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Biological Control of Diptera Calliphoridae: A Review

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ABSTRACT

The Calliphoridae family (order: Diptera), also known as blowflies, has a holometabolous cycle which spans across three larval instars. During development, they appear vermiform and exhibit necrophagous habits. It has been suggested that these organisms are responsible for the development of myiasis and transmission of pathogens to humans and animals. Biological control refers to the regulation of the number of pests by their respective natural enemies. It is an event that occurs naturally in the environment or due to mass creation and subsequent release of the controller in the laboratory. The objective of this review was to study the main biological controllers of the Calliphoridae family through a bibliographical survey of the last 10 years (2007-2017). The use of biological controllers avoids damages to the environment. One form of biological control of blowflies is to use plant extracts, which provides a great diversity of sustainable species to choose from. Additionally, it poses no harm to human health. Parasitoids, predators, bacteria and entomopathogenic fungi have also been tested. Though the results are promising, further studies are necessary before implementation in the society.

Key words: Entomopathogenic microorganisms, Parasitoids, Predators, Plant extracts.

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INTRODUCTION

Biological control refers to the management of agricultural pests, transmitter or disease-causing insects using natural enemies such as predators, parasitoids, and microorganisms. This method of control is more suitable than chemical pesticides since it leaves no residue in nature and is harmless to human health, thus promoting sustainability and protection of the environment (Parra, 2002).

Entomopathogenic microorganisms form the basis of bio-insecticides or biological insecticides. The advantages of its use are: high specificity, low risk to environment and human health, lower induction of insect resistance to the compound, and increased possibility of reproduction of the microorganism in the environment. The main disadvantages include greater susceptibility to environmental conditions and shorter shelf life, which may be minimized through good formulations (Angelo, Vilas-Bôas, & Castro-Gómez, 2010).

Commonly used microorganisms are *Bacillus thuringiensis* (Berliner, 1915) (Bt), *Wolbachia pipientis* (Hertig, 1936), and *Beauveria bassiana* (Balsamo-Crivelli & Vuillemin, 1912), and the fungus *Metarhizium anisopliae* (Metchnikoff & Sorokin, 1883). The entomopathogenicity of Bt is due to the crystal proteins (Cry) produced during their sporulation. These Cry proteins interact with and paralyze the microvilli of an insect's digestive tract, leading to death by starvation, general paralysis of the muscles or by septicemia (Angelo et al, 2010). *W. pipientis* acts by reducing the longevity of the insect, thus preventing it from acting as a carrier for the transmission of diseases such as dengue, zika, chikungunya and malaria (Oliveira & Moreira, 2012).

Spores of *B. bassiana* penetrate the insect cuticle thereby gaining access to its internal organs. Upon infection, the bacteria release toxins that interfere with the development of the insect leading to death (Feijó et al, 2008). The effect of *M. anisopliae* is similar to that of *B. bassiana*, where the spores colonize the insect through hyphal growth, followed by release of toxins that lead to death of the host (Mahdi, 2015).

The Calliphoridae family of the order Diptera, popularly known as blowflies, cause serious damage to livestock, as well as human health. These necrophagous insects are vectors of several diseases and cause primary and secondary myiasis in humans and animals. In a study conducted by Hadi (2013), 15 species of intestinal parasites, eggs, cysts and oocytes were isolated and identified from two species of Calliphoridae (*Chrysomya albiceps* (Wiedemann, 1819) and *Chrysomya megacephala* (Fabricius, 1794)). Eight of these were identified as nematode eggs and two as protozoan cysts.

According to Sandeman et al (2014), several studies in the last 50 years have been directed toward the control of *Lucilia cuprina* (Wiedemann, 1830), a major infestation affecting the sheep industry in Australia. Although, the use of chemical insecticides resolved the problem initially, the insect has begun to show resistance to several of these products making it necessary to come up with newer and better control methods.

The objective of this work was to carry out a bibliographical survey of the articles published in the last 10 years on the biological control of Diptera: Calliphoridae blowflies that cause primary and secondary myiases, and transmission of pathogens to humans and animals.

MATERIALS AND METHODS

A bibliographic survey was carried out on the biological control of the Calliphoridae family of the order Diptera, using the key words (Calliphoridae, fungi and entomopathogenic bacteria, biological control, predators, parasitoids and plant extracts) of articles published in Portuguese and English in the last 10 years (2007-2017) through the following platforms: *Portal Capes*, Science direct and Google Scholar. The most relevant literature were referred for the development of the review.

RESULTS

Thirty-one relevant scientific papers were selected for the development of the review, of which twenty-seven were published between the period 2007 and 2017, and four were prior to 2007 (Table 1).

Table 1. Number of scientific papers related to Biology of Calliphoridae, Biological control, Entomopathogenic microorganisms, Predators and Parasitoids, totaling thirty-one papers.

Papers	**Number of papers**
Biology of Calliphoridae	**8**
Biological control	**1**
Entomopathogenic microorganisms	**8**
Predators	**1**
Parasitoids	**7**
Plant extracts	**6**
Total	**31**

DISCUSSION

Calliphoridae

From the Calliphoridae family, 1020 species have been recognized worldwide, of which around 100 are Neotropical. The thorax and abdomen of these insects show metallic coloration, usually blue, green, or black. The antenna is tri-segmented, having feathery edges and well-developed calypters (Carvalho & Ribeiro, 2000). The female insects rely on decomposing matter for their protein needs, required for both nutrition and oviposition (Chaiwong et al, 2012).

Blowflies show a holometabolous life cycle, with three larval instars. Female blowflies can deposit up to 2000 eggs throughout its life. The time required for development is temperature dependent and accelerated with increase in temperature. The larvae of the Calliphoridae family show vermiform appearance and necrophagous habits (Donato & Liria, 2016).

The economic importance of blowflies is varied because of its ability to infest vertebrates (myiasis). The *L. cuprina* species is responsible for estimated losses of up to $170 million per year in the sheep industry in Australia (Sanderman et al, 2014). Apart from damage to livestock, some species also cause myiasis in humans, such as *Cochliomyia hominivorax* (Coquerel, 1858) and *Chrysomya bezziana* (Villeneuve, 1914) which cause primary myiasis, and *Cochliomyia macellaria* (Fabricius, 1775) which cause secondary myiasis (Zumpt, 1965). Another problem caused by these blowflies is the transmission of pathogens that cause diseases in animals and humans. The genera *Cochliomyia* sp. and *Chrysomya* sp. are known to act as vectors of infectious bacteria (Maldonado & Centeno, 2003; Paraluppi, Vasconcelos, Aquino, Castellón, & Silva, 1996). Due to their necrophagous habits, microorganisms of the genus *Mycobacterium* sp. (Lehmann & Neumann, 1896) can stay in the insect, making it a vector. The bacterium that causes paratuberculosis in pigs, birds and cattle (*Mycobacterium avium*) have been isolated from blowflies in different experiments (Fischer et al, 2001).

Due to these characteristics, several studies have been aimed at the biological control of blowflies. Most of these studies are related to the use of entomopathogenic microorganisms as a means of biological control.

Biological control

Biological control is a naturally occurring event in which the number of animals belonging to a particular species is limited through the action of their respective natural enemies. Humans observed this natural phenomenon and began to use it to their advantage, by studying the relation between pests and their natural enemies, followed by mass creation and release of these enemies in order to control the pests (Parra, 2002).

Biological control can occur in two ways. First, without human interference (natural event) and second, human-mediated, either by manipulating or introducing organisms capable of controlling the pest. Biological control can be categorized into four types: (i) artificial control, where man interferes positively for the increase of the natural enemies; (ii) classical control, which occurs through importation and colonization of natural enemies for the control of exotic pests; (iii) natural control, which refers to the population of naturally occurring enemies found in the location; and (iv) applied control, which deals with the dissemination of natural enemies created or mass-produced in the laboratory (Parra, 2002).

On the basis of the relationship with natural enemies, a parasite can be defined as an organism that needs a host to complete its life cycle, but most of the time do not lead to host mortality. On the other hand, parasitoids need a host to complete only part of their developmental evolution; parasitoids emerging as adults from the hosts cause host mortality. Predators lead a free life and feed on pests, and entomopathogens are microorganisms (fungi, viruses, bacteria, nematodes, and protozoa) that are used to control insect pests (Parra, 2002).

Biological control of Calliphoridae

Entomopathogenic bacteria

Several works have been carried out using entomopathogenic bacteria against Diptera: Calliphoridae flies. These include the use of bacteria such as *Brevibacillus laterosporus*, *Bacillus thuringiensis* and *Wolbachia pipientis*. Studies by Carramaschi, Pereira, Queiroz, & Zahner, 2015; Carramaschi et al, 2017 made use of strains of *Brevibacillus laterosporus* that showed control activity against *Chrysomya megacephala*, where a 70% mortality of the larvae could be achieved at a concentration of 1×10^8 spores/g diet. The activity of this bacterium resembles that of Bt, where after the ingestion of the spores by the insect, released toxins interact with intestinal receptors, causing death of the insect by starvation, septicemia and paralysis of the organ. This alteration in the intestine was confirmed by transmission electron microscopy. Pessanha, Carramaschi, Mallet, Queiroz, & Zahner (2015) also used strains of *Brevibacillus laterosporus* to control larvae of *Lucilia cuprina*, describing results ranging from 29% to 54% mortality.

Using different commercial concentrations of *Bacillus thuringiensis israelensis*, Mehdi & Noshee (2015) determined mortality of *C. albiceps* second instar larvae as 30% and 63.33% for 100 and 2000 parts per million (ppm), respectively. Larvae treated with 1000 ppm of the bacterial formulation had a 30% mortality rate after 2 days and 72.96% after 12 days while adult mortality of 6.67 and 73.33% occurred when flies were treated with 100 and 200 ppm respectively, thus indicating the potential of *B. thuringiensis* for the control of *C. albiceps*.

Wolbachia bacteria are known to cause reproductive abnormalities in their hosts, such as cytoplasmic incompatibility, feminization, parthenogenesis, and lethality in males. According to Mingchay et al. (2014), endosymbiotic bacteria were detected in natural populations of Calliphoridae, Sarcophagidae and Muscidae flies in different regions of Thailand. *Wolbachia* supergroups A and B were identified by PCR, which can be used for future research on fly control programs.

The application of entomopathogenic bacteria against Calliphoridae presents efficient results of mortality in laboratory, manly with *B. thuringiensis* and *B. laterosporus*. However, we observed only commercial formulations of Bt in the studies that we reviewed here, which suggests that additional studies are necessary on bacteria that are useful.

Entomopathogenic fungi

Like the use of bacteria, fungi present themselves as an alternative in the control of the Calliphoridae family. The main species used are *Metarhizium anisopliae* and *Beauveria bassiana*.

According to Mahdi (2015), *Metarhizium anisopliae* was grown in rice, potato and wheat broths; its secondary metabolites were filtered and then applied under the larvae and adults of *Chrysomya albiceps*. The results showed that the potato filtrate was the most efficient, with 46.67% mortality in second instar larvae, while the rice and wheat filtrates obtained accumulated mortality of 36.67% for larvae and 40% for pupae. Adults treated with potato filtrate had a mortality rate of 66.67%, reaching 90% after one week.

The use of *Beauveria bassiana* on eggs, larvae and adults of *C. albiceps* demonstrated that the fungus does not present a great inhibition in the rate of hatching of eggs, which the authors explained as non-specificity to the host in addition to the physical barrier posed by the chorion. Third-instar larvae infected with the fungus did not present significant differences in relation to the control during pre-pupal and pupal periods, due to the salivary action of these immature developmental stages. However, the rate of emergence and longevity of adults was reduced with increasing concentration of the microorganism. Thus, the fungus presents potential for control of this fly but further studies focused on the standardization of inoculum concentration must be carried out to develop products that could be commercially viable (Feijó et al, 2008).

The re-isolation of *Beauveria bassiana*, after passage under eggs, larvae and adults of *C. albiceps*, was carried out and the cytological aspects of the fungus were studied. The results show that it did not present significant differences related to its behavior and cytological aspects, indicating its high degree of adaptability, exhibiting its preference for insect-pest control (Feijó, Lima, Alves, & Lima, 2007).

The evaluation of the pathogenic action of *Metarhizium anisopliae*, *Beauveria bassiana* and *Paecilomyces fumosoroseus* on egg, larva and adult stages of *C. putoria* was performed and presented the following results. The fungi *B. bassiana* and *M. anisopliae* showed 100% mortality of the larvae at the concentration of 10^8 conidia/mL. The isolate JAB 07 of *B. bassiana* when inoculated into pupae significantly reduced the emergence of adults. In relation to adults, there were only survival reductions at the concentration of 10^8 conidia/mL of *M. anisopliae* and *B. bassiana* (Yoshida, 2007).

Similar to bacteria, fungi exhibit a good control of the blowflies, but we did not observe any commercial formulation, so the use of this form of biological control is not available commercially. Therefore, further studies are necessary to determine the efficiency of fungi when exposed to the environment.

Predators

Botteon, Neves, & Godoy (2016) used third instar larvae of *Chrysomya putoria* as diet for the predator widely used in biological control, *Podisus nigrispinus* (Hemiptera: Pentatomidae), concluding that these larvae serve as a diet for predator breeding. This result indicates that biological control by means of this predator presents potential to be used on a large-scale.

The use of predators on Calliphoridae is scarce in literature. Our survey showed only one piece of evidence regarding the usage of *P. nigrispinus*, although it did not confirm that *P. nigrispinus* would predate the maggots. Thus, predators remain less explored with regard to different forms of biological control of Calliphoridae.

Parasitoids

The use of parasitoids is one of the most recognized forms of biological control. Several studies show the use of *Nasonia vitripennis* in relation to the control of blow flies.

Mello, Borja, & Aguiar-Coelho (2009) evaluated the exposure of different dosages of *Nasonia vitripennis* parasitoid females to the host *Chrysomya megacephala*. It was observed that parasitoids per host tended to greatly decrease with higher dosages of *N. vitripennis*, and thus, higher rates of parasitism were found in treatments 3:1 and 5:1, and an increase in the percentage of pupae was not observed with increase in the number of females per host, which is justified by superparasitism.

The exposure of *Nasonia vitripennis* to immature *Chrysomya megacephala* at different exposure times- 24, 48, 72 and 96 hours showed that in 72 hours, the development period of *C. megacephala* increased, and consequently the number of viable pupae decreased with the increased exposure time to the parasitoid (Mello, Sabagh, & Aguiar-Coelho, 2007; Mello et al, 2009; Mello, Borja, & Coelho, 2010). Similar results were described for *Cochliomyia macellaria* (Barbosa, Couri, & Coelho, 2008).

Mello (2012) evaluated the parasitoid *Nasonia vitripennis* on pupae of *Chrysomya megacephala* at different pupation depths. The following depths were used: from 0 cm to 5 cm with intervals of 0.5 cm and two exposure times, 48 hours and 72 hours. The results showed that the parasitism rate decreased as the depth increased and parasitism up to 2 cm and 3 cm were observed at 72 and 48 h, respectively. These results demonstrate that for effective biological control, the pupation habit of each insect is extremely important.

Research related to new parasitoid species of Calliphoridae has also been carried out in the last 10 years.

Chin et al (2009) reported that the parasitoids *Exoristobia philippinensis* (Hymenoptera: Encyrtidae) and larvae of *Ophyra spinigera* (Diptera: Muscidae) on pupae of *Chrysomya rufifacies* were collected from carcasses of monkeys in Malaysia. Marchiori & Miranda (2011) collected synanthropic Diptera from chicken feces and observed natural parasitoids of these insects. The parasitism rate was 28.4%. The following species were identified: *Muscidifurax raptorellus, Nasonia vitripennis, Pachycrepoideus vindemmiae, Spalangia cameroni, S. drosophilae, S. endius, S. nigra, S. nigroaenea, Spalangia* sp. (Hymenoptera: Pteromalidae) and *Tachinaephagus zealandicus* (Encyrtidae), with the *P. vindemmiae* species having the highest incidence. These data serve as a basis for future assessments of biological control of the Calliphoridae family by parasitoids.

The use of parasitoids is widely studied and have high efficiency against different insects. *Nasonia vitripennis* is the principal parasitoid which is used as a biological control of blowflies and presents good results. Other species have been focus of research, thus, indicating promise in this area.

Plant extracts

Another form of control of blowflies is the use of plant extracts, an area that presents a great variety of species to be used and great potential for a sustainable and harmless control of human health.

Banumathi et al (2017) used plant extracts for the control of second instar larvae of *Lucilia sericata*. The extraction was carried out using ethanol and the *Lobelia leschenaultiana* species at 60 mg/L (LC50 = 3.4 mg/L) obtained 100% control. In this way, the researchers selected this extract for the formulation of ZnO nanoparticles for use against the insect.

The evaluation of the crude extract of leaves of the plant *Pouteria sapota* (Jacq.) H.E. Moore & Stearn, Sapotaceae, altered the post-embryonic development of *C. putoria*. In the concentrations of 5, 10 and 25% of the extract, there was a decrease in the pupal period and the period between hatching and emergence of adults, as well as the decrease in weight when compared to the control. The 5% extract showed lower viability for the insect (47.5%) (Carriço et al, 2014).

The evaluation of the lyophilized latex activity of *Parahancornia amapa* (Huber) Ducke (Apocynaceae) under the post embryonic development of *Chrysomya megacephala* showed a change in insect development. At a concentration of 1% and 3% of latex, there was a change in the insect cycle, being the viability of 53% in 3% of latex, indicating that high concentrations of *P. amapa* latex present control potential for the species in question (Mendonça, Lima, Albuquerque, Carvalho, & Queiroz, 2011).

Abdel-Shafy, El-Khateeb, Soliman, & Abdel-Aziz (2009) evaluated the viability of control of 4 extracts of plants, extracted by different solvents, under third instar larvae of *Chrysomya albiceps*. Results showed that all extracts were effective in the control, presenting deformed pupae and further histological analyzes identified alterations in intestinal epithelial cells. Plant preparations of *A. herba-alba* and *A. monosperma* were considered more promising for insect control.

Khater & Khater (2009) observed the insecticidal effect of *Trigonella foenum-graecum*, *Apium graveolens, Raphanus sativus* and *Brassica campestris* against third-instar larvae of *Lucilia sericata*, and determined that these plants cause abnormalities in larvae, pupae and adults, as well as an imbalance in sexes, interfering in the medium term during the reproduction of these flies.

Nogueira, Mello, Kato, & Cabral (2009) determined the effect of tetrahydrofuran lignin grandisin against *C. megacephala* larvae. This component resulted in changes in the rates of development. The observed changes were 30% for eggs, 38% for larvae besides reducing their weight. These results confirmed the control exerted over populations of *C. megacephala* by tetrahydrofuran lignin grandisin.

In the last 10 years, there has been extensive research involving alternative forms to the chemical control of Calliphoridae family, recognized for causing serious damage to livestock and human health. We highlight the research involving entomopathogenic microorganisms, parasitoids and plant extracts that demonstrate great controlling potential. However, the studies are still under study and are restricted to the laboratory. Therefore, further studies are required to evaluate viable formulations of these agents of biological control when exposed to diverse environmental conditions.

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