Beauveria bassiana (Bals.-Criv.) Vuill. – A biocontrol agent with more than 100 years of history of safe use

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History of *Beauveria bassiana (Bals.-Criv.) Vuill.*

The origins of microbial pest control date back to the early nineteeth century, when the Italian scientist Agostino Bassi spent more than 30 years studying white muscardine disease in silkworms (*Bombyx mori* L.). He identified *Beauveria bassiana* (Bals.-Criv). Vuill., named in his honour, as the cause of the disease. His discovery not only laid the foundation for microbial pest control, but also significantly influenced the work Louis Pasteur, Robert Koch and other pioneers of microbiology (Ainsworth, 1956; Porter, 1973; Van Driesche & Bellows, 1996). Bassi himself recognized the potential to use organisms such as Beauveria bassiana to control insect pests (Bassi, 1836; cit. in Van Driesche & Bellows, 1996) and by the early 20th century, field trials had been conducted with *B. bassiana*, *B. brongniartii* (Sacc.) Petch, and *Metarhizium anisopliae* (Metschn.) Sorokin. Today, over 100 years later, there are no known reports of significant adverse effects that can be attributed to the use of these organisms in biocontrol.

Pathogenicity/Infectivity of *B. bassiana*

An extensive literature search was conducted to evaluate risks related to human exposure to *Beauveria bassiana.* A total of 8 distinct reports naming the genus *Beauveria* Vuill. as the alleged cause of fungal infections and disease of humans were identified, but only 4 of these reports could be conclusively attributed to species of the genus *Beauveria*.

Like any micro-organism, *Beauveria bassiana* has the potential to act as an opportunistic pathogen, but as the literature study demonstrates, *Beauveria* infections are extremely rare events. A detailed analysis of case reports allegedly involving *Beauveria bassiana* reveals that extraordinary circumstances, such as a severely compromised immune system or a history of surgery/injury, are required for a *B. bassiana* infection to occur.

The most severe human cases of *Beauveria* infections are two recent reports of disseminated mycoses (Henke *et al.*, 2002; Tucker *et al.*, 2004). Both of these infections occurred in severely immuno-compromised patients with acute leukemia. Prior the development of mycoses, one patient underwent 4 full cycles of chemotherapy, the other was in her first cycle of chemotherapy and had been diagnosed with *Streptococcus viridans* in her bloodstream. Despite their poor health, both patients responded well the antimycotic treatments and fully recovered from their mycoses. Further reports of *Beauveria*-related deep tissue mycoses (Freour *et al.*, 1966a-c, Drouhet & Dupont, 1980) could not be substantiated.

While there are some reports of *Beauveria* spp. isolated from patients with corneal keratitis, the *B. bassiana* can certainly not be considered a significant eye pathogen. Of four reports linked to *Beauveria*, only two (Sachs *et al.*, 1985; Kisla *et al.*, 2000) can be conclusively attributed to *Beauveria bassiana*. In these cases the affected eye had undergone surgery following traumatic injury to the eye, and in all reported cases, the therapy of the injured eye involved corticosteroids and antibiotics, which, according to Sachs *et al.* (1985) can predispose the eye to fungal infections by otherwise non-pathogenic fungi.

None of the studies conducted for the registration of *B. bassiana* strain GHA in the US (acute oral toxicity/pathogenicity; acute dermal toxicity; acute pulmonary toxicity/pathogenicity; acute intraperitoneal toxicity/pathogenicity) showed any pathogenicity of the test organism against the tested mammals (US EPA, 2006). Likewise, eye irritatiaton studies (US EPA, 2006) and literature reports (Ishibashi *et al.*, 1987; Begley & Waggoner, 1992) show no unacceptable effects related *B. bassiana* exposure to the healthy eye. Furthermore, it has been used in biocontrol for over 100 years with no reports of illness related to exposure to *B. bassiana* strains used in biocontrol.

These considerations allow the conclusion, that the label compliant use of B. bassiana based products such as Botanigard® will not result in unacceptable risks for applicators and consumers.

Metabolites

Species of the genus *Beauveria* have been reported to produce the secondary metabolites bassianin, bassiacridin, beauvericin, bassianolide, beauverolides, tenellin and oosporein (Strasser *et al.*, 2000; Vey *et al.*, 2001; Quesada-Moraga & Vey, 2004).

It is important to note that the discovery of a certain metabolite during liquid cultivation of a specific strain cannot be extrapolated to all strains of the species. Moreover, it cannot be assumed that these substances will also be produced under natural conditions in the soil or in the target host. Further, it should be kept in mind that entomopathogenic fungi naturally cause epizootics similar to those resulting from artificial inoculations. There are no reports of metabolites entering the food chain or accumulating in the environment as a result of such natural or artificicial epizootics or natural metabolite secretion (Vey *et al.*, 2001). In contrast, numerous studies have documented environmental accumulation and food chain contamination with chemical pesticides and antibiotics used in agricultural production.

Specifically, no metabolites of concern have been detected in end-use formulations of Botanigard® products, which are based on *B. bassiana* GHA spores, and toxicological tests (see pathogenicity section), have not shown any adverse effects related to either pathogenicity or toxicity.

The ubiquitious natural occurrence of the species *B. bassiana,* its long history of use in biocontrol, and the fact that Botanigard® products have been safely used in the United States and other countries for more than 10 years, demonstrate that no unacceptable risks are expected to result from the use of these products.

Ecotoxicology

No negative effects of Botanigard® were found in ecotoxicology studies with mammals, birds or fish (US EPA, 2006). Literature reports of *B. bassiana* infections in captive reptiles (Georg *et al.*, 1962; Fromtling *et al.*, 1979; Gonzalez *et al.*, 1995) can be attributed to inappropriate captivity conditions, and no reports of any vertebrates infected by *B. bassiana* in the wild were found. Further, *B. bassiana* is not known to cause adverse effects to plants or earthworms.

Compared to the second species of the genus, *Beauveria basssiana* has a wider host range and a therefore, in theory, a somewhat higher potential to affect non-target arthropods. It is, however, important to recognize the difference between the physiological and the ecological host range of an organism, i.e. if a non-target species can be infected in the lab this cannot be directly translated into potential adverse effects in the field. Based on the natural occurrence of *B. bassiana* and the low toxicity profile demonstrated by ecotoxicology studies conducted with Botanigard® products, the ecological risk due to exposure to this microorganism is expected to be minimal (US EPA, 2006).

Efficacy

Levels of control reached with biological organisms are generally more dependent on environmental conditions, such as climatic factors, than those achieved with conventional pesticides. *Beauveria bassiana* has been tested in a wide range of pest control scenarios and has been successfully used in many countries. While, under suitable conditions, efficacy rates of Botanigard® can exceed 90 %, in many instances, a considerably lower level, but longer-term suppression can be sufficient to prevent crop damage. It is important to recognize that biological control agents such as *B. bassiana* significantly differ from chemical pesticides in their properties and this should be taken into account when designing and reviewing efficacy studies.

Comparison of safety with synthetic plant protection products used

Chlorpyrifos is a common insecticide used against the some of same targets as *B. bassiana*. This substance acts as neurotoxin (acetylcholine esterase inhibitor) and is therefore highly toxic to a wide range of non-target organisms, including mammals and birds, aquatic organisms and bees. Chlorpyrifos can leach into the ground water or enter the food chain through agricultural produce. The risks of this pesticide have been judged acceptable by the European Union, yet *Beauveria bassiana,* which is by far less problematic than chlorpyrifos, is still awaiting approval for inclusion in Annex I of Directive 91/414/EEC.

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