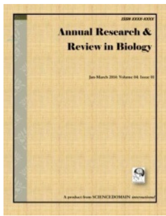

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Bioformulations in Pest Control – A Review

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Authors' contributions

This work was carried out in collaboration between all authors. Authors APAPS and EALAL designed the work and managed the literature searches. Author APAPS organized figures and conducted the search. Authors APAPS and RTA prepared the initial draft. Author VLML managed cross-opinion corrections, reviewed and proof read the manuscript. All authors read and approved the final manuscript

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ABSTRACT

Biotic and abiotic factors limit the action of entomopathogens and interfer for reaching the expected results. Moreover, the requirement of import and export markets for good quality foods with low content of toxic waste has increased. In this sense, new organic products have been developed in order to improve the stability, virulence and efficacy of entomopathogenic agent in the field. The aim of this paper is to report on the use of formulations with entomopathogenic fungi to control pests. About 12 species or varieties of fungi have been used as active ingredients in formulations of mycopesticides. A formulation can be defined as the combination of an active ingredient (such as entomopathogen), an inert carrier and an adjuvant which will improve the performance of the product, and also will be ease for handling and application. The *Metarhizium anisopliae* and *Beauveria bassiana* are the most used fungi in formulations worldwide. The synergistic effect of fungal interactions with the phytosanitary product has attracted the attention of several researchers

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due to their potential to cause high mortality of the target insect, becoming a tool for deployment in integrated pest management.

Keywords: Formulations with entomopathogens; biological Control; Metarhizium anisopliae; Beauveria bassiana.

1. INTRODUCTION

Over several decades, the widespread use of chemical insecticides to control pests caused side effects such as environmental imbalance, toxic residues in foods, diseases in humans and other animals and the development of resistance mechanisms in insects [1]. A viable alternative to chemical control is the use of natural enemies, a practice known as biological control. This method enables the maintenance of insect populations in balance, limiting their rapid multiplication without causing harm to other organisms [2,3].

The entomopathogenic fungi are widely used in Biological Control because they are the main pathogens of insects, causing more than 80% of their diseases [4]. Under favorable conditions, they can cause outbreaks and enzootic diseases in natural species of Hemiptera, Lepidoptera and Coleoptera. They specialize in penetration via the tegument and may infect different stages of host development [5-7].

In relation to the Integrated pest management, which recommends the combination of different techniques and resources to maintain a population of insect pests below the economic injury level [8,9], several researches have been designed to enhance the action of entomopathogenic formulations, which contribute to the development of stability, virulence and efficacy of the entomopathogenic agent in the field [10,11]. This study aimed to present a review of the use of formulations containing entomopathogenic fungi to control pests.

2. BIOLOGICAL CONTROL WITH ENTOMOPATHOGENIC FUNGI

Among the entomopathogenic fungi, *Metarhizium*, *Beauveria*, *Paecilomyces*, *Lecanicillium*, *Nomurea*, *Aschersonia*, *Hirsutella* and *Entomophthora* are considered the most important [4] genus. Most of the entomopathogenic fungi have been distributed throughout many decades in the Hyphomycetes class. These organisms were called anamorphic (group of fungi that have no phylogenetic relationship with others, being considered as perfect) [12]. The anamorph has its counterpart in sexual teleomorph, which corresponds to the sexual phase of Ascomycota or more rarely, Basidiomycota. Subsequently, the *Metarhizium* genus was described as the anamorph of Ascomycota *Cordyceps brittlebankisoides* [13], since the anamorph *Metarhizium anisopliae* var. *majus* (= *M. anisopliae*) [12-15] was isolated from the larvae of Coleoptera (Scarabaeidae). Molecular phylogenetic studies have supported this classification and currently the teleomorph of *Metarhizium* was assigned to *Metacordyceps* [16,17].

Among the entomopathogenic fungi, *Metarhizium anisopliae* (Metsch.) Sorokin and *Beauveria bassiana* (Bals.) Vuilemin stand out due to their wide geographic distribution and host range. The most successful biological control program with *M. anisopliae* was from 1970-1991, when the fungus was applied in several acres of sugar cane fields infested by *Mahanarva posticata* Stal (*Hemiptera*: *Cercopidae*), sugar cane leafhopper in the northeast of Brazil [4]. Since then, several researches have been conducted proving the pathogenicity of *M. anisopliae* to other species of agricultural importance such as *Coptotermes formosanus* Shiraki (termites) [18], *Schistocerca gregaria* Forck (grasshoppers) [19], *Hylobius abietis* (pine weevil) [20], *Rhipicephalus (Boophilus) microplus* Canestrini (cattle tick) [21] and *Anthonomus grandis* Boheman (boll weevil) [22].

B. bassiana, an anamorph of *Cordyceps* Fr [23], is one of the most established entomopathogenic fungi taxonomic, and it has widespread occurrence in all countries, being more frequent on insects and soil samples, in which it can survive for long time in saprogenesis [24]. The *B. bassiana* fungus has become internationally known by the Soviet product called Boverin, a wettable powder formulation [25] recommended for the control of *Leptinotarsa decemlineata* Say (potato beetle) and other species. Research conducted with different species showed that *B. bassiana* is pathogenic and virulent to various pests and parasites as *Haematobia irritans* L. (horn fly) [26]; *Psoropets ovis* Hering (rabbit parasite) [27]; *Laniifera cyclades* Druce (cactus pest) [28]; *Tribolium castaneum* Herbst (red flour beetle) [29]; *Atteva sciodoxa* Meyrick (medicinal plants caterpillar) [30]; *Hyalomma anatolicum* Koch [31] and *Rhipicephalus (Boophilus) microplus* (ticks) [32], and *Zaprionus indianus* [33].

The mechanism of action of entomopathogenic fungi involves several processes until the insect is completely colonized and killed. First, the mechanical force exerted by the pressure of the hyphae, by breaking the membranous or sclerotic areas of the cuticle of the host, followed by the start of the enzymatic process that results from the release of enzymes, especially proteases, chitinases and lipases, which alter the surface tegument, releasing peptides that serve as nutrients for the fungus and facilitate the penetration into the insect [6,34]. These features are unique to fungi and puts them at an advantage compared to other pathogens that depend on the intake of their propagules to initiate infection [35,7]. Thus, the fungi can infect different host development stages including the stages, which are not fed such as eggs and pupae. After infection, the externalization of the fungus appears on the body surface of the parasitized insect, and the morphologic appearance of the colonization depend on the specie of the entomopathogenic fungi, an example is shown in Fig. 1 for larvae of *Diatraea saccharalis*, the sugarcane borer, killed by *M. anisopliae* (Fig. 1a) and *B. bassiana* (Fig. 1b) colonization. The sick insect dies of a set of modifications on the hemocele, tissues and internal organs. The cycle is completed when sporulation occurs in the body of the parasitized insect allowing horizontal transmission of the pathogen by spreading the propagules among the insect population, as well as to the environment, so that resulting in spreading infection [36,37].

3. FORMULATIONS OF ENTOMOPATHOGENIC FUNGI

The preservation time and viability of conidia are the main obstacles to its use in a large scale. Generally, biotic and abiotic factors (temperature, solar radiation, moisture, predation competition, among others) limit the fungi action on the field and can have a direct effect on the growth, germination and infective potential of entomopathogenic fungi [38-44]. In this sense, several products have been developed in order to increase stability, virulence and efficacy of the entomopathogenic agent (Fig. 2). Among these products, formulations based on emulsifiable adjuvant oil have been widely studied due to the facility for storage under controlled temperature and relative humidity (25±1ºC; 70±10%), and protection of conidia against UV rays, with consequent increase persistence in the field and ease of implementation [10].

Several aspects should be considered prior to develop a formulation with entomopathogenic fungi. Firstly, it is necessary the addition of certain compounds that improve the performance of the fungus in the field. Secondly, the formulation should be easier to handle and apply; and finally, that allows a longer time of storage under conditions that minimize the cost, and also with the minimal loss of the quality of the product. These components should also increase the persistence of the product, adhesiveness on the insect and attractiveness to the pest [45] (Fig. 2). In addition to the active ingredient (pathogen) and inert/vector, the formulations can contain adjuvant, components that are used to optimize the action of the active ingredient and improve the characteristics of the formulated product (Fig. 2), for example, their ability to spread on hydrophobic surfaces. Among other properties, they have photoprotective, phagostimulant and anti-evaporation functions.

About 12 species or subspecies (varieties) of entomopathogenic fungi have been used as active ingredients in mycopesticides, for the control of insects and mites, *such as M. anisopliae var. anisopliae, M. anisopliae var acridum, M. flavoviride, B. bassiana, B. brongniartii, Verticillium lecanii, Paecilomyces fumosoroseus, Isaria farinosa, Sporothrix insectorum, Hirsutella thompsonii, Nomuraea rileyi* and *Cladosporium cladosporioides*. Among the most common products developed worldwide containing entomopathogenic fungi as the active ingredients, *M. anisopliae* and *B. bassiana* are in first place, each one representing 33.9% of the total, followed by *Lecanicillium* spp. (9.4%), *Isaria fumosorosea* (5.8%) and *B. brongniartii* (4.1%). In the inventory of products and formulations, around 26% are substrate colonized by the fungi, 20.5% are wettable powders and 15.2% oil dispersions [45,46].

The most used types of formulation containing entomopathogenic fungi are wettable powder, granules, water dispersible granule, bait, sprinkle powder, powder for contact, oil dispersion, suspension concentrate, miscible suspension concentrate in oil, and suspension in ultra-low volume. The formulation of wettable powder type is applied after dilution with water, such as Boveril®, a commercial product which is used for control of pests in crucifers [47]. The sprinkle powder is applied by dusting, whilst powder for contact is by direct application on the pest [45,48]. The granules are a kind of solid formulation of uniform size, but cereal grains such as rice are not included in this type of formulation, because they are considered as technical concentrated or non-formulations [49]. On the other hand, water dispersible granule disintegrates in water before application, such as PFR97 TM® having *Paecilomyces fumosoroseus* as the active ingredient [50].

The type bait formulation was developed to attract the pest and be consumed by it, as traps Termitrap® used for subterranean termites, water based and sugar cane molasses [51]. The oily dispersion contains the active ingredient (entomopathogenic fungus) in surfactant for using after dilution in water [52]. In this type of formulation are included suspensions in oil emulsion, for example Met52 EC®, a commercial product (Novozymes Biologicals, Inc., USA) that must be diluted prior to use in the laboratory or in the field, e.g. against the sweet potato beetle *Cylas formicarius* [53]. The suspension concentrate is already an active ingredient in water, and may be diluted further prior to be applied [54]. The miscible suspension concentrate in oil is a suspension containing the active ingredient in a fluid for dilution in organic liquid, in this formulation it is included the Metarril SP Organic® produced by Koppert Biological Systems [55]. The suspension in ultra-low volume comes ready to use or may need small dilution, but it requires special sprayers equipment for application [56], in order to avoid blockage in the outlet nozzle of the applicators; most of ultra-low volume sprayers utilize a small electric pump that can be very finely adjusted to vary droplet size and flow rate, so that meet the desired specific spray application.

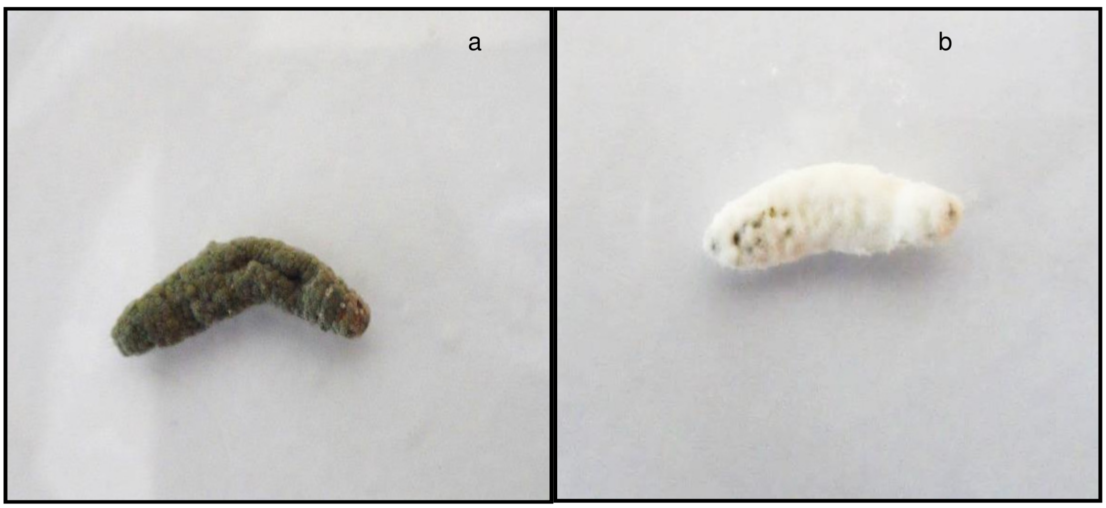

Fig. 1. Larvae of *Diatraea saccharallis* colonized by *Metarhizium anisopliae* (a) and *Beauveria bassiana* (b)

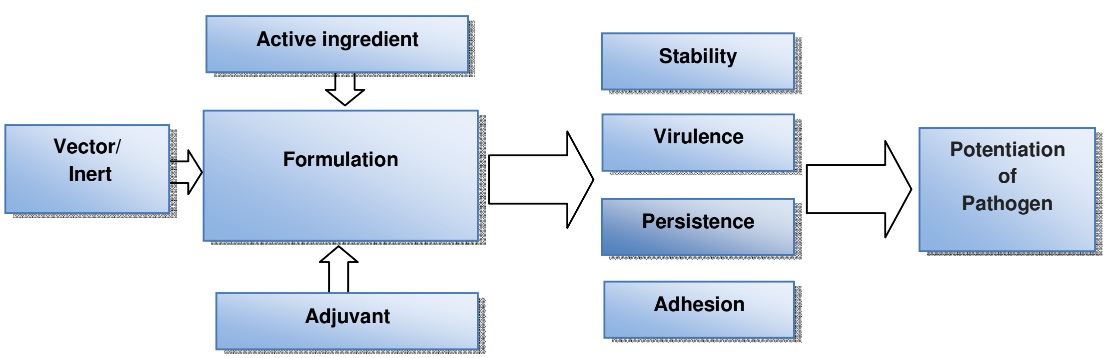

Fig. 2. Schematic representation of development of formulations and their advantages

4. GLOBAL SCENARIO OF FORMULATIONS

In Brazil, products based on *M. anisopliae* represent 55% of commercially available products or in the registration process, followed by *B. bassiana* (30%), *Lecanicillium* spp. (7.5%) and *Sporothrix insectorum* (7.5%). Most of these Brazilian mycopesticides have no record; of these, 2.5% are marketed as pure conidia, 72.5% are technical concentrates (liquid or solid substrates, colonized by fungi) and only 25% are in fact oil dispersion formulations. In this sense, studies with formulations based on vegetable oils and emulsifiers have being carried out in several countries and have produced interesting results for example, when the neem oil (*Azadirachta indica*) is associated with the fungus *B. bassiana*, causing over 90% mortality, on nymphs of *Bemisia tabaci* (whitefly), in the United States of America [57]. Experiments in the South Africa with *Tetranychus urticae* Koch exposed to *B. bassiana* in an oil emulsion obtained 61% of mortality of mites after seven days [58], demonstrating the potential of the formulation for field-testing.

Test formulations of the fungus *M. anisopliae* and *B. bassiana* in oil, for the control of malaria mosquito (*Anopheles* sp.) have showed that formulated conidia were more effective in controlling larvae than non-formulated ones, in addition to persisting longer under field conditions, in the Netherlands [59]. Other studies have also confirmed the high mortality of insects (*Plutella xylostella* L.) when in contact with castor oil (*Ricinus communis* L.) added with *B. bassiana* [60].

Therefore, one can notice that the synergism of the association between fungus and oil, indicate its potential against the target pest in the field. The availability of products on the market formulated with high concentration and viability of infective structures, easy handling and application, greater efficiency and with competitive price is essential for establishing the use of entomopathogenic fungi for pest control in large-scale [61,62].

5. CONCLUSION

Among the main advantages of using bioformulations with entomopathogen fungi for biological control of insect pests we can point out the easiness of production of its infective units on a commercial scale, the simplicity of usage in field conditions, the low cost of its utilization, and mainly, the reduction on environmental impact [63-67]. Interactions between entomopathogenic fungi with phytosanitary products, such as chemical insecticides (e.g. Decis OC), fungicides (e.g. Manzate 800) or herbicides (e.g. Granoxone) are important to evaluate new formulations, since it can be positive when an additive or synergistic action occurs with the entomopathogen and the product. However, a negative interaction may appear when an antagonistic effect is caused by the inhibition of one of the components, which usually is the active ingredient or entomopathogen. Therefore, prior to consider joint implementation as an effective formulation, there is a need for compatibility testing, seeking more selective products and able to promote the conservation of the pathogen in the field for a longer period of time [68,69].

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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