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Review

Advances and Perspectives of the Use of the Entomopathogenic Fungi Beauveria bassiana and Metarhizium anisopliae for the Control of Arthropod Pests in Poultry Production

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ABSTRACT

Global poultry production is plagued by a wide variety of arthropods. The problems associated with their chemical control have led to an increasing search for control alternatives, and entomopathogenic fungi seem to be a promising strategy. Despite the large number of insects and mites considered as important pests in animal production, studies on the use of entomopathogenic fungi for their control are still scarce compared with agricultural pests, particularly in Brazil. This article reviews some damages and control aspects of the main arthropod pests that affect Brazilian poultry production, including house flies, lesser mealworms, and feather mites, by the use of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae*. Studies published in the last 20 years were reviewed, and the main problems and limitations of that pest-control strategy are discussed.

INTRODUCTION

Insects and mites are commonly present in animal production environments. Intensive animal production is characterized by housing a large number of individuals in a relatively small area, permanent production of organic waste (derived from feed and manure), and by the presence of shelter and adequate temperature, luminosity, and humidity conditions, providing a favorable environment for an exponential growth of arthropod populations (Axtell & Arends, 1990; Axtell, 1999). This proliferation may damage productivity, and consequently economic profitability, animal health, as well as the health of workers and consumers.

Many arthropod pests affect meat and egg poultry production, in particular (Hinton & Moon, 2003). They are found in the excreta accumulated underneath layer cages, in broiler house litter (in wood shavings or other plant materials used as bedding) (Axtell & Arends, 1990), on the birds' body surface and feathers, in cracks and gaps in the facilities, or galleries built by the arthropods themselves in the poultry houses.

In the context, in the class of the Insecta, order Diptera , some representatives of Calliphoridae and Sarcophagidae families, which potentially cause myiasis, the species *Musca domestica* and *Stomoxys calcitrans*, of the Muscidae family, are particularly important (Geden & Hoqsette, 2001; Taylor *et al.*, 2010). Some flea species (Siphonaptera) of the Pulicidae and Ceratophyllidae families, and several mite species (Phthiraptera) of the Philopteridae and Menoponidae families, especially the latter, with the species *Menacanthus stramineus* and *Menopon gallinae* are highlighted (Geden & Hoqsette, 2001; Taylor *et al.*, 2010). More recently, *Alphitobius diaperinus* (Tenebrionidae), known as lesser mealworm, was described (Geden & Hoqsette, 2001; Chernaki-Leffer *et al.*, 2001).

The main Acari found are burrowing mites *Knemidocoptes* spp. (Knemidocoptidae), feather mites *Syringophilus* spp. (Syringophilidae) and quill mites *Dermoglyphus* spp. (Dermoglyphidae), mites of the trachea and air sacs *Sternostoma tracheacolum* (Rhynonissidae), and the hematophagous mites *Dermanyssus gallinae* (Dermanyssidae), *Ornithonyssus sylviarum*, and *Ornithonyssus bursa* (Macronyssidae), as well as several tick species of the Argasidae and Ixodidae families (Geden & Hoqsette, 2001; Taylor *et al.*, 2010).

Among the aforementioned pest species, the most important in poultry production worldwide are the house fly, the lesser mealworm, and hematophagous mites. It must be noted that those species are considered important from the veterinary perspective globally, but there are no published surveys on the main pests, their geographical regions, and their impact on poultry meat and egg production in Brazil. However, based on scientific studies (Gianizella & Prado 1998; Lopes *et al.*, 2006; Pinto *et al.*, 2007; Japp *et al.*, 2010; Rezende *et al.*, 2013) and poultry technical publications (Pedroso-de-Paiva 1998; Palhares & Kunz 2001; Avila 2004; Mazzuco *et al.*, 2006; Avila *et al.*, 2007) in Brazil, it is possible to infer that those species are also the most important in Brazilian poultry production, and their implications will be presented and discussed below.

CHARACTERIZATION AND DAMAGES CAUSED BY HOUSE FLIES, LESSER MEALWORMS, AND HEMATOPHAGOUS MITES

These pests cause damage as ectoparasites, stress birds, and are vectors of several pathological agents (Axtell & Arends 1990; Hazeleger *et al.*, 2008; Omalu *et al.*, 2011). In addition, they are environmental pests, and may cause severe structural damage, disturbing farm management, and upsetting farm workers.

Musca domestica is a synanthropic species, and it is usually the most common fly found in poultry houses (Bruno *et al.*, 1993; Mariconi *et al.*, 1999). Its life cycle ranges between 6-10 days in hot temperature. Egg masses are laid on layer excreta and on the litter of poultry houses (both in layer and broiler houses), where the fly develops all its immature stages up to the emergence of the adults in the excreta (Pedroso-de-Paiva 2000).

In addition of upsetting people and birds, the house fly also transmits several pathogenic microorganisms, such as *Salmonella pullorum*, *S. Typhimurium*, *Pasteurella multocida*, *Erysipelothrix rhusiopathiae*, *Staphilococcus* sp, as well as fowl cholera and other enteric bacteria, protozoan oocysts, and several viruses (Pedroso-de-Paiva 2000; Malik *et al.*, 2007; Omalu *et al.*, 2011). House flies also mechanically carry helminths' eggs and are intermediate hosts of tapeworms (*Hymenolepis carioca*, *Raillietina cesticillus*, *Choanotaenia infundibulum*). They are also considered structural pests because their feces on the surface of lamps reduce illumination and stain the house paint and the eggs of layers (Crespo & Lecuona, 1996).

The lesser mealworm *A. diaperinus* rapidly adapts to poultry house environmental conditions, and colonize the litter (Axtell & Arends, 1990). It was probably first introduced in poultry farms by contaminated feed, as it is a secondary pest of meals, feeds, and by-products of stored grains (Pacheco & Paula, 1995). In cold regions, where the use of thermal insulation is required in poultry houses, that insect makes perforations, causing structural damage in up to 30% of the insulating material (Steelman 1996). Lesser mealworms may also be vectors of viruses, bacteria, fungi, protozoa, and helminths that cause diseases in poultry, as shown by several studies (Chernaki-Leffer *et al.*, 2002; Bates *et al.*, 2004; Segabinazi *et al.*, 2005; Hazeleger *et al.*, 2008; Agabou & Alloui, 2010; Chernaki-Leffer *et al.*, 2010; Alborzi & Rahbar, 2012). Crippen *et al.* (2012), in particular, demonstrated that they are vectors of *Salmonella*, one of the main pathogens found in poultry houses.

In Brazil, the infestation of commercial layers with the hematophagous mite *Dermanyssus gallinae*, *Ornithonyssus sylviarum*, and *Ornithonyssus bursa* was reported. The species *O. sylviarum* and *Derma. gallinae* are the most frequently found (Faccini, 1987; Tucci *et al.*, 1998; Rezende *et al.*, 2013). However, there are no reports of hematophagous mites in broiler houses in Brazil.

The mite *O. sylviarum*, commonly called northern fowl mite, occurs in all temperate regions of the world and it is a serious pest in commercial layer production (Soares *et al.*, 2008). They have a short life cycle, of up to five days, which is completed on the host (Rassette *et al.*, 2011), and does not require direct contact among birds to disseminate (Mullens *et al.*, 2001).

In the United States, *O. sylviarum* is commonly found in commercial poultry farms. In Brazil, its presence was registered only in the state of São Paulo, affecting at least 48% of the farms visited in the survey of Tucci *et al.* (1998); however, it is probably disseminated in all Brazilian poultry-producing regions. The survey also reported that *Derma. gallinae* was found in more than 60% of the evaluated farms.

Globally, the prevalence of *Derma. gallinae* in layer houses ranges between 20 and 90%, depending on the country and production system (Sparagano *et al.*, 2009). Commonly known as poultry red mite, this species is the most important ectoparasite in layer production (Chauve, 1998). Its morphological characteristics and size are very similar to *O. sylviarum*, but its life cycle may take 7-10 days, and occurs mostly outside the host's body. This mite hides in cracks and gaps in the building, seeking the birds only to feed, usually during the night (Chauve, 1998; Meyer-Kuhling *et al.*, 2007).

Birds infested by these mites may present anemia and behavioral changes, such as increased feather pecking (Kilpinen *et al.*, 2005), which may cause skin lesions. In addition, the infestation may reduce egg production and livability, and cause high mortality in young birds (Rezende *et al.*, 2013). *Derma. gallinae* may be a vector of viruses and bacteria, particularly of *Salmonella* (Valiente Moro *et al.*, 2007), indicating that its presence in poultry farms is a cause of concern.

In addition of the direct losses reported, these pests may negatively affect flock health as they are vectors of several pathogens. These indirect losses are difficult to measure, and these pests, even when present at low levels in the facilities, may harbor pathogens than can be transmitted to the following flocks, despite the constant use of disinfectants, antibiotics, and vaccines.

PROBLEMS IN PEST CONTROL IN POULTRY HOUSES

Each poultry production system (for meat or eggs) presents specific housing structures, such as different poultry house design, drinkers and feeders, waste disposal practices, and environmental management equipment and activities, and therefore, taking into account the arthropods species that parasite poultry, pest management practices should be adapted to each specific production system (Axtell & Arends, 1990).

The insect and mite pest control in animal production has been traditionally based on chemical insecticides. Organochlorine insecticides were the first to be used, but now are forbidden. These were followed by carbamate, organophosphate, chlorophosphate, pyrethroid, growth regulator, formamidine, avermectin, and neonicotenoid compounds. More than 35 chemical have been reported for mite control (Chauve 1998; Wall 2007; Rezende *et al.*, 2013). These products are typically applied as the only control strategy, and treatments include spraying the entire animal or animal immersion in the product, injection, addition of chemicals to the feed or water, as well as treatment of the housing facilities and the litter.

Chemical products are often inadequately using, making this strategy increasingly less feasible in poultry production, because they harm the health of farmers, consumers, and birds themselves. In addition, the environment and non-targeted organisms may be affected. Another consequence is the selection of pest populations resistant to specific chemical principles, as previously reported (Beugnet *et al.*, 1997; Chernaki-Leffer 2004; Lambkin, 2005). This situation may become even worse when the application of chemical insecticides is not associated with pest monitoring. For instance, the treatment against lesser mealworms is often preventive, and not made only after the presence of high populations of this insect are detected.

The application of chemical products and pest control practices in poultry facilities also present difficulties. For instance, products are usually sprayed as liquids, because powder formulations are difficult to apply and may irritate birds' eyes and respiratory system. However, in layer houses, battery cages are often too close, making it difficult to spray and to obtain uniform product distribution (Axtell & Arends 1990).

In broiler houses, birds are also exposed to the treatment applied in the facilities, and therefore, the problems reported with the use of chemical insecticides and acaricides may be even worse; in addition, their meat can be contaminated, despite the controversy on the presence or not of chemical residues. The cryptic behavior of lesser mealworms and red mites also prevents their effective treatment.

Therefore, alternative strategies have been sought, and biological insecticides have received particular attention in the last few years, particular entomopathogenic fungi (Pinnock & Mullens, 2007; Hajek & Delalibera, 2010). Biological products are natural, less harmful to the environment, potentially cheaper, and the selection for resistance is less likely (Whipps & Lumsden, 2001) as the pathogen-host relationship is in a process of permanent co-evolution.

THE USE OF ENTOMOPATHOGENIC FUNGI AS A PEST CONTROL MEASURE

Entomopathogenic (or acaropathogenic) fungi usually infect their hosts using specialized structures (spores or conidia) that adhere, germinate, and penetrate the hosts' integument. After penetration, the fungus multiplies inside the host's tissues and hemocoel, and the host dies usually 3-10 days after the infection due to water loss, nutrient deprivation, mechanical damage, and/or by the action of toxins (St Leger, 1993).

Despite accounting for only 10% of the global market of biopesticides, those fungi are the most frequently used microbial agents for pest control in Latin America (Faria & Wraight 2007), particularly the species *Beauveria bassiana* (Ascomycota: Cordycipitaceae) and *Metarhizium anisopliae* (Ascomycota: Clavicipitaceae). These species are also the most frequently researched for pest control in general due to their wide geographic distribution, broad range of affected hosts, and to the natural, enzootic or epizootic, conditions where they are found (Alves *et al.*, 1998). The genomes of both species were recently sequenced (St. Leger & Wang, 2010), considerably increasing the possibility of their application.

The characteristics of those fungi, together with their commercial production, allow their potential use in poultry houses. Also, some poultry house conditions may favor the development of fungi, increasing or maintaining the presence of those control agents in the environment.

Despite the difficulties presented by pest control measures, including chemical agents and the possible stability of fungus populations in poultry houses, there are few studies on the use of enthomopathogenic fungi in poultry production compared with their application in crops. Some strategies for the use of those fungi in poultry production are promising, as discussed below; however, studies are very recent and most were not performed under field conditions.

Moreover, there is no information available on the economic viability of those strategies. In addition to environmental benefits, biological products must have competitive costs relative to chemicals to allow their use by farmers.

Tests carried out with the entomopathogenic fungi *B. bassiana* and *M. anisopliae*

Trials with Musca domestica under laboratory conditions

There are several literature reports on the use of fungi for the control of *Musca domestica*, particularly under laboratory conditions, such with the fungi *Entomophthora muscae* (Geden *et al.*, 1993), *B. bassiana* (Steinkraus *et al.*, 1990), and *M. anisopliae* (Barson *et al.*, 1994). Other fungus species were studied by Al-Olayan (2013), who evaluated the pathogenicity and virulence of *Acremonium cephalosporium*, *Aspergillus niger*, *Penicillium chrysogenum*, *Trichoderma viride* and *Verticillium albo-atrum*.

For the specific control of house flies as poultry house pests, there are studies only with the fungi *B. bassiana* and *M. anisopliae*, but further aspects still need to be evaluated in order to use them in control strategies.

The first reports on the occurrence of entomopathogenic fungi associated with *Musca domestica* were published in 1990. Steinkraus *et al.* (1990) recorded for the first time the occurrence of *B. bassiana* in adult flies in farms in the state of New York, USA, with a maximum prevalence of 0.86% in the field. Testes performed with isolate against house flies reared in the laboratory demonstrated its pathogenicity against adults and 3rd instar larvae. In 1995, Geden *et al.* tested an feeding attractant bait containing *B. bassiana* (at 10^5-10^8 conidia/100mg attractant), and six days later, there was 100% mortality when flies fed on the bait with the highest fungus concentration.

Watson *et al.* (1995) evaluated two *B. bassiana* isolates for pathogenicity to adult and larval house flies. The isolates were formulated as a dust or aqueous solution and applied to plywood surfaces. The authors reported 56 and 48% mortality of larvae with the two isolates, and more than 90% mortality of adults with the dust formulation.

More recently, Mishra & Malik (2012) evaluated five *B. bassiana* against house fly larvae and adults, and obtained satisfactory results with both stages, but the highest mortality (100%) was obtained in adults, with a lethal time (LT_{50}) of three days with the isolated HQ917687.

Siri *et al.* (2005) were the first to report the natural occurrence of *B. bassiana* in adult house flies in the Neotropical region, finding a prevalence of 0.4 and 1.45% in poultry houses of the region of La Plata, Argentina. They applied the fungus isolated from house flies obtained in poultry houses in laboratory adults, and obtained 94% mortality after 14 days of incubation.

Barson *et al.* (1994) evaluated the susceptibility of house fly adults and larvae to six fungus species at different concentrations and found that *M. anisopliae* presented the highest virulence, killing 100% of the adults six days after application, under laboratory conditions. The authors evaluated different compounds for product formulation (vegetable oils, minerals oils, and adjuvants) and verified that the fungus formulated with linseed oil presented the best results, with 100% mortality of adult flies in three days, half of the time observed with water solution.

Also working with *M. anisopliae*, Carswell *et al.* (1998) evaluated the effect of the fungus applied in water suspension on the abdomen of adult flies under different incubation temperatures (20, 25, or 30°C). It was observed that 25 conidia were sufficient to cause 100% mortality up to nine days after application when incubated at 25 and 30°C.

In the experiment carried out by Lecuona *et al.* (2005), the authors tested isolates previously selected against adult house flies, offering baits with feeding and sexual attractant impregnated with B. bassiana in a 33m^3 room. The authors observed an average longevity of two weeks and 90 mortality index.

The interaction of *M. anisopliae* with subdoses of the chemical insecticide spinosad was evaluated by Sharififard *et al.* (2011), aiming at the control of house flies in poultry houses. The authors observed up 72% mortality in adults when the fungus was applied alone and 95% mortality with the combined treatment with the chemical insecticide. Larval mortality reached 100% with the combined treatment at the highest doses, and synergy was demonstrated in all evaluated treatments.

In Brazil, there are reports of studies performed only under laboratory conditions. In the scientific note of Bernardi *et al.* (2006), evaluating the effect of B. bassiana and *M. anisopliae* (only one isolate of each) on the emergence of house flies, only *M. anisopliae* affected this parameter. In the study of Fernandes *et al.* (2010), the only enthomopathogenic fungus species tested was *M. anisopliae*, which caused more than 80% mortality of 3rd instar larvae of house flies, depending on the concentration. However, neither study explores or discusses in depth the obtained results. To date, there is no reference study in literature carried out in Brazil on biological control of house flies.

It must be noted that, in general, laboratory results suggest that the entomopathogenic fungi could be used in the field for house fly control strategies because, as reported above, dead flies with these fungi were found in poultry houses and high mortality was obtained under laboratory conditions, demonstrating the susceptibility of house flies in different development stages. Also, their efficacy when applied in combination with other control methods, such as chemical insecticides, was reported.

Control of Musca domestica under field conditions

The main study on this subject was carried out by Kaufman *et al.* (2005), who evaluated the effect of an oil formulation of B. bassiana on the mortality of adult house flies in layer houses. The authors compared houses treated with the fungus with control houses, which were later treated with a chemical insecticide (pyrethrin). House fly pupal hymenopteran parasitoids *Muscidifurax raptor* and *Musci. raptorellus* (Hymenoptera: Pteromalidae) were released in all facilities to evaluate the applied fly management strategies. The number of adult flies recovered from the facilities treated with B. bassiana was significantly lower (43% lower) than in pyrethrin-treated facilities. In addition, in the general collection of arthorpods, the population the beetle *Carcinops pumilio* (Coleoptera: Histeridae), a predator of fly larvae, was higher in the fungus-treated facilities, demonstrating that beneficial organisms were maintained in the environment. This highlights the importance of selective strategies, which may reduce pest populations in the long run.

Trials with A. diaperinus under laboratory conditions

Steinkraus *et al.* (1991) were the first to report the natural occurrence of *B. bassiana* in commercial poultry houses in the United States. After isolation and multiplication, the effect of this fungus on lesser mealworm adults and larvae was evaluated. High mortality (up to 98%) was observed, with higher efficacy when the water suspension was directly applied on larvae than on the litter or insulating material. Adult mortality did not reach 30% in none of the tests, indicating that susceptibility is influenced by the life stage of the insects.

Subsequent studies with *B. bassiana* in the laboratory (Crawford *et al.*, 1998; Geden *et al.*, 1998) evaluated different fungus formulations and application strategies and reported that larvae are more susceptible than adults, the dust formulation was more efficient than the liquid suspension, and litter treatment presented the lowest insect mortality rates. This information was used as subsidies for subsequent studies that demonstrated the same effects on the field.

Many studies were carried out on the use of fungi for the control of lesser mealworms in Brazil. Alves *et al.* (2004, 2005) were the first to report the presence of entomopathogenic fungi in lesser mealworms in commercial poultry houses in the state of Paraná. The first study recorded the occurrence of *M. anisopliae* in dead adult beetles found on the floor of the poultry house. In the second study, in addition of recording the epizootic occurrence of B. bassiana in 74% of the larvae and in 40% of the adults, the fungus was isolated and submitted to Koch's postulates to confirm its pathogenicity.

Rohde *et al.* (2006) tested more than 100 isolates of *B. bassiana* and *M. anisopliae* against lesser mealworms and reported that the isolate Unioeste 04 presented high killing efficiency and excellent production characteristics.

Alexandre *et al.* (2006) evaluated the effect of environmental temperature and litter type on the germination, vegetative growth, virulence, and production of conidia of *B. bassiana* and *M. anisopliae* isolates. The authors found that high environmental temperature (32°C) and used litter influenced fungi virulence and other parameters, especially of *B. bassiana* isolates. These data complement the findings of Crawford *et al.* (1998) and Geden *et al.* (1998), specifically demonstrating how abiotic factors influence that fungus under field conditions.

Chernaki-Leffer *et al.* (2007) observed in two isolates of *M. anisopliae* values higher than 104 conidia for larvae and 10^5 conidia for adult lesser mealworms. The study showed that, under the evaluated conditions, a large amount of conidia was required to kill the insects, which poses a problem for field application.

Haas-Costa & Alves (2007) investigated biosafety aspects of the utilization of *B. bassiana* using caged broilers. Birds were daily fed with diet containing the fungus (10^9 conidia/g feed) and were compared with a control group. No bird mortality or changes in performance or behavior were observed. After sacrifice, histological examination also did not detect any heart, kidney, liver, small intestine or lung changes, demonstrating that *B. bassiana* was safe for broilers under the conditions of the experiment.

Alves *et al.* (2008) evaluated the efficacy of B. bassiana in the control of lesser mealworms relative to formulation (powder or liquid suspension), litter material (new or reused), and soil application, and verified that the powder formulation was more efficient in killing the insect and that litter and soil may affect fungus efficiency. This study, using Brazilian isolates, confirms the findings of Crawford *et al.* (1998) and Geden *et al.* (1998). Another important finding of that study was that the longer the contact of insects with the substrates (new and reused litter and soil), the lower the mortality.

Santoro *et al.* (2008) selected *B. bassiana* isolates and evaluated their lethal concentrations for the control of the lesser mealworm, and determined a LC50 of 8×105 conidia/mL for the isolate Unioeste 04, which was selected by Rohde *et al.* (2006) and used in the studies of Alexandre *et al.* (2006), Haas-Costa & Alves (2007), and Alves *et al.* (2008).

The study of Cassiano *et al.* (2008) should be also mentioned. In addition of evaluating lesser mealworm mortality caused by *M. anisopliae* (74% in larvae after 48h), the authors studied the relationship between the time of contact of the lesser mealworm with conidium adhesion, establishing a statistical model that can be used for this evaluation.

Gindin *et al.* (2009) carried out different laboratory studies in Israel, demonstrating that the application of *M. anisopliae* on new litter caused 90% of lesser mealworm larvae mortality, and had a residual effect of up to 14 days. New litter consists only of wood-shavings spread on the poultry house floor, whereas reused litter contains waste generated during growout that negatively affect fungus efficiency.

In general, laboratory trials have shown that entomopathogenic fungi cause lesser mealworm mortality at different virulence levels (up to 98%), and their development may be strongly influenced by environmental factors, such as of compounds present in the reused litter and environmental temperature. These factors must be considered when designing control strategies in the field.

However, information on the lethal dose of fungi are still lacking; only the aforementioned study of Chernaki-Leffer *et al.* (2007) investigated the number of conidia required to kill 50% of the insects. As for *B. bassiana*, no studies on its lethal dose were found. Moreover, there is also lack of information on the effect of different fungal formulations on lesser mealworm mortality and on the protection of fungi from abiotic effects, warranting further studies on these subjects.

Control of A. diaperinus under field conditions

Perez *et al.* (1999) evaluated the efficacy of a product based on *M. anisopliae* applied directly on excreta collection dumps of layer houses and observed that lesser mealworm populations were reduced 40 days after the application. However, the reduction was numerical, and no statistical analysis was presented by the authors, preventing the interpretation of the results.

Geden *et al.* (2003), after a previous selection of isolates, evaluated the effect of three *B. bassiana* formulations on lesser mealworm control in layer houses. The treatment with the highest fungal concentration (3×10^9 conidia/m^2) in the granulated formulation applied twice with a 7-day interval significantly reduced the population of larvae in 60-70%. However, the authors reported that population suppression was short-lived, as population numbers increased again the following week, reaching the same levels as the non-treated controls. Therefore, the strategy of fungus application in reused litter was not effective.

Alves (in press)[1] carried out several field studies showing the efficacy of an oil emulsion formulation of *B. bassiana* applied on the soil and in the facilities of poultry houses during downtime. The author observed that, with only one application of 1000L of the suspension at a concentration of 1×10^6 conidia/mL in the total area, the population of lesser mealworm adults was significantly reduced (in 73%) up to five months after application. This is a promising result, considering the conditions observed in the poultry houses and the cost of the control of that pest, as a single application was efficient in the medium term.

Trials with hematophagous mites under laboratory conditions

There are much less studies involving mite pathogens relative to insects, and most of those published until 2000 are merely descriptive (Poinar & Poinar, 1998) or report experiments carried out under laboratory conditions.

The work on the evaluation of fungi for the control of mites is very recent, and this strategy is not mentioned in the reviews of Chauve (1998) or Chandler *et al.* (2000) or in the reference document of the workshop "Workshop on Livestock Pest Management: To Assess National Research and Extension Needs for Integrated Pest Management of Insects, Ticks, and Mites Affecting Livestock and Poultry" (Geden & Hoqsette, 2001). To date, few studies on this issue were published, and the results reported do not provide parameters for the use of this strategy as an effective control measure.

The first results with the use of fungi for the control of poultry mite parasites were obtained in the laboratory by Steenberg & Kilpinen (2003), who tested the effect of *B. bassiana*, *M. anisopliae*, and *Isaria fumosorosea* (Ascomycota: Cordycipitaceae) on the mortality of *Derma. gallinae*. The authors reported that the most effective were *B. bassiana* isolates, with 90% mortality, and I. fumosorosea isolates, with approximately 85% mortality, both after 16 days. However, the results were reported to be preliminary, and the methodology applied was direct contact in the path of the mite, and therefore it is difficult to accurately determine the tested concentration or dose. In addition, the applied isolates (name, data bank) or the type of mortality were not identified, making it difficult to use this study as a reference.

In a subsequent study, Steenberg *et al.* (2005) presented further results obtained in the lab and under what the authors called 'semi-field' conditions, testing isolates of *B. bassiana* and *M. anisopliae* against poultry red mite. In addition of mortality, the effects on female mite fertility, fungus vertical transmission, and persistence of the fungi in a poultry house-like environment were tested. However, only a summary was published, and there are no details on the methodology applied or on the results obtained.

On the other hand, Tavassoli *et al.* (2008) reported that three *M. anisopliae* isolates efficiently killed *Derma. gallinae* adults, and that their virulence depended on the mites' stage of development, isolate, isolate concentration, and time of evaluation. Mortality rates higher than 90% were obtained after 10 days of incubation at all mite developmental stages and at the highest concentration (106 conidia/mL) of all isolates.

Kaoud (2010) evaluated the effects of the fungi *B. bassiana* and *Trichoderma album* (Ascomycota: Hypocreaceae) and of the bacteria Bacillus nigateria israelensis on the mortality of Derma. gallinae. The results showed that the three tested agents were pathogenic for red mites, causing high mortality rates. This is the first record of *T. album* and *B. nigateria israelensis* pathogenicity against that mite species, according to the author. However, the type of mortality (total or confirmed in the case of fungi) was not specified and the treatment methodology was walking on a plate with pathogen culture, which does not allow evaluating the actual fungus concentration. Moreover, it is difficult to explain the high mortality observed with the use of the bacterium, as it affects insects and mites only by ingestion, and not by contact.

Except for Tavassoli *et al.* (2008), the studies mentioned above present serious methodology and interpretation problems. These are indeed pioneering studies, but caution must be taken not to make wrong inferences. It should also be noted that the published laboratory studies involve only the species *Derma. gallinae*. To date, there are no records of studies evaluating the susceptibility of *O. sylvarium* to fungi under laboratory conditions, stressing the importance of evaluations with this species.

[1] Luis Francisco Angeli Alves, 2013: unpublished data.

Control of hematophagous mites under field conditions

The recently study of Rassete *et al.* (2011) compared under field conditions the efficacy of *B. bassiana* in the control of *O. sylviarum* and of a chemical insecticide registered for the control of this mite in the United States (based on tetrachlorvinphos-dichlorvos). At a dose of 2.9 x 10^{10} conidia per bird, the number of mites in the birds was significantly reduced with use of *O. sylviarum* relative to the control treatment 21 days after application, but the fungus treatment did not outperform the chemical treatment.

Tavassoli *et al.* (2011) evaluated the efficacy of three *M. anisopliae* isolates for the control of *Derma. gallinae* in oil formulations. The suspensions contained 1 x 10^7 and 1 x 10^9 conidia/mL, and 180 mL were applied per cage. The number of mites was determined using traps. At the highest concentration, pest population significantly decreased relative to the control group after one month, but the treatment was not significant when the low concentration was used. In this study, the control group was treated with distilled water combined with sunflower oil (which was used in all treatment), and this may pose a methodological problem and may have influenced the final result. The results may have been different if the control group was treated only with water, as vegetable oil by itself may have mite-control effects, as they block the spiracles, causing asphyxia (Rodrigues & Childers 2002). Oil formulations should be considered in the control of mites in poultry production. Oil formulations can intensify the effects on pest mortality and also protect the pathogens against environmental effects (Wraight *et al.*, 2001).

Considerations on the use of entomopathogenic fungi in poultry production

Considering that the specific occurrence of the pests in different types of poultry houses, the development of research and production of knowledge in different areas may aid the design of control strategies. Each species have their own peculiarities, and based on the mentioned literature studies, some practices may be suggested.

House flies should be mainly controlled by farm management, as this pest proliferates especially when litter is not adequately managed. Therefore, good litter management practices during the growout and changing litter between flocks is essential to prevent the establishment of favorable conditions for fly multiplication (Pedroso-de-Paiva, 2005).

The correct use of chemical insecticides and the application of entomopathogenic fungi may complement litter management programs. Also, fly-development monitoring programs could also be applied, with effective sampling technics, allowing the use of feeding attractants and pheromones. Further studies on the fungi *B. bassiana* and *M. anisopliae* should be carried out to determine their lethal dose for house flies. Other entomopathogenic fungi should also be tested under poultry house conditions, such the species *Entomophthora muscae* (Entomophthorales: Entomophthoraceae), which was tested for the control of house flies in other environments.

Entomopathogenic fungi could also be an alternative for the control of the lesser mealworm, provided care is taken when treating the poultry house facilities to preserve the fungi in the environment, such as using selective products for poultry house disinfection. However, specific studies on these disinfectants are required. The disinfectants applied during downtime, together with high ammonia concentrations in the litter prevent the survival of entomopathogenic fungi in the poultry house environment. The direct contact with the litter is extremely harmful to those fungi (Geden *et al.*, 1998; Alves *et al.*, 2008). The use of oil formulations was shown to be more efficient to protect fungi from abiotic factors (McClatchie *et al.*, 1994; Hedimbi *et al.*, 2008; Lopes *et al.*, 2011), and their application on the soil during downtime showed better results, as observed with other lesser mealworm control practices (Santos *et al.*, 2009).

The application of fungi for the control of *O. sylviarum* seem to be less efficient, as these mites are present on the body surface of birds, which body temperature is higher than that required for fungus development. In this case, control measures should be focused on the host, with localized product application, which represents an economic advantage. Chemical control may be a better alternative in this case, with the use of more selective products that are less harmful to the environment and the birds.

On the other hand, entomopathogenic fungi may be an excellent strategy for the control of *Derma. gallinae*, which spends most of its life cycle in the environment, away from the host, particularly during the first life stages. In this case, measures to control red mites should focus on the places where they hide in facilities during the day; traps containing the fungi can also be used. Further studies should mainly evaluate the dynamics of the disease in mites, such as determining the most virulent isolates, time of infection and death, lethal concentrations, and most susceptible stages. In the lesser mealworm, fungi stability in the poultry house environment, formulations that protect beetle pathogens, and possible application strategies to effectively kill the pest should also be investigated.

As previously shown in Brazil, research on the use of entomopathogenic fungi for the control of some poultry pests is still in the initial stages, including laboratory studies. The number of studies on this subject is small, and this is mostly due to deficiencies in extension services. It is difficult to obtain the support of poultry companies in Brazil to apply research results in the field, and thereby to establish control practice recommendations. Testing alternative control strategies in commercial poultry houses requires that the conventional chemical control is not applied. This may increase pest populations, resulting in performance losses and flock exposure to pathogens transmitted by arthropods. This is one of the main reasons why poultry companies are reluctant in supporting such studies. Another reason is that the companies usually determine guidelines for the use of chemical insecticides as part of their management packages for the farmers, and the companies may reduce their profits if those products are not sold.

In this context, it is difficult to find the ideal balance between research and commercial interests. This problem needs to be overcome to allow research development and contribution for the Brazilian poultry sector.

CONCLUSIONS

As discussed in this article, there are several obstacles for pest control in poultry production, and the advance of research using entomopathogenic fungi depends on overcoming those barriers. Important aspects still need to be elucidated, and other actions need to be promoted.

It is essential for research to support extension services and to establish partnerships with companies, allowing the production of knowledge and the transference of the developed technologies.

The absence or the small number of studies on the prevalence and population dynamics of the pest species, their financial impact and average cost of control measures hinders the further development of research in this field. This information is essential for the design of pest management strategies and good egg and broiler production practices.

The way forward is to perform these studies in poultry-producing regions in Brazil to understand the current situation and to develop and implement integrated pest management programs both in egg and broiler production, including monitoring (methods have been already described) and different control practices associated with adequate management of the facilities, equipment, and waste. In this context, the use of entomopathogenic fungi is highlighted, presenting real possibility of contributing for the reduction of pest populations.

The key to the success of pest control is the application of combined strategies, because when there are high pest populations, not even the best strategy using fungi can be effective.

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