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Ocurrence of Entomopathogenic Fungus from Flea *Ctenocephalides canis* (Siphonaptera: Pulicidae)

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Abstract

The objective of this study was to isolate entomopathogenic fungus from fleas (*Ctenocephalides canis* Curtis) collected from *Cannis familiaris* specimens. From 60 collected fleas, two dead insects presented white mycelia growth on the body surface, changing to a creamy color as time passed; the insects presented a mummified aspect, typical characteristic caused by entomopathogenic fungus. This fungus was identified as *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin by their mycelia characteristics, macro- and micro-morphology. Up to our knowledge, this is the first report of entomopathogenic fungus isolated from adult *C. canis* fleas.

Keywords

Ectoparasite, Biological Control, Dog, *Beauveria bassiana*

1. Introduction

The dog flea, *Ctenocephalides canis* Curtis, is a hematophagous ectoparasite distributed across the world [1].

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The infestation of this ectoparasite is a common and recurring problem in dogs, causing discomfort, manifestations of anemia and allergies, also due to its feeding habits; it possesses the efficient ability to be a vector of different pathogens. The flea also inoculates substances and allergens that produce in the host, skin irritation and eventual allergic dermatitis [2] [3].

The control of adult fleas on animals has been carried out through the use of insecticides, which have yielded very effective results for the control of infestations, even in extreme climates, demonstrating controls of up to 100% [4]. In the environment, the different flea-life cycles can also be controlled, and chemicals commonly used are growth regulators and even repellents [3]. Despite of the effectiveness and efficiency of synthetic chemical products, the adverse effects in the environment are widely and publically known. In the last five decades, many problems with the indiscriminate use of chemically synthetic insecticides in agriculture and public health programs have been identified; and the inconvenience of pesticide resistance, environmental pollution, effects on non-objective organisms and human toxicity, specifically on the vulnerable population (pregnant women, elderly and children) have been observed [5]. All insects have natural enemies in the environment, those include predators, parasitoids, parasites and pathogens; and the ability to recognize and respond defensively to the different challenges is an important characteristic of insect behavior [6].

A successful epizooty in insects depends of the optimal interaction among the host-pathogen-environment; and the previous statement is necessary to understand the factors that make an insect susceptible or resistant to a pathogen that will be used as a biological control agent [7]. All insects possess a complex defense system where chemical and immunologic mechanisms, which will either succeed or fail in such a way that the relationship host-pathogen-environment is maintained at a balance and any variation in the environment, will result in the host or pathogen success, just like it has been demonstrated with different genus of mites and insects. For example: in *Reticulitermes flavipes* Kollar (Isoptera: Rhinotermitidae), 24 hours from the inoculation of the entomopathogenic fungus *Metarhizium anisopliae* Metschnikoff, Sorokin, the insect increases its circulating hemocytes that favors the pathogen encapsulation [8]; without a doubt, the environmental conditions and the host defense mechanisms prevent that the entomopathogenic fungus will express its ability to control *R. flavipes* in an outstanding manner. This result could be different if the entomopathogenic fungi are provided with the adequate environmental conditions for fully develop their pathogenic abilities [8]. For instance, [9] evaluated entomopathogenic fungi on *Ixodes scapularis* Say (Acari: Ixodidae) adult ticks in laboratory and field, and it was discovered that *M. anisopliae* was more prone to infect the mites in laboratory conditions than under field conditions, suggesting that field conditions limited the ability of entomopathogenic fungi to control pests. The use of native entomopathogens to the regions and host could present advantages regarding to non-native isolations and non-associated to the pest being studied. The objective of this study was to isolate entomopathogenic fungi from fleas collected from *C. familiaris* specimens. To the best of our knowledge, there are no reports of the presence of entomopathogenic fungus associated to *C. canis* fleas in published scientific literature, so this present study reports the first entomopathogenic fungus isolated in adult fleas of *C. canis*.

2. Materials and Methods

As received in the laboratory of Parasitology and Biological Control of the Life Sciences Division of the Irapuato-Salamanca Campus of the University of Guanajuato, 71 specimens of fleas for their morphologic identificationwith the main objective of detect species present; all were collected from a domestic canine (*Canis lupus familiaris* Linnaeus) of Cocker Spaniel breed.

The 71 specimens were collected at 21˚7'31.43"N and 100˚55'10.30"W, approximately 1950 m.a.s.l., in a building with easy access for wild fauna; they were later placed in sample containers for transporting. At the laboratory, the 71 specimens were transferred into Petri dishes, and to make manipulation easier they were maintained for a minute at 4˚C ± 1˚C. Once the fleas were immobilized, they were transferred to the Petri dishes (90 × 10 mm) with a double layer of Whatman #1 filter paper. After 48 hours of being captured, fleas were morphologically identified as *C. canis* according to the keys provided by [10]. Also, mycelia growth was observed on several flea bodies, for that reason, humidity was kept at 25˚C under a regimen of 16/8 h light/dark [11]. Eight days later, once the mycelia growth was completely developed on the flea's surface, it was subcultured on Agar Dextrose Sabouraud (Dextrose, Casein-peptone Meat), having Yeast Extract [12] [13] and 500 ppm chloramphenicol [14]. The culture was repeated until a pure culture, once the pure fungus were obtained, the entomopathogenic fungus were identified at the Parasitology and Biological Control laboratory of the University of Guanajuato, using the [15] identification keys, by its macro morphologic aspects; like color, diameter and mycelia texture, while the optical microscope was used to identify the mycelia characteristics and conidial micromorphology [16].

3. Results

Two entomopathogenic fungi, associated to *C. canis* adult fleas, were isolated following the experimental procedures. From 71 collected fleas (**Figure 1**), two dead insects presented white mycelia growth on the body surface, changing to a creamy color as time passed; the insects presented a mummified aspect, typical characteristic cause by entomopathogenic fungi (**Figure 2**). These fungi were identified as *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin by its mycelia characteristics, macro and micromorphology (**Figure 3**).

Figure 1. *C. canis* flea collected from dog.

Figure 2. *C. canis* flea infected from *B. bassiana*.

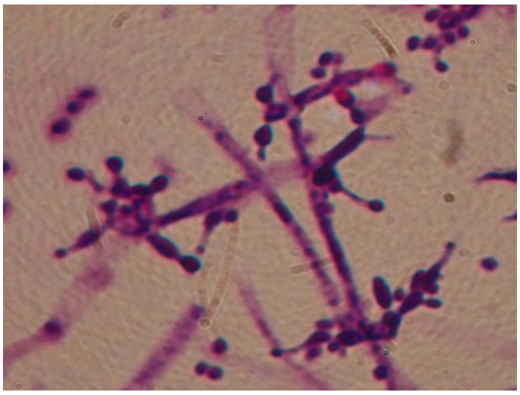

Figure 3. Mycelia characteristics, gential violet 100×.

Up to our knowledge, the present study is the first report of entomopathogenic fungus isolated from adult *C. canis* fleas. These entomopathogenic fungi will be evaluated on their capacity and rate of *in vitro* infestation for other flea's species; likewise, they will be applied and measured as a sustainable system for the biological control of fleas in the field.

4. Discussion

Up to our knowledge, there are no reports of entomopathogenic fungus naturally infecting adult *C. canis* fleas; nonetheless, [17] demonstrated that strains of *B. bassiana* were capable of killing adult cat fleas (*Ctenocephalides felis* Bouche). This discovery suggests that it is greatly recommended to search for entomopathogenic fungi from *C. canis* fleas, besides evaluating the isolated fungi in the present study. Likewise, it is important to scout other entomopathogens from several origins, because probably, the pest could have defense mechanisms that would not allow the entomopathogens to develop and perform a biological regulation under field conditions; findings that were recently reported by [8] on termites.

There are numerous reports on the isolation of entomopathogenic fungi from dead arthropods in natural habitats [18] [19], belonging to different species, and animal parasites, out of which some of them are promising candidates to be used as agents for the biological control of the species where they were originally isolated [20]. These results will allow, in a near future, reducing the need to apply chemical products on animals and the environment, but the influence of entomopathogenic fungus in fleas should be further investigated, and whether the entomopathogenic fungus could bring any negative effect for animal health should also be considered.

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