. 59–87.

SCHLAPBACH, F. A. Control integrado de moscas. **Producción porcina**, p. 1–18, 2007.

SILVA, I. Sistemas de produção de galinhas poedeiras no Brasil. p. 3–40, 4 jul. 2019.

SILVA-DÍAZ, H. et al. Coccidiosis intestinal en niños admitidos en un hospital de Perú y comparación de dos métodos para la detección del *Cryptosporidium spp*. **Revista Peruana de Medicina Experimental y Salud Pública**, v. 33, n. 4, p. 739, 13 dez. 2016.

TANG, R. et al. Identification and testing of oviposition attractant chemical compounds for *Musca domestica*. **Scientific Reports**, v. 6, 26 set. 2016.

TRIPLEHORN, C.; JOHNSON, N.; BORROR, J. **Study of insects**. 7. ed. Belmont, CA.: Thompson, 2005. v. 1

TUDI, M. et al. Agriculture Development, Pesticide Application and Its Impact on the Environment. **International Journal of Environmental Research and Public Health**, v. 18, n. 3, p. 1112, 27 jan. 2021.

WATSON, D. W.; WALDRON, J. K.; RUTZ, D. **Integrated management of flies in and around dairy and livestock barns**. New York State, New York State: Cornell University, Integrated Pest Management, 1994.

WORLD AND HEALTH ORGANIZATION. Chapter 6: Houseflies. Em: ROZEENDAL (Ed.). **Vector control methods for use by individuals and communities**. 2. ed. Genebra: WHO, 1997. v. 1p. 302–323.

ZAHRADNÍK, J.; CHVALA, M. **La gran enciclopedia de los insectos**. 1. ed. Praga: Susaeta ediciones s.a., 1990. v. 1

CHAPTER I

Toxicity of different insecticides to two field-collected populations of *Musca domestica* L. (Diptera: Muscidae)

ABSTRACT

Housefly *Musca domestica* L. (Diptera: Muscidae) is a major domestic, medical and veterinary pest. In poultry farms, its control is relying on insecticides application as principal approach. However, the overreliance on such strategy frequently led to cases of control failure and selection of resistant individuals. Here, we assessed the toxicity of ten insecticides to two housefly populations (POPUFLA and POPNEP) collected in Lavras' region (Brazil). The chemicals used included chlorpyrifos, imidacloprid, thiamethoxam, deltamethrin, lambda-cyhalothrin, azadirachtin, lambda-cyhalothrin + thiamethoxam, chlorfenapyr, cyantraniliprole and spinetoram. Adults' flies were offered dental cottons imbibed with insecticidal solution in 200ml glass pots under controlled conditions (T°: 25±2ºC, RH:60±5% and photophase 12L:12D). Mortality was assessed after 48 hours and dose-response curves determined. Larvae (contact with impregnated filter paper) and pupae (submersion in insecticide solution) were exposed to recommended doses. Four replicates of 15 adults, 10 larvae and 10 pupae were used for each insecticide x concentration. The results showed that the POPNEP presented high LC50 than POPUFLA to four insecticides. The resistance ratios (LC_{50} POPNEP/ LC_{50} POPUFLA) were 20.5; 13.1; 12.4 and 12.2 folds for deltamethrin, thiamethoxam, lambda-cyhalothrin + thiamethoxam and lambda-cyhalothrin indicating potential insecticides' resistance occurrence in POPNEPO. The other insecticides were effective against the adults of the two populations. Larval and pupal mortalities ranged from 50 to 100% in the two populations. Chlorpyrifos, chlorfenapyr and imidacloprid were the most toxic and lambda-cyhalothrin was the least toxic for larvae while only imidacloprid presented high toxicity for pupae of the two populations. These findings may explain the reported outbreaks of housefly populations in the region and resistance mechanisms in POPNEP should be further investigated.

Keywords: Housefly control, Poultry farm, Insecticide resistance.

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1 INTRODUCTION

Brazil is one of world's top poultry producing countries participating in the satisfaction of the increasing demand for animal protein from the growing world population (AUGÈRE-GRANIER, 2019). Intensive production entails to the generation of large amounts of waste that are an attractive source of food and breeding material for insects like the house fly (KEIDING, 1986), microorganisms that cause gastric diseases in humans such as enteritis due to *Escherichia coli*, salmonellas, shigellas and enterobacteria (MORRIS OSTROLENK; HENRY WELCH, 1941) and diseases in poultry such as New Castle, infectious bronchitis, infectious bursitis (HABIBI; FIROUZI; ROHOLLAHZADEH, 2018) and H5N1 known as bird flu (WANARATANA; PANYIM; PAKPINYO, 2011) as the most common.

The development of the poultry industries generates an environmental and social impact. The waste generated by the large poultry farms when poorly managed may become a spot of flies' proliferation. Proliferation also occurs in settlements adjacent to egg-producing poultry activities and in those where there is an absence or deficiency of public cleaning and sewage services. Such a situation occurred in the State of Minas Gerais; one of the largest producers of poultry products in Brazil; where flies populations outbreaks have been frequently reported. This situation leads to economic losses, the occurrence and dissemination of diseases as well as discomfort for the inhabitants of the areas affected by this insect pest (EPTV 2, 2019; LAVRAS TV, 2019).

The control strategies of adult houseflies in aviaries are diverse. Such strategies include the exclusion of the winged adults by means of air curtains that prevent their entrance to the production enclosures by up to 80% (HINKLE; HOGSETTE, 2021) and electric lamps that attract and kill insect by small electric shock (URBAN; BROCE, 2000). However, chemical control is often the preferred option in situations where immediate removal of the pest is required

The proliferation of flies within farms above threshold levels makes the use of insecticides unavoidable. When insecticide application is carried out without any technical guidance, much less a pre-established integrated pest management and for several applications over time, resistance problems can emerge (CISNEROS, 2019).

In this context, urgent action measures for the benefit of poultry farms and surrounding communities need to be considered starting from the understanding of the reported outbreaks of house fly populations. So, the present toxicological tests were used

to determine and compare the degree of insecticide sensitivity of a population of flies, presumed to have a history of exposure to insecticides, collected in a country side (POPNEP) with geographic coordinates 21°12'18.6"S 45°14'19.1"W and another population collected in an area without a history of insecticides application (POPUFLA) with geographic coordinates 21°13'28.4"S 44°58'23.9"W. The distance between the two points is 27.55 km (17.12 mi).

2 OBJECTIVES

2.1 General objective

-To evaluate the toxicity of ten different synthetic insecticides selected in adults of the two populations of *M. domestica*. and establish dose-response curves for each insecticide.

2.2 Specific objective

- To evaluate the toxicity of different synthetic insecticides selected in larvae of two populations of *M. domestica*.

- To evaluate the toxicity of different synthetic insecticides selected in pupae of the two populations of *M. domestica*.

- Identify the differences in the biological parameters of the life cycle between the two populations.

3 HYPOTHESIS

-H_1: POPUFLA and POPNEP have different susceptibility profiles to synthetic insecticides

-H_0: POPUFLA and POPNEP do not present differences in mortalities caused by the synthetic insecticides

-H_1: Some synthetic insecticides still have good toxicity for flies from POPNEP population.

-H_0: POPNEP is not susceptible to any of the tested insecticides.

4 MATERIAL AND METHODS

4.1 Location of experiments

The Insect rearing and toxicological bioassays were carried out at the Laboratory of Molecular Entomology and Ecotoxicology in the Department of Entomology at the Federal University of Lavras (DEN-UFLA) under controlled conditions of temperature (25±2ºC), relative humidity at 60±5% and photophase for 12:12 h (L:D).

4.2 Origin and maintenance of the colony of *Musca domestica*

The first population originated from wild adults, collected in the campus of the Universidade Federal de Lavras and presumed to be without a history of insecticide application. This population was named as POPUFLA. The second one was obtained near the surroundings of a conventional poultry farm in Nepomuceno commune, which is presumed to have a history of previous exposure to insecticide applications. This population was named as POPNEP.

The parental house flies (100 individual) of each population were caught with an insect-collector-net, and after being properly identified as *Musca domestica*, were reared separately in acrylic entomological cages of 30 x 30 x 60 cm conditioned with a voile sleeve to facilitate air circulation, the handling and maintenance of the colonies, avoiding contaminations (Figure 4).

Following the modified methodology proposed by Embrapa's "Manual for breeding house flies" (2008), three containers were used in each cage. The first for water supply, containing a moistened cotton. The second contained a mixture of sugar and powdered milk in a ratio of 1:1. The third container was for the oviposition and contained a mixture of water and wheat bran in proportions of 1:1 leaving the substrate loose, avoiding caking for a correct oviposition of the females. The laboratory was maintained under controlled conditions of temperature (25±2ºC), relative humidity (60±5%) and photophase (12:12 h L:D) (BRITTO et al., 2008).

Once the house fly eggs were obtained, a mixture of wheat bran (as a source of cellulose and carbohydrates) with previously ground dog food (as a source of protein) and water in proportions of 3:1:3 was prepared. This pasty mixture was added to the pot containing the eggs and covered with a voile mesh to allow air flow and adjusted with an

elastic band to prevent the larvae from escaping. The pasty mixture is used for larvae development until the pupa stage, that will be and put into a new oviposition cage, to start new generation, in amounts of 300 pupae every 48h, to avoid overpopulation and to continue the reproductive cycle (BRITTO et al., 2008).

Before starting the tests, three generations were bred in order to standardize the individuals and to calibrate the methodology for maintaining the colonies. In addition, the cleaning of cages, the supply of food and fresh water, and the removal of eggs were carried out at two days interval (ABBAS; HAFEZ, 2021).

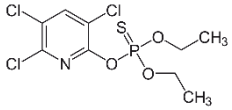
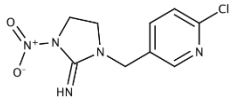
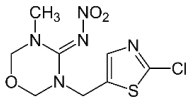
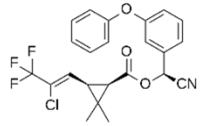
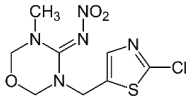
Figure 4– Laboratory rearing of the of *Musca domestica* colony

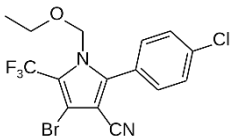
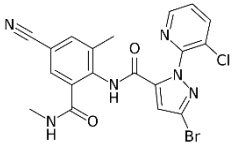
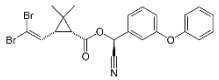
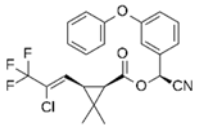
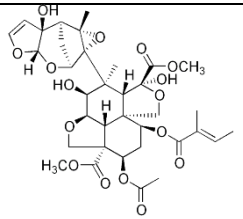
a) Entomological cage for adults' maintenance. Adults mate and then oviposit in the wheat bran and water mixture substrate (1:1), b) Eggs collected, every two days, the oviposition substrate with the eggs inside are collected, c) the substrate used for larvae rearing, it contains wheat bran, dog food and water (3:1:3), where the larvae remain until they pupate, then the pupae are introduced into a new entomological cage for adults' emergence.

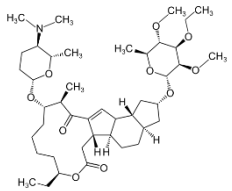
4.3 Insecticides

The bioassays were carried out using synthetic insecticides registered in Brazil for pest control (MAPA, 2022), with the trade name of Capataz, Evidence, Engeo pleno, Pirate, Benevia, Deltamax, Azamax, Delegate, Actara and Termimax (Table 1). Those were preparate according to the manufacturer's instructions, to reach an initial concentration equal at the maximum field recommended dose (MFRD) from which serial dilutions were elaborated, using a 20% sugar solution was used as a solvent and also as a control solution (IRAC, 2011).

Table 1 – List of insecticides used in this research and their modes of action

Commercial name	Active ingredient	Structural formula	Chemical group	Insecticide mode of action
Capataz	CHLORPYRIFOS		Organophosphates	They act on the nervous system, permanently blocking the activity of acetylcholinesterase by non-competitive inhibition. That is, an accumulation of acetylcholine, hyper stimulating the receptors of the sodium channels so that they remain open and produce erratic firing in the nerves. Causing the death of the insect by exhaustion
Evidence	IMIDACLOPRID		Neonicotinoids	They act on the nervous system, permanently blocking the activity of acetylcholinesterase by competitive modulators of the nicotinic acetylcholine receptor. That is, introduce an acetylcholine mimetic that binds to the acetylcholine receptor in synaptic sodium channels and causes them to remain open, producing erratic nerve activity and the insect death.
Actara	THIAMETHOXAM			
Engeo pleno	LAMBDA-CYHALOTHRIN + THIAMETHOXAM	 + 	Pyrethroids + Neonicotinoids	Composed of two active ingredients that have complementary characteristics: Thiamethoxam (neonicotinoid) Lambda-Cyhalothrin (pyrethroid)

Pirate	CHLORFENAPYR		Pyrroles	Pro-insecticide for ingestion, of synthetic origin from pyrroles isolated from *Streptomyces fumarus*, which acts by uncoupling oxidative phosphorylation, that is, by increasing the membrane permeability to ions, which collapses the proton gradient by allowing H+ passes without passing through ATP synthase, impairing ATP synthesis.
Benevia	CYANTRANILIPROLE		Diamides	It acts on ryanodine receptors (intracellular calcium channels). They induce the release of calcium accumulated in the muscles, which produces a generalized muscular contraction of the insect and its death due to exhaustion.
Deltamax	DELTAMETRINE		Pyrethroids	Of botanical origin, from chrysanthemum flowers, but later chemically synthesized, they cause hyper-stimulation of sodium channels by synthetic pyrethrin at the axon level, where sodium channels keep them open, causing hyper-depolarization of the membrane due to excess sodium ions, with loss of electrical activity, causing the death of the insect.
Termimax	LAMBDA-CYHALOTHRIN			
Azamax	AZADIRACHTIN		Botanicals	It is a secondary metabolite that is extracted from the seeds of the Neem tree. It is a growth regulator, interrupting the molt in insects, in addition to causing lesions in the Malpighian tubules, being anti-feeding and sterilizing.

Delegate	SPINETORAM		Spinosyns	Is a semisynthetic insecticide composed of two modified spinosyns that are from bacterial origin (*Saccharopolyspora spinosa*) This ingested neurotoxin acts on nicotinic acetylcholine receptors, over-exciting the nervous system due to alterations in nicotinic function and GABA ion channels. It does not interact in the same sites of action of neonicotinoid, fipronil and abamectin insecticides, therefore, the appearance of cross-resistance is not expected.

4.4 Toxicological bioassays with adults

According to the modified methodology proposed by the Insecticide Resistance Action Committee (IRAC, 2011): The bioassays were carried out in 200ml glass jars with foam caps. Dental cottons moistened with a 2 ml insecticide solution were placed inside them, using 20% aqueous sucrose to dilute the insecticides in concentrations logarithmically spaced by 1%; 0.1%; 0.01% and 0.001% of the MFDR (IRAC, 2011). At the same time, the control groups consisted of the same 20% sucrose solution without any insecticide and under the same conditions of temperature, humidity and photoperiod. The individuals to be used were uniform adults (adults of three-days-old), which that were introduced in groups of 15 flies per glass jar, making 4 replicates per concentration. These pots were covered with their respective foam caps and taken to the BOD chambers under conditions of 25 ± 2 °C, 50% RH and photoperiod of 12L:12D (Figure 5).

Mortality was recorded after 48 hours The flies were assessed according to the following classification: (a) alive or not affected, giving a normal response when gently stimulated (making coordinated movements), or (b) dead or affected, giving an abnormal response to stimulation (showing uncoordinated or absent movements). The mortality of the control flies was also recorded (IRAC, 2011).

Figure 5 – Experimental units of bioassays with adults.

Glass jars with dental cottons moistened with 2 ml of insecticide solution where 15 flies were introduced and covered with foam plugs.

4.5 Toxicological bioassays with larvae

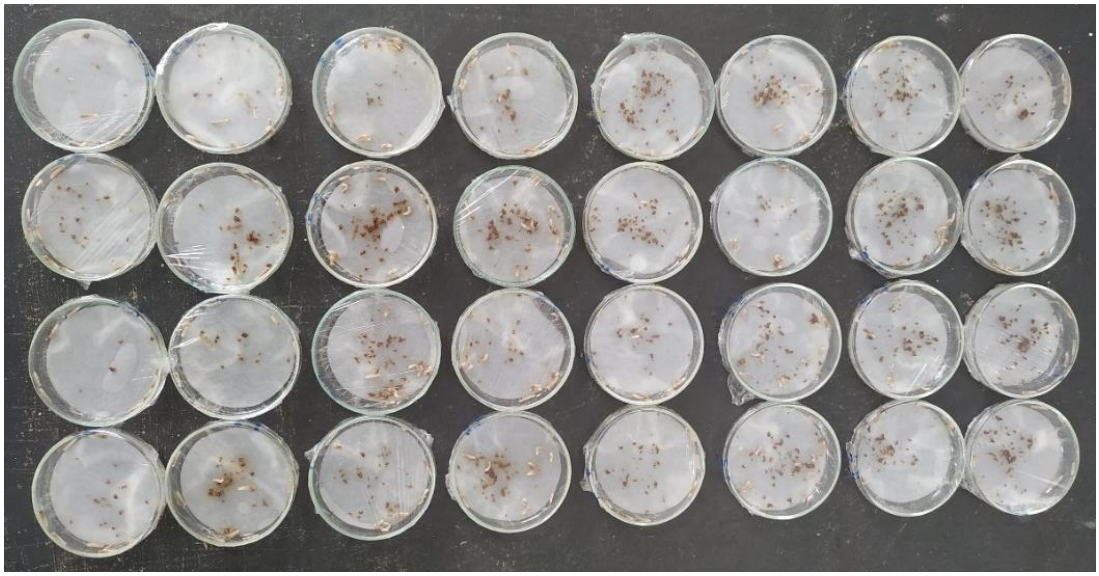
Following the modified methodology proposed by Melo (2014), for larvae bioassays, were used the dose concentrations recommended by the manufacturer for each insecticide.

The treatments used a 5 cm diameter filter paper disc placed on the bottom of the 5 x 1.5 cm Petri dish. The filter papers were moistened with 500 µL of the insecticide solution to be tested. The plates were kept open for 10-20 minutes to air dry. Then, ten third-instar-larvae (L3) were added to each plate. The plates were closed with plastic film and small holes were made in the plastic with a sewing pin to allow gas exchange. The control group consisted of water with 20% sugar only. Four replications were performed for each insecticide. The experimental groups were kept in climatized chambers (25±2°C and 60±10% RH) (Figure 6).

After the first 24 hours, approximately 2 g of larval food were supplied on each plate to the survivor larvae, the food was prepared 24h before to reach certain degree of fermentation. Mortality assessments of treated larvae were performed daily (Figure 7).

The pupating individuals were removed and evaluated for defects (Figure 8) and maintained until adult emergence. Adults were also evaluated based on the defect criteria. Any pupa that has given rise to a healthy adult individual was considered viable. Pupae were considered defected when they present a striated surface in dorsolateral view. Sometimes they will be elongated, curved and striated. Pupae were considered dead or nonviable if an adult does not emerge after 9 days of pupa formation. An adult is considered deformed if it is unable to detach itself from the pupal exuviae or if it does not have normal wings.

Figure 6 – Experimental units of bioassays with larvae at the assembly moment



Petri dishes with filter paper moistened with the insecticide solution containing 10 third-stage larvae and covered with plastic film

Figure 7 – Experimental units of bioassays with larvae after adding the larval substrate.

24 hours after the start of the experiment, larval substrate was added to each petri dish to provide food and shelter for the larvae.

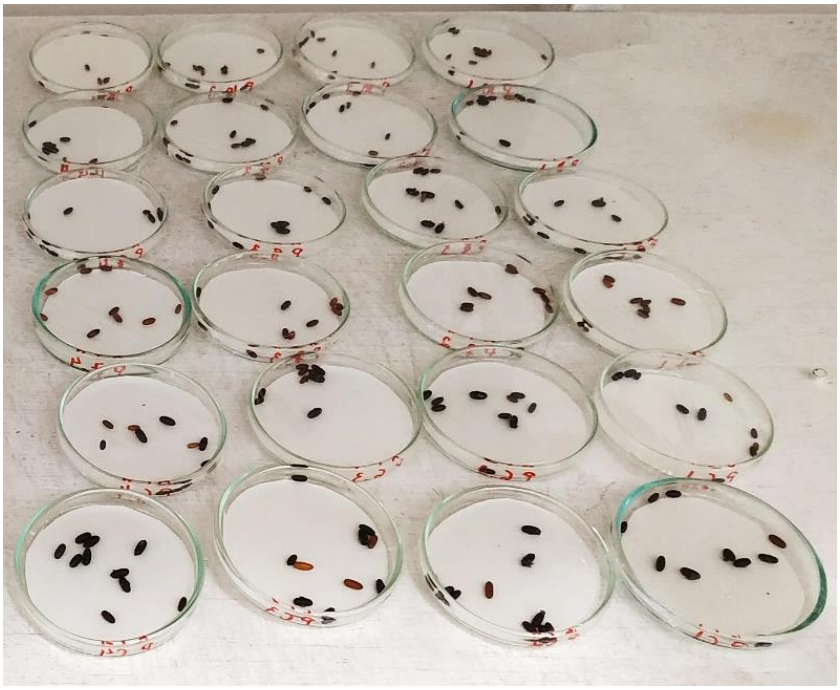
Figure 8 – Larvae that pupate defectively and did not reach the adult stage.

Some larvae started the pupation process but did not complete it efficiently due to exposure to insecticides that gave rise to deformed pupae, and their subsequent death, without being able to reach the adult stage.

4.6 Toxicological bioassays with pupae

For bioassays with pupae, the methodology proposed by Melo (2014) was modified. The maximum field recommended dose (MFRD) was used; also 5 x 1.5 cm diameter Petri dishes and 5 cm diameter circles of filter paper inside each Petri dish (MELO, 2014). Ten one-day-old individuals per repetition and four repetitions per insecticide were taken. The pupae were submerged in the insecticide solution for 10 seconds, then excess of moisture was removed by placing the pupae on absorbent paper and then placed in the petri dish until the adults emerged. The percentage of defects of emerged adults was evaluated. An affected or non-viable pupa was considered when at the end of 9 days, no healthy adult emerged (MELO, 2014) (Figure 9).

Figure 9 – Experimental units of bioassays with pupae.



The ten pupae that were previously submerged in the insecticide solution and then arranged in the Petri dishes with a disc of filter paper at the bottom and covered with plastic film.

4.7 Data analyses

Toxicity bioassay data were analyzed using the Probit analysis, followed by the construction of dose-response curves both through the statistic software SAS 9.0 and SigmaPlot 14.5, managing to determine the values of median lethal concentration (LC50) and to plot the dose-response graphics (SAS INSTITUTE INC., 2021; SYSTAT SOFTWARE, 2003).

The difference in susceptibility between POPUFLA and POPNEP populations was evaluated by calculating the resistance ratio (RR) of each insecticide by dividing the LC_{50} (concentration necessary to cause 50% adult mortality) of the resistant population (POPNEP) by the LC_{50} of the most susceptible population (POPUFLA) as the following formula (SIQUEIRA et al., 2001). $RR = \frac{LD50\ of\ POPNEP}{LD50\ of\ POPUFLA}$.

The resistance levels were staggered based on the Resistance Ratio (RR) criteria, where RR = 1 as susceptibility, RR = 2 to 10 as low resistance, RR = 11 to 30 as moderate resistance, RR = 31 to 100 as high resistance and RR > 100 as very high resistance (ABBAS; HAFEZ, 2021; AHMAD; IQBAL-ARIF; AHMAD, 2007).

For the bioassays carried out with larvae and pupae, the data were analyzed using One-Way ANOVA or Two-Way ANOVA analysis and in case of non-parametric distribution, the data were compared by Kruskal-Wallis tests, using the previously mentioned programs.

5 RESULTS

5.1 Dose-response curves

The results of the adulticide evaluation of field strains, based on the criterion of failure of 95% CI, showed that there was a significant difference between the populations for most of the confrontations with the insecticides resulted in Population factor (F= 10.20; *df*=1; *P*= 0.002), Insecticidal factor (F= 10.64; *df*= 8; *P*<0.001) and Population x insecticidal factor (F= 10.55; *df*= 8; *P*<0.001), declaring that The LC_{50} of the strain POPNEP was significantly higher than those of the strain POPUFLA (Table 2). Indeed, the resistance relationship between the populations ranged from 0.92 to 20.46 folds. The slopes of the concentration-mortality curves for Spinetoram, Chlorpyrifos and Cyantraniliprole were relatively similar between both house flies' populations falling in the classification of not resistant (RR= 0.92 to 1.25). The dose–response calculations indicated that the tested toxicities were as follows: Deltamethrin > Thiamethoxam > Thiamethoxam with Lambda-Cyhalothrin mixture > Lambda-cyhalothrin > Chlorfenapyr > Imidacloprid > Cyantraniliprole > Chlorpyrifos > Spinetoram (Figure 10). Those insecticides can be suggested for the control of flies in the Nepomuceno city. Finally, none of the adult populations showed susceptibility to Azadirachtin.

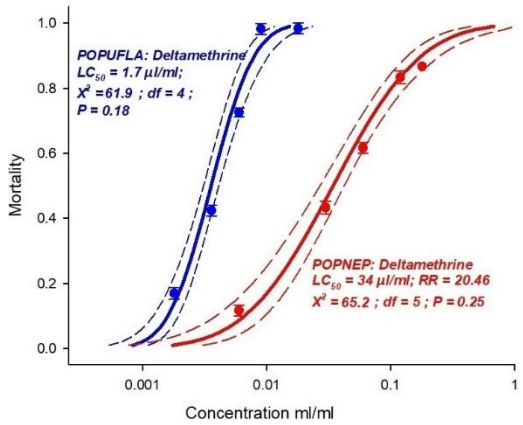
Table 2 – Lethal effect of nine insecticides to adults of two field collected populations of house flies POPUFLA and POPNEP. Arranged in descending order according to the Resistance Ratio (RR).

Commercial name	Active Ingredient	MFRD*	POPNEP			POPUFLA			Resistance
			LC_{50} *	χ^2	P	LC50*	χ^2	P	Ratio (RR)
Deltamax[X]	DELTAMETHRIN [a]	60	34.4	0.25	65.2	1.7	0.18	61.9	20.46
Actara[Y]	THIAMETHOXAM [b]	20	3.7	0.36	54.4	0.3	0.16	92.1	13.03
Engeo Pleno [X]	THIAM+LAM.CIAL. [b]	26	6.7	0.12	99.9	0.5	0.92	0.46	12.40
Termimax [X]	LAMBDA-CYHALOTHRIN [b]	75	740	0.99	0.45	60	0.98	0.99	12.24
Pirate [X]	CHLORFENAPYR [c]	1500	2.3	0.12	56.6	0.9	0.33	33.9	2.69
Evidence[Y]	IMIDACLOPRID [c]	10	1.9	0.99	0.45	0.8	0.29	61.2	2.34
Benevia [X]	CYANTRANILIPROLE [d]	38	2.1	0.54	21.5	1.7	0.80	0.99	1.25
Capataz [X]	CHLORPYRIFOS [d]	10	0.7	0.03	0.99	0.6	0.96	18	1.06
Delegate [X]	SPINETORAM [d]	5	0.2	0.05	111	0.2	0.42	27.8	0.92

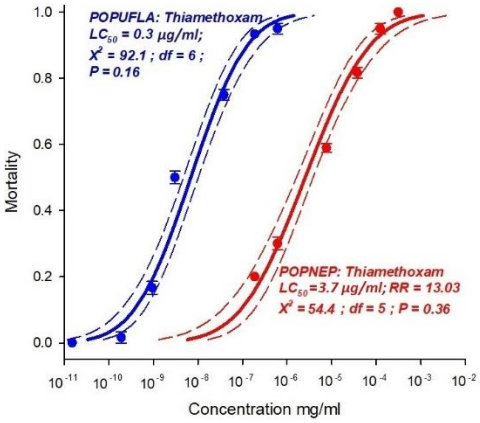
*Quantities express the amount of commercial product necessary to formulate 10ml of the applied dose; X = µl; Y = mg.
N = 60, for each bioassay.
Different letters in each in each table cell indicate significant differences between populations reaction to treatments.
The comparisons by the population factor determined P=0.002, thus there is a significant statistical difference between the populations (P<0.050).

Figure 10 – Dose–response curves showing the lethal effects of series of diluted and concentrate doses based on the respective MFRD, resulted with significative difference (p<0.050) between POPUFLA and POPNEP a) Deltamethrin, b) Thiamethoxam c) Thiamethoxam with Lambda-Cyhalothrin mixture d) Lambda-Cyhalothrin; and without significative difference, e) Chlorfenapyr, f) Imidacloprid, g) Cyantraniliprole h) Chlorpyrifos i) Spinetoram. Data are based on the assessed mortality after 24 hours to adults been exposed to the product. The goodness of fit of the curve was based on the R2 values.

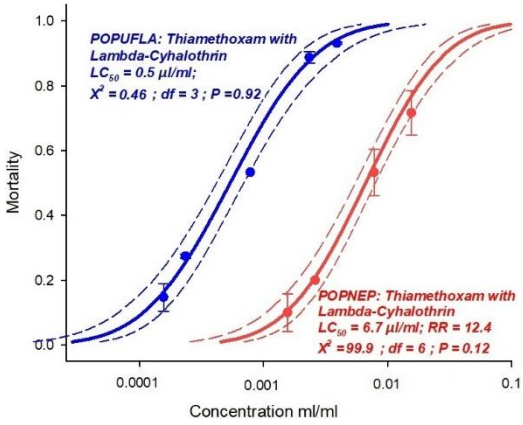
a) DELTAMETHRIN



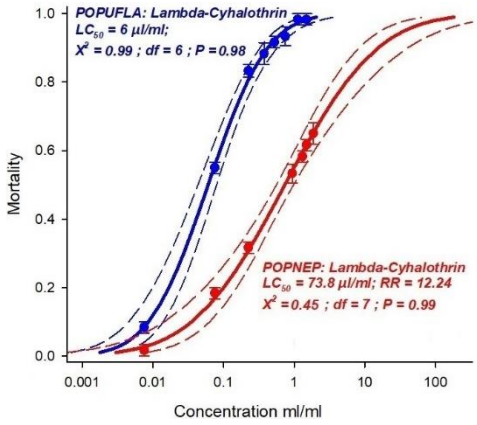
b) THIAMETHOXAM



c) THIAMETHOXAM WITH LAMBDA-CYHALOTHRIN

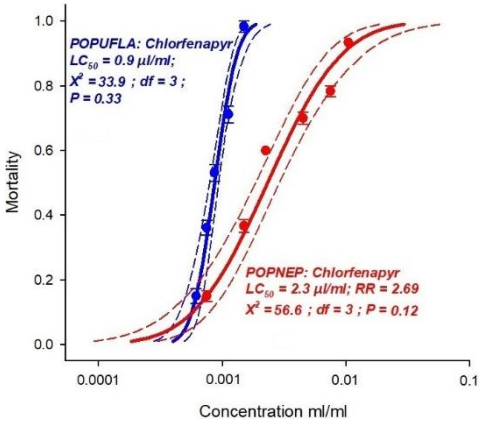


d) LAMBDA-CYHALOTHRIM

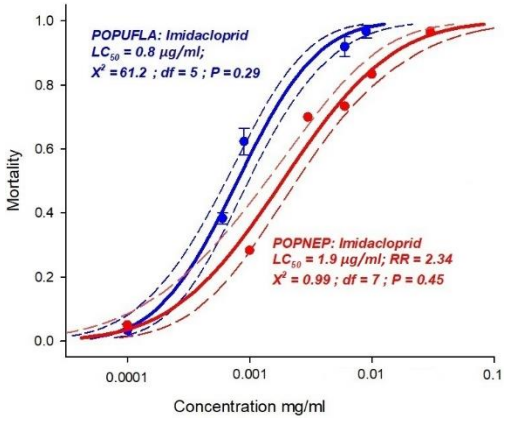


The populations POPUFLA and POPNEP presented significant differences between these insecticides (p<0.050).

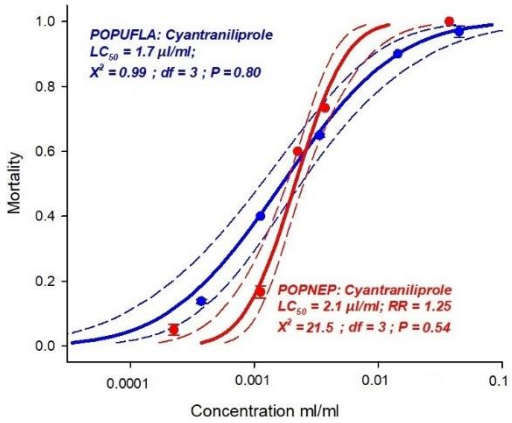
e) CHLORFENAPYR



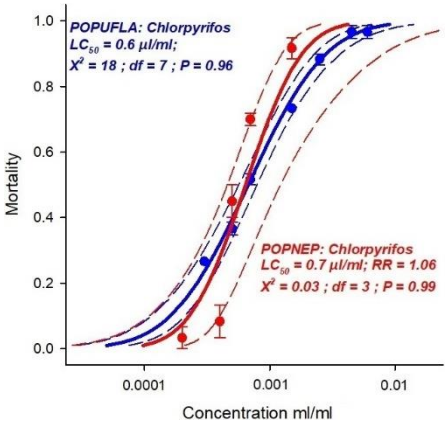
f) IMIDACLOPRID



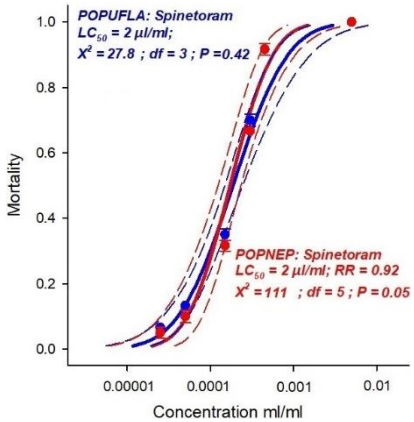
g) CYANTRANILIPROLE



h) CHLORPYRIFOS



i) SPINETORAM



The populations POPUFLA and POPNEP did not presented significant differences to these insecticides ($p>0.050$).

5.2 Toxic effect on larvae

The Anova-2 analysis showed the existence of statistically significant differences for the population factor (F= 5.215; *df*=1; *P*= 0.026) and for the insecticidal factor (F= 4.552; *df*= 9; *P*<0.001). For the strain POPUFLA, was showed highest susceptibility to the treatments with Chlorpyrifos, Imidacloprid, Thiamethoxam with Lambda-Cyhalothrin mixture and Chlorfenapyr with a larvae mortality of 100%, following in descending order the treatments Spinetoram 95%> Deltametrine 90% > Cyantraniliprole 80%, Azadirachtin 80% > Lambda-Cyhalothrin 75% > Thiamethoxam 60%. Meanwhile for the strain POPNEP showed less susceptibility reaching 100% of larvae mortality only with the treatments Chlorpyrifos, Imidacloprid and Chlorfenapyr, followed in decreasing order by Thiamethoxam with Lambda-Cyhalothrin mixture 85% > Cyantraniliprole 85% > Spinetoram 70% > Deltametrine 65% = Azadirachtin 65% > Thiamethoxam 60%, > Lambda-Cyhalothrin 50%.

Table 3 – Lethal effect of ten insecticides on third instar larva of two field collected populations of house flies POPUFLA and POPNEP.

Commercial name	Active Ingredient	Median mortality ± SEM %		P
		POPUFLA*	POPNEP*	0.026
Capataz	CHLORPYRIFOS [a]	100±0	100±10	0.46
Evidence	IMIDACLOPRID [a]	100±0	100±0	1.0
Engeo Pleno	THIAM+LAM.CIAL. [a]	100±0	85±6	0.27
Pirate	CHLORFENAPYR [a]	100±0	100±0	1.0
Benevia	CYANTRANILIPROLE [a]	80±4	85±5	0.18
Deltamax	DELTAMETRINE [a]	90±4	65±16	1.8
Delegate	SPINETORAM [a]	95±5	70±13	1.8
Azamax	AZADIRACHTIN [a]	80±9	65±5	0.35
Termimax	LAMBDA-CYHALOTHRIN [a]	75±20	50±17	0.71
Actara	THIAMETHOXAM [a]	60±10	60±17	0.58

*Quantities express the median mortality in percentage.
N=40, individuals from the same cohort for each bioassay
SEM= Standard errors of means.
Same letters indicate no significant differences between the ten treatments.
that reached the adult stage up to a maximum of 9 days after to reach the pupae stage.

5.3 Toxic effect on pupae

The Anova-2 analysis showed the existence of statistically significant differences for the Insecticidal factor (F= 4.53; *df*= 9; *P*<0.001) and for the Population *x* Insecticidal factor (F= 3.36; *df*= 9; *P*<0.002). For the strain POPUFLA, also showed highest susceptibility tan POPNEP. The pupae treatments arranged in decreasing order of susceptibility, were: Imidacloprid 100% > Lambda-Cyhalothrin 95% > Cyantraniliprole 90% > Thiamethoxam 90% > Deltamethrin 85% > Chlorpyrifos 65% = Azadirachtin 65% > Spinetoram 60 % > Chlorfenapyr 45%. Meanwhile for the strain POPNEP the pupae treatments arranged in decreasing order of susceptibility, were: Spinetoram 100% > Imidacloprid 90% > Deltamethrin 85% > Chlorpyrifos 80% = Azadirachtin 80% > Lambda-Cyhalothrin 75% > Chlorfenapyr 60% = Cyantraniliprole 60% > Thiamethoxam 50%.

Table 4 – Lethal effect of ten insecticides on pupae of two field collected populations of house flies POPUFLA and POPNEP.

Commercial name	Active Ingredient	Median mortality ± SEM %		P
		POPUFLA*	POPNEP*	0.5
Capataz	CHLORPYRIFOS [a]	65±9	80±5	0.25
Evidence	IMIDACLOPRID [a]	100±0	90±6	0.25
Pirate	CHLORFENAPYR [a]	45±5	60±13	0.25
Benevia	CYANTRANILIPROLE [a]	90±11	60±6	0.68
Deltamax	DELTAMETRINE[a]	85±9	85±5	0.81
Delegate	SPINETORAM[a]	60±10	100±3	0.013
Azamax	AZADIRACHTIN[a]	65±10	80±8	0.35
Termimax	LAMBDA-CYHALOTHRIN[a]	95±7	75±6	0.17
Actara	THIAMETHOXAM [b]	90±9	50±10	0.001

*Quantities express the median mortality in percentage.
N=40, individuals from the same cohort
SEM= Standard errors of means.
Different letters in each in each table cell indicate significant differences between the populations.
Data are based on the assessed mortality calculated from the initial number of pupae minus the number of individuals that reached the adult stage up to a maximum of 9 days

6 DISCUSSION

The present research, exhibited that the POPNEP adults presented high LC_{50} than POPUFLA to four insecticides, classified as moderate resistance (ABBAS; HAFEZ, 2021; AHMAD; IQBAL-ARIF; AHMAD, 2007) to Deltamethrin (RR= 20.46), Thiamethoxam (RR= 13.03), Thiamethoxam with Lambda-Cyhalothrin mixture (RR= 12.40) and Lambda-Cyhalothrin (RR=12.24), indicating potential insecticides' resistance occurrence in POPNEPO.

Meanwhile shown low resistance (ABBAS; HAFEZ, 2021; AHMAD; IQBAL-ARIF; AHMAD, 2007) for Chlorfenapyr (RR= 2.69) and Imidacloprid (RR= 2.34), and when exposed to Spinetoram (RR=0.92), Chlorpyrifos (RR= 1.06) and Cyantraniliprole (RR= 1.25) was shown as susceptible (ABBAS; HAFEZ, 2021; AHMAD; IQBAL-ARIF; AHMAD, 2007), besides that the CL_{50} of these last three is less than its own MFRD for each insecticide. We could recommend the interspersed application of these insecticides, having in their favor that they have different modes of action and that a smaller amount of product will be needed to control adult flies.

Some authors reported lower susceptibility in bioassays of larvae with treated substrate (ACEVEDO; ZAPATER; TOLOZA, 2009; FU-XING; MO; BEN-HUA, 2002; KEIDING; JESPERSEN; EL-KHODARY, 1991) different from the results obtained in the present investigation, where the larvae shown a greater susceptibility, this can be explained due to the 24-hour starvation period that the larvae were submitted after the treatment was applied.

On the contrary, other author reported a greater mortality of the larvae, which reached 100%, where no substrate was provided to the larvae and the treatment was topical, that is, the solutions (essential oil of *Lippia sidoides* 15mg/ml, thymol 30mg/ml and carvacrol 10mg/ml) were applied directly on the body of the larvae (MELO, 2014). All the insecticides tested on POPUFLA an POPNEP larvae achieved mortality greater than 50%, including azadirachtin, that previously didn't show any effect on adults, this for being a growth regulator, it does not show apparent affections (can cause inapparent affections like sterility) in the adult stage, which is the end of the biological cycle, but it can noticeably affect the juvenile stages (KRAISS; CULLEN, 2008).

Perhaps, this is possible thanks to the thin integument of the larvae that allows the passage of insecticides towards the hemolymph, in relation to the pupae that have a more structured integument with less spiracles (MULLEN; DURDEN, 2019). The use of

insecticides that reached LC_{90} in larvae, using only the MFRD, which are Imidacloprid, Chlorfenapyr and Chlorpyrifos, could be recommended as larvicides; however, these data need to be complemented with field studies, since chicken manure, the substrate where larvae grow in chicken farms, represents an additional variable to assess.

Where the best results for pupicidal control in POPNEP were obtained with the insecticide Spinetoram that reached CL_{90} only using the MFRD. Since imidacloprid is a synthetic insecticide specially formulated for the control of house flies and stable flies, it is not surprising that it has good activity as a pupicidal.

There is an interesting relationship regarding the POPNEP strain, that correlates with the studies presented by Cariño (1994), where they describe the house fly chromosome II as the main importance, which controls the other metabolic resistance genes related to P450, which together with the juvenile hormone I, present in larvae, its presence is directly proportional to modifying the response to insecticides, according to its production levels in each strain (CARIÑO et al., 1994), this would explain the direct relationship of the resistance of larvae to insecticides Deltamethrin, Lambda-Cyhalothrin and Thiamethoxam, as well as adults in POPNEP and pupae, but the latter to a lesser extent, since, as indicated by Cariño (1994), they do not have the same level of molecular activity.

However, due to the fact that the results showed different activity of the insecticides on the different life stages of the house fly and since the organic mass of rearing of these insects can be an impediment for the products to reach their aim, the author recommends only the use of insecticides for the control of adult flies, while good sanitation of the facilities is preferred for control the other stages.

7 CONCLUSIONS

-The insecticide with active ingredient Chlorpyrifos has the potential to reduce the house fly infestation, and can be used as an adulticide, larvicide or pupicidal to control our problem population (POPNEP), as it is consistent with the mortality of the three stages of the insect, followed by the active ingredients Spinetoram and by Imidacloprid; that should be applied in rotation in Pest Management Strategies**.**

- There is a correlation in the resistance results observed for POPNEP, since Deltamethrin and Lambda-Cyhalothrin belong to the chemical group of pyrethroids, while Thiamethoxam belongs to the group of neonicotinoids, both groups have

complementary activity on the sodium channels of the nervous system, the first at the axonal level and the second at the level of the receptor of the synapse.

- Although Imidacloprid belongs to the group of neonicotinoids, POPNEP turned out to have very low resistance compared to POPUFLA, due to the fact that there could be differences in the resistance mechanism, although it shares the same objective with Thiamethoxam.

REFERENCES

Abbas, N., & Hafez, A. (2021). Resistance to insect growth regulators and age-stage, two-sex life table in *Musca domestica* from different dairy facilities. *PLOS ONE*, *16*(4), 1–19. https://doi.org/10.1371/journal.pone.0248693

Acevedo, G. R., Zapater, M., & Toloza, A. C. (2009). Insecticide resistance of house fly, *Musca domestica* (L.) from Argentina. *Parasitology Research*, *105*(2), 489–493. https://doi.org/10.1007/s00436-009-1425-x

Ahmad, M., Iqbal-Arif, M., & Ahmad, M. (2007). Occurrence of insecticide resistance in field populations of *Spodoptera litura* (Lepidoptera: Noctuidae) in Pakistan. *Crop Protection*, *26*(6), 809–817. https://doi.org/10.1016/j.cropro.2006.07.006

Augère-Granier, M. (2019). The EU poultry meat and egg sector. *European Parliament*, 1–23. https://doi.org/10.2861/33350

Britto, L. G., Oliveira, M. C., Giglioti, R., Barbieri, F., Netto, F., Chagas, A., & Celestino, O. (2008). Manual de identificação, importância e manutenção de colônias estoque de dípteras de interesse veterinário em laboratório. *Embrapa Documento 125*, 30.

Cariño, F. A., Koener, J. F., Plapp, F. W., & Feyereisen, R. (1994). Constitutive overexpression of the cytochrome P450 gene CYP6A1 in a house fly strain with metabolic resistance to insecticides. *Insect Biochemistry and Molecular Biology*, *24*(4), 411–418. https://doi.org/10.1016/0965-1748(94)90034-5

Carvalho, B. (2016). *Avaliação da toxicidade de quatro solventes e um surfactante, e dos fenilpropanoides eugenol e (e)-cinamaldeído sobre larvas e pupas de* Musca domestica *Linnaeus, 1758 (*Diptera: Muscidae*)* [Universidade Federal de Juiz de Fora]. https://doi.org/10.1088/1751-8113/44/8/085201

Cisneros, F. (2019). *Control químico de las plagas agrícolas* (1st ed., Vol. 1). Sociedad Entomológical del Perú.

EPTV 2. (2019, February 6). *MP apura irregularidades em aviário por infestação de moscas em Nepomuceno, MG*. Journal News. https://g1.globo.com/mg/sul-de-minas/noticia/2019/02/06/mp-apura-irregularidades-em-aviario-por-infestacao-de-moscas-em-nepomuceno-mg.ghtml

Fu-xing, Z., Mo, W., & Ben-hua, T. (2002). DIFFERENCES IN SUSCEPTIBILITY TO INSECTICIDES BETWEEN ADULTS AND LARVAE OF HOUSEFLY, *Musca domestica* (L.). *Insect Science*, *9*(2), 23–27. https://doi.org/10.1111/j.1744-7917.2002.tb00466.x

Habibi, H., Firouzi, Sh., & Rohollahzadeh, H. (2018). THE FLIES' AS A MECHANICAL VECTOR OF AVIAN VIRAL PATHOGENS. *International Journal of Agriculture, Environment and Bioresearch*, *3*, 221–227.

Hinkle, N. C., & Hogsette, J. A. (2021). A Review of Alternative Controls for House Flies. *Insects*, *12*(11), 1042. https://doi.org/10.3390/insects12111042

IRAC. (2011). *IRAC BIOTECH Susceptibility Test Methods Series IRAC BIOTECH Susceptibility Test Methods Series*. 2–3.

Keiding, J. (1986). The Houseflies Biology and control. *Insects Biology*, 302–323.

Keiding, J., Jespersen, J. B., & El-Khodary, A. S. (1991). Resistance risk assessment of two insect development inhibitors, diflubenzuron and cyromazine, for control of the housefly *Musca domestica*. *Pesticide Science*, *32*(2), 187–206. https://doi.org/10.1002/ps.2780320207

Kraiss, H., & Cullen, E. M. (2008). Insect growth regulator effects of azadirachtin and neem oil on survivorship, development and fecundity of aphis glycines (Homoptera: Aphididae) and its predator, *Harmonia axyridis* (Coleoptera: *Coccinellidae*). *Pest Management Science*, *64*(6), 660–668. https://doi.org/10.1002/ps.1541

Lavras TV. (2019, February 7). *MP apura irregularidades em aviário por infestação de moscas em Nepomuceno*. News Article. https://lavras.tv/site/2019/02/07/mp-apura-irregularidades-em-aviario-por-infestacao-de-moscas-em-nepomuceno/

MAPA. (2022). *AGROFIT*. https://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons

Melo, D. (2014). Atividade do óleo essencial de *Lippia sidoides* (Verbenaceae) e dos monoterpenos timol e carvacrol sobre larvas e pupas de *Musca domestica* LINNAEUS, 1758 (DIPTERA: MUSCIDAE). In *Universidade Federal de Juiz de Fora* (Thesis No. 1; Vol. 1, Issue 12). Universidade Federal Juiz de Fora.

Morris Ostrolenk, & Henry Welch. (1941). The common House fly (*Musca domestica*) as a source of pollution in food establishments. *British Birds*, *1938*.

Mullen, G. R., & Durden, L. A. (2019). *Medical and Veterinary Entomology* (G. R. Mullen & L. A. Durden, Eds.; 3rd ed., Vol. 1). Elsevier, Academic Press, Book Aid International.

SAS Institute Inc. (2021). *SAS/STAT 15.1 User's Guide*. User Guide. https://documentation.sas.com/doc/en/pgmsascdc/9.4_3.4/statug/titlepage.htm

Siqueira, H. A. A., Guedes, R. N. C., Fragoso, D. B., & Magalhaes, L. C. (2001). Abamectin resistance and synergism in Brazilian populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *International Journal of Pest Management*, *47*(4), 247–251. https://doi.org/10.1080/09670870110044634

Systat Software. (2003). *SigmaPlot 12.5 User's Guide* (pp. 1–455). Systat Software. chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/viewer.html?pdfurl=http%3A%2F%2F131.130.57.230%2Fclarotest190%2Fclaroline%2Fbackends%2Fdownload.php%3Furl%3DL1N0YXQvU2lnbWFQbG90MTI1LVVzZXItR3VpZGUucGRm%26cidReset%3Dtrue%26cidReq%3D300358WS18&clen=12407695&pdffilename=SigmaPlot125-User-Guide.pdf

Urban, J. E., & Broce, A. (2000). Killing of flies in electrocuting insect traps releases bacteria and viruses. *Current Microbiology*, *41*(4), 267–270. https://doi.org/10.1007/s002840010132

WANARATANA, S., PANYIM, S., & PAKPINYO, S. (2011). The potential of house flies to act as a vector of avian influenza subtype H5N1 under experimental conditions. *Medical and Veterinary Entomology*, *25*(1), 58–63. https://doi.org/10.1111/j.1365-2915.2010.00928.x

CHAPTER II

Comparative life tables of two field collected populations of the house flies *Musca domestica* L. (Diptera: Muscidae)

ABSTRACT

Musca domestica L. (Diptera: Muscidae); is a ubiquitous dipteran of economic and sanitary importance reported to easily develop resistance to insecticides. Insecticide resistance is widely assumed to carry fitness costs. Here, life histories of a susceptible (POPUFLA) and a neonicotinoid and pyrethroid resistant population (POPNEP) were comparatively assessed through an Age-stage, two-sex and fertility life-table. Cohorts of 70 one-hour-old housefly eggs of each population were randomly collected from different egg batches and individualized in glass tubes. The eggs were observed under a stereoscope, 1.5 g of rearing substrate was added to the tube after larvae hatching and the larvae were monitored through their development until adult's emergence. The biological parameters evaluated until adult emergence included egg, larvae, pupa and adult durations, oviposition period, sex ratio. At adult stage, 20 couples of virgin flies were formed for each population and their survival and females' fertility assessed to construct the fertility life table. The results revealed significant statistical differences between the two populations for the egg (H= 46.99; *df* =1; *P* <0.001) and the third larval stage (H= 33.91; *df* =1; *P* <0.001) durations, which prolonged the total juvenile mean duration of POPNEP (15.71 ± 0.38 days) compared to POPUFLA (15,69 ± 0.25 days). Furthermore, the female flies of POPNEP presented higher intrinsic rate of increase (rm = 0.197 females / female/ day) but longer mean generation time (T = 33.21 days) compared to POPUFLA (rm= 0.192; T= 31.36). Therefore, under laboratory conditions, our findings suggest that although the resistant population POPNEP presented a fitness cost in terms of longer development times, it presents reproductive advantage over the susceptible population POPUFLA that may explain its reported uncontrolled growth under natural conditions. These encountered developmental cost and reproductive advantage should further be studied and verified under field conditions.

Keywords: Fitness cost, Reproductive advantage, House flies.

1 INTRODUCTION

Musca domestica L. (Diptera: Muscidae) is a ubiquitous insect pest of poultry and livestock in general, attracted to human activities, their food and waste (KEIDING, 1986). These behavioral habits make them extraordinary disease vectors, capable of spreading more than a hundred diseases in humans and animals (ZAHRADNÍK; CHVALA, 1990) just like as bacteria that cause diarrhea, dysentery, anthacnosis, typhoid fever, anthracnose, ophthalmic infections, they are also vectors of eggs of the most common parasitic helminths such as *E. vermicularis, S. stercoralis, T. trichiura* and *T. canis*, *Hymenolepis* and taenias also protozoans such as *Trichomonas duodenalis* or *Entamoeba histolytica* (ISSA, 2019). In poultry were reported diseases such as New Castle, infectious bronchitis, infectious bursitis (HABIBI; FIROUZI; ROHOLLAHZADEH, 2018) and the viruses H5N1 known as bird flu (WANARATANA; PANYIM; PAKPINYO, 2011) as the most common. Selection for resistance to insecticides is a constant problem in poultry and livestock activities, due to the inappropriate use of the pyrethroids and neonicotinoids (LEVCHENKO et al., 2019).

Life tables are made up of comprehensive data sets regarding the survival, development and fecundity of a population, that is, it records the probabilities of organisms in the population to live, die and reproduce at different stages of their lives (CAREY, 2001). In this study, the growth characteristics of two populations of houseflies were evaluated, one of them without a history of exposure to insecticides (POPUFLA) and the other with a history of exposure to insecticides (POPNEP), which will be key to implement an efficient Integrated Pest Management, in balance with the environment (BLOUQUY et al., 2021), this helped us to understand the effects and fitness costs that have appeared after insecticide resistance have developed in POPNEP (ABBAS; HAFEZ, 2021). For instance, in the phenomenon called population compensation, where the few individuals who survive exposure to a toxicant, having more available resources, experience a population explosion that covers the niche left, in a few generations, with the advantage of being a less susceptible population, to that toxic (STARK; SUGAYAMA; KOVALESKI, 2007). The influence of insecticides can be analyzed through the biological parameters of life tables such as the reproductive rate (R_0) that is the contribution of newborn females per generation to the next generation, the mean generation time (T), the intrinsic rate of increase (rm) that is the rate of natural increase in a cohort, the finite rate of increase (λ) which represents the number of individuals added

to the population per unit of time, and the doubling time (D) that is the time required for the population to double in number of individuals (STARK; SUGAYAMA; KOVALESKI, 2007).

The Age stage two-sex life table include both sexes by age stage. When all males are ignored, valuable information about the species is lost, since many species of insects, both male and female, cause damage, making decision-making to control that pest inefficient, because it is based on a percentage of individuals smaller than the real one (CAREY, 2001).

The parameters commonly evaluated in the age-stage, two-sex life tables of insects are egg, larvae, pupa and adult durations, oviposition period, sex ratio, female fertility, female and male survival rates (ROCKSTEIN; LIEBERMAN, 1959).

In order to have standardized life tables, it is necessary to collect cohorts of individuals of the same age. Thus, for house fly, cohort collection should start with the eggs laid in the same hour to then continue through the consequent phases of development (ROCKSTEIN; LIEBERMAN, 1959).

This theoretical knowledge should not be underestimated since it is useful to understand the state of resistance in the development of this pest and develop control strategies not only momentarily, but also to be applied in long-term control (LI; HUANG; YUAN, 2018).

2 OBJECTIVES

2.1 General objective

To comparatively evaluate the life histories of *M. domestica* of a susceptible population (POPUFLA) and a population resistant to neonicotinoids and pyrethroids (POPNEP) through an Age-stage, two-sex, and fertility life-table.

2.2 Specific objective

- Record the development parameters of all the life stages on POPUFLA and POPNEP.
- Record and evaluate the fertility life table on POPUFLA and POPNEP.

3 HYPOTHESIS

-H1: POPNEP, resistance to synthetic insecticides, is affected in their biological parameters when compared to the non-resistant population.

-H0: The biological parameters were similar between POPUFLA and POPNEP

4 MATERIAL AND METHODS

4.1 Location of experiments

The experiments were carried out at the Laboratory of Molecular Entomology and Ecotoxicology in the Department of Entomology at the Federal University of Lavras (DEN-UFLA) located in Lavras, Minas Gerais – Brazil.

4.2 Origin and maintenance of the colony of *Musca domestica*

The first population originated from wild adults, collected on the campus of the Universidade Federal de Lavras and presumed to be without a history of insecticide application. This population was named POPUFLA. The second one was obtained near the surroundings of a conventional poultry farm in the Nepomuceno commune, which is presumed to have a history of previous exposure to insecticide applications. This population was named POPUFLA.

The parental house flies (100 individuals) of each population were caught with an insect-collector-net, and after being properly identified as *Musca domestica*, were reared separately in acrylic entomological cages of 30 x 30 x 60 cm conditioned with a voile sleeve to facilitate air circulation, the handling, and maintenance of the colonies, avoiding contaminations, where they were breaded.

4.3 Life table construction

Following the modified method of Abbas et al (2021), to construct the life table of the house fly, for each population, 70 one-hour-old house fly eggs were randomly collected from different egg batches and placed singly, using a fine paintbrush, inside a 9-cm-high flat-bottomed test tube. Each egg represents a replica, that is, 70 replicas were

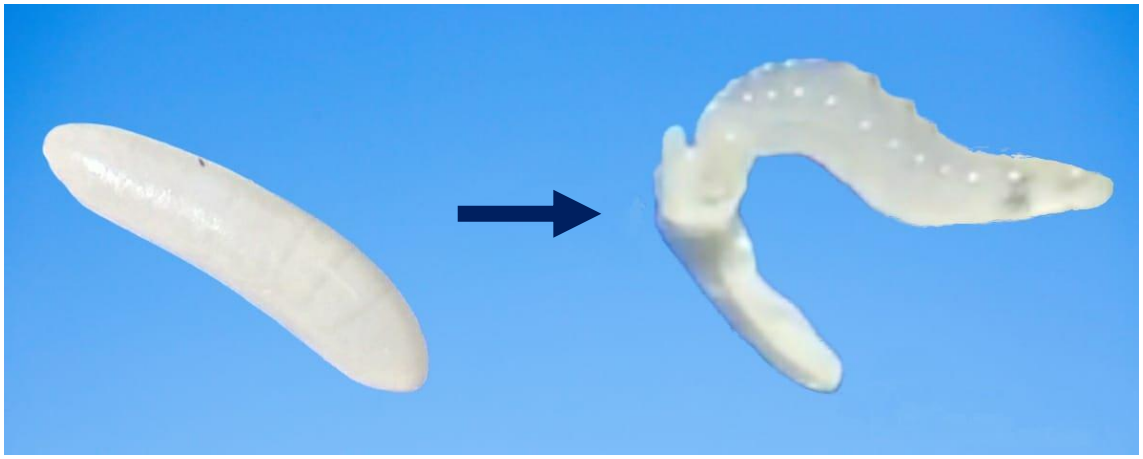
installed per population (ABBAS; HAFEZ, 2021). The eggs were observed under a stereoscope until hatching (Figure 11), recording the duration of the egg phase.

Immediately after the hatching, 1.5 g of the larval rearing substrate, prepared 24 hours in advance, was added to the test tube. The tubes were covered with voile fabric and fastened with an elastic band (ABBAS; HAFEZ, 2021) (Figure 12).

The larvae were observed through their development recording individually the duration of the first, second, and third larval stages and the pupae, the weight of the pupae, the date of emergence of the adults, and the weight of the exuviae.

The adults, were sexed and paired, forming couples of one male and one female from the same population and placed in mini-cages made of plastic pots and voile sleeve. The total number of couples obtained were 20 for POPUFLA and 22 for POPNEP. They were supplied with water, food and substrate for oviposition (ABBAS; HAFEZ, 2021) (Figure 13). Subsequently, the eggs of each pair were collected every day and counted until the death of the female, recording the number of eggs for each collection, the number of times that the oviposition occurred and the total sum of eggs per couple. The eggs of the couples were reared in plastic glasses with larval rearing substrate, until the emergence of adults, counting the number of individuals that completed their development up to adults and sexing them (ABBAS; HAFEZ, 2021).

Figure 11 – Exact time of larva emergence. The time of the egg phase was delimited by two events, the first was the time of oviposition and the second was the time of emergence of the L1 larva from the egg.



The eggs were observed under a stereoscope until the time of hatching was recorded.

Figure 12 – Experimental units used for larvae growing.

The eggs that were previously isolated, when the larva emerged, were immediately supplied with the necessary substrate for its complete growth.

Figure 13 – Mini-cages with house fly couples (virgin male and female)

Once sexed, they were placed in couples with a source of food, water and oviposition substrate.

4.4 Data analyses

Data were analyzed using One Way Analysis of Variance and in case the presumptions of ANOVA were not met the non-parametric Kruskal-Wallis test was used. For the offspring, average cumulative numbers of female progeny emerged daily were analyzed using non-linear regressions. The statistical analyses were carried out in SigmaPlot 14.5 (SYSTAT SOFTWARE, 2003).

To estimate the parameters of the Fertility table, the following algorithms were used: reproductive rate [$R_0 = \sum$ lx.mx], the mean generation time [$T = (\sum x.lx.mx) / (\sum lx.mx)$], the intrinsic rate of increase [rm= Ln(Ro) / T], the finite rate of increase [$\lambda = erm$] and doubling time [D= ln(2) / rm] (BUSATO et al., 2004).

5 RESULTS

Analysis of Kruskal-Wallis showed that the resistant population POPNEP had a significant statistical difference in relation to the susceptible population POPUFLA. Starting with the development time of the egg that for POPNEP was longer (H= 46.99; *df*=1; *P* <0.001).

The difference in development time became more evident during the LIII stage, where again for POPNEP was longer (H = 33.911; *df* =1; *P* <0.001). Both accumulated differences affected the final time of the total development of the flies that were used as progenitors (H = 47.857; *df* =1; *P* <0,001).

Indeed, the progenitors of POPNEP had delayed development with respect to POPUFLA. Regarding the other parameters such as duration of Larva I, duration of Larva II, duration of the pupa, Longevity of females, Longevity of males and Fecundity there was not statistically significant difference (Table 5).

Table 5 – Biological parameters of age-stage, two-sex life tables of two field collected populations of house flies POPUFLA and POPNEP.

Biological parameters	N	POPUFLA median ± SEM	N	POPNEP median ± SEM	*df*	*P*
Egg duration (days)	40	0.69 ± 0.01	48	0.71 ± 0	1	≤ 0.001
First larval stage duration (days)	40	1 ± 0	48	1 ± 0	1	1
Second larval stage duration (days)	40	2 ± 0.08	48	2 ± 0	1	0.27
Third larval stage duration (days)	40	3 ± 0.27	48	4 ± 0.24	1	≤ 0.001
Pupa duration (days)	40	9 ± 0.24	48	9 ± 0.33	1	0.28
Total duration egg to adult (days)	40	15.69 ± 0.25	48	15.71 ± 0.38	1	≤ 0.001
Longevity female (days)	20	42 ± 7.1	22	38 ± 6.3	1	0.37
Longevity male (days)	20	23.4 ± 6.2	22	24.9 ± 5.6	1	0.6
Fecundity/female (number of eggs per day)	16	464 ± 136	19	318 ± 635	1	0.08
Sex ratio*	16	52.6 ± 9	19	66.6 ± 11.7	1	0.49
Pupal weight (milligrams)	40	20 ± 0	48	19 ± 0	1	0.9
Exuvial weight (milligrams)	40	1.2 ± 0	48	1.3 ± 0	1	0.23
Adult weight (milligrams)	40	18.3 ± 0	48	17.8 ± 0	1	0.083

* = Number of females/Total number of progeny
SEM= Standard errors of means

Below are the values of the fertility table that allows understanding and comparing the population dynamics, survival and reproduction rates of POPUFLA and POPNEP. The differences between the populations are statistically significant (H= 4.50; *df*=1; *P*= 0.034), Where the POPNEP population shows a clear reproductive advantage compared to POPNEP, because in POPNEP a female can produce a greater number of offspring during her lifetime (R_0), with higher intrinsic and finite growth rates, which requires less time to double her population with respect to POPUFLA and whose only disadvantage is having a mean generation time (T) greater than POPUFLA. (Table 6).

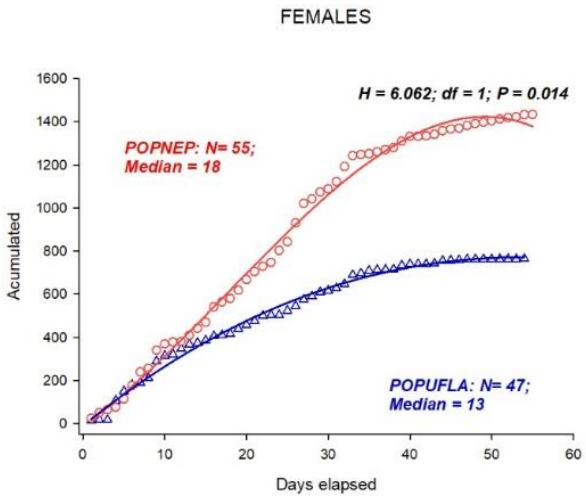
Table 6 –Fetility life table for two field collected populations of house flies POPUFLA and POPNEP

	Reproductive rate R_0 (females)	Mean generation time T (days)	Intrinsic rate of increase r_m (females / female/ day)	Finite rate of increase λ (females / day)	Doubling time D (days)
POP UFLA	423.25a	31.363a	0.192a	1.211a	3.61a
POP NEP	698.73b	33.211b	0.197b	1.217b	3.51b

-Means in column followed by the same letter are not significantly different by Tukey method

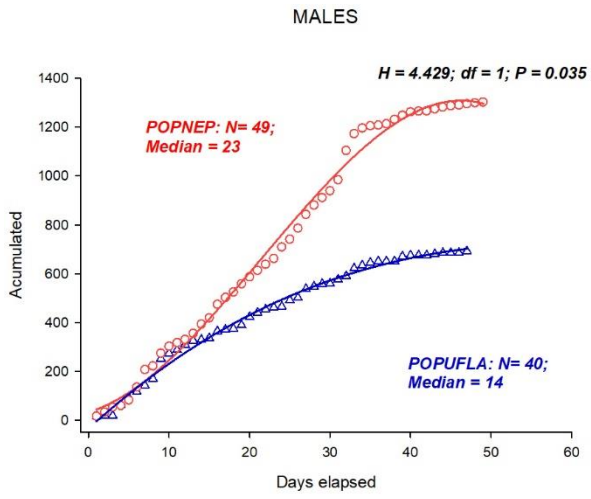
As for the offspring, there was a statistically significant difference, for both, female (H= 6.06; *df* =1; *P* = 0.014) and male (H= 4.43; *df* =1; *P* = 0.035) progeny. As shown in Figure 14, corresponding to the progeny of females, a marked ascending red curve, corresponding to POPNEP that separates above the female curve from POPUFLA, clearly showing a greater number of adult females.

Figure 14 – Cumulative average of female progeny emerged daily.



As for Figure 15, corresponding to the progeny of males, a marked ascending red curve can be seen, corresponding to POPNEP that separates above the male curve from POPUFLA, clearly showing a greater number of adult males for POPNEP, in a clear population advantage of the progeny of POPNEP over POPUFLA.

Figure 15 – Cumulative average of male progeny emerged daily.



6 DISCUSSION

The findings that have been exposed are consistent to other studies, where populations with a history of insecticides show different biological parameters than those that do not have a history of insecticides, affecting significantly the development, survival, and reproduction of house fly populations.

Exposure to sublethal doses of insecticide leads to altered gene expression that modifies the level and activity of proteins and, consequently, the physiology of the insects; These effects can be transgenerational, such is the case of sublethal concentrations of Cyantraniliprole, that decreased the growth speed of *Agrotis ipsilon* (BANTZ et al., 2018). This can explain the important variation in the duration of the egg's development and also the growth of the third instar larva between POPUFLA and POPNEP, which may mean an adaptive cost manifested in the total time of development of the POPNEP.

As for the duration parameter of the larvae III, since both populations were fed with the same substrate and raised under the same conditions of temperature and humidity, this result cannot be attributed to the nutritional quality. According to Abbas (2021), in addition to the sublethal effects, could be due to the difference in climatic conditions at the sampling sites where the parental house flies were collected (ABBAS; HAFEZ, 2021).

Another differential parameter to take into account is the ability to produce a greater number of POPNEP offspring (females and males) over POPUFLA, which is consistent with the reproductive rate (R_0) from the fertility life table, when POPNEP (R_0) = 698,73 > POPUFLA (R_0) = 243.25; explaining its pest condition. In the same direction, the demographic parameters of POPNEP shows that each female is replaced by 698.73 females in an average time of 33.21 days, higher than POPUFLA where each female is replaced by 423.25 females in an average time of 31.36 days. Consistent with the claims of other studies that mention that species can promote epigenetics to trigger adaptation to insecticides, that the more offspring appear with the inherited condition, the faster their offspring will adapt and survive (BANTZ et al., 2018)

Since the "Doubling time (D)" is 3.61 and 3.51 for POPUFLA and POPNEP respectively, while the "Mean generation time (T)" is 31.363 and 33.211, there will be an overlapping of generations, once the age reproductive. Fortunately, the climatic

conditions of nature and its predators prevent the potential reproductive capacity from reaching its maximum levels in reality.

Regarding the longevity of the adult flies, the females of both populations outlived the males, with differences in the POPUFLA adults being longer than those of POPNEP. It is known that females generally only copulate once (KEIDING, 1986), so it is understood that, taken into reality, male flies will continue to generate the same level of economic and health damage and nuisance in humans and animals, even when their reproductive function has been completed.

7 CONCLUSIONS

-The fertility life table can be used to differentiate the populations of *M. domestica*, regardless of the collection site, providing basic information for the understanding of its life cycle, reproductive capacity and survival in controlled laboratory conditions for both populations.

-It is evident that POPNEP has managed to develop a greater reproductive potential than POPUFLA, despite its delayed development time, attributed to its previous history of contact with insecticides. Molecular studies are needed for confirmation.

-Since the development of resistance depends on the type and period of application of insecticides, and the fact of the insecticides used in the bioassays, only Imidacloprid had specifications for use on house fly control, while the other nine are for general agricultural use, which does not exclude their possible use in the control of house flies, but it is of responsibility of the personnel in charge of the elaboration of the Integrated Pest Management, to select the correlation of the insecticides that will be applied over time, the times that they will be applied before changing to the next one and especially is expected, that the formulation to be used, have the least possible impact on the environment.

GENERAL CONCLUSIONS

-The dose-response curves and the resistance ranges obtained in the first chapter confirm the existence of a difference in susceptibility to insecticides, with POPUFLA being the most sensitive and POPNEP the least sensitive, and when evaluating the biological parameters of the second chapter we found a "fitness cost" evidenced in the delayed development and in the greater Mean generation time (T) of POPNEP; which is based on the molecular-bases that explain that sublethal exposures to insecticides induce physiological changes that are transgenerational, which while allowing them to trigger detoxification mechanisms, that generates a cost in another aspect of their physiology.

- The biological parameters recorded will be useful in estimating the size of the population that will have to be controlled over time, while the lethal concentrations recorded and their respective insecticides should allow to establish monitoring and sampling plans for this important pest of poultry farms as a first step in developing IPM programs

-Studies with synergists such as piperonyl butoxide (PBO), diethyl malonate (DEM) and triphenyl phosphate (TPP) need to be implemented to complement the findings with real data at the molecular level.

REFERENCES

Abbas, N., & Hafez, A. (2021). Resistance to insect growth regulators and age-stage, two-sex life table in *Musca domestica* from different dairy facilities. *PLOS ONE*, *16*(4), 1–19. https://doi.org/10.1371/journal.pone.0248693

Bantz, A., Camon, J., Froger, J.-A., Goven, D., & Raymond, V. (2018). Exposure to sublethal doses of insecticide and their effects on insects at cellular and physiological levels. *Current Opinion in Insect Science*, *30*, 73–78. https://doi.org/10.1016/j.cois.2018.09.008

Blouquy, L., Mottet, C., Olivares, J., Plantamp, C., Siegwart, M., & Barrès, B. (2021). How varying parameters impact insecticide resistance bioassay: An example on the worldwide invasive pest *Drosophila suzukii*. *PLOS ONE*, *16*(3), e0247756. https://doi.org/10.1371/journal.pone.0247756

BUSATO, G., GRÜTZMACHER, A., GARCIA, M. S., GIOLO, F.; ZOTTI, M., & MAGALHÃES, T. R. (2004). TABELA DE VIDA DE FERTILIDADE DE POPULAÇÕES DE *Spodoptera frugiperda* (J.E. SMITH, 1797) (LEPIDOPTERA: *NOCTUIDAE*) EM FOLHAS DE MILHO E ARROZ IRRIGADO. *R. Bras. Agrociência*, *10*(4), 449–455. file:///C:/Users/Admin/Downloads/1005-1424-1-PB.pdf

Carey, J. R. (2001). INSECT BIODEMOGRAPHY. *Annual Review of Entomology*, *46*(1), 79–110. https://doi.org/10.1146/annurev.ento.46.1.79

Habibi, H., Firouzi, Sh., & Rohollahzadeh, H. (2018). THE FLIES AS A MECHANICAL VECTOR OF AVIAN VIRAL PATHOGENS. *International Journal of Agriculture, Environment and Bioresearch*, *3*, 221–227.

Issa, R. (2019). *Musca domestica* acts as transport vector hosts. *Bulletin of the National Research Centre*, *43*(1), 73. https://doi.org/10.1186/s42269-019-0111-0

Keiding, J. (1986). The Houseflies Biology and control. *Insects Biology*, 302–323.

Levchenko, M. A., Silivanova, E. A., Balabanova, G. F., & Bikinyaeva, R. H. (2019). Insecticide susceptibility of house flies (*Musca domestica*) from a livestock farm in Tyumen region, Russia. *BULGARIAN JOURNAL OF VETERINARY MEDICINE*, *22*(2), 213–219. https://doi.org/10.15547/bjvm.2027

Li, Q., Huang, J., & Yuan, J. (2018). Status and preliminary mechanism of resistance to insecticides in a field strain of housefly (*Musca domestica*, L). *Revista Brasileira de Entomologia*, *62*(4), 311–314. https://doi.org/10.1016/j.rbe.2018.09.003

Rockstein, M., & Lieberman, H. M. (1959). A life table for the common house fly, *Musca domestica*. *Gerontology*, *3*(1), 23–36. https://doi.org/10.1159/000210828

Stark, J. D., Sugayama, R. L., & Kovaleski, A. (2007). Why demographic and modeling approaches should be adopted for estimating the effects of pesticides on biocontrol agents. *BioControl*, *52*(3), 365. https://doi.org/10.1007/s10526-006-9040-6

Systat Software. (2003). *SigmaPlot 12.5 User's Guide* (pp. 1–455). Systat Software. chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/viewer.html?pdfurl=http%3A%2F%2F131.130.57.230%2Fclarotest190%2Fclaroline%2Fbackends%2Fdownload.php%3Furl%

3DL1N0YXQvU2lnbWFQbG90MTI1LVVzZXItR3VpZGUucGRm%26cidReset%3Dtrue%26cidReq%3D300358WS18&clen=12407695&pdffilename=SigmaPlot125-User-Guide.pdf

WANARATANA, S., PANYIM, S., & PAKPINYO, S. (2011). The potential of house flies to act as a vector of avian influenza subtype H5N1 under experimental conditions. *Medical and Veterinary Entomology*, *25*(1), 58–63. https://doi.org/10.1111/j.1365-2915.2010.00928.x

Zahradník, J., & Chvala, M. (1990). *La gran enciclopedia de los insectos* (M. Lang, Ed.; 1st ed., Vol. 1). Susaeta ediciones s.a.