

REVIEW

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Toward the efficient use of *Beauveria bassiana* in integrated cotton insect pest management

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Abstract

Background: For controlling the resistance to insects, in particular carpophagous and phyllophagous caterpillars, using chemical pesticides has led to contamination of cotton area in Benin. Facing this problem, alternative methods including the use of entomopathogenic fungi as biopesticide could be a sound measure to preserve the environment, biodiversity and ensure good quality of crops. Previous studies have revealed the insecticidal potential of the entomopathogenic *Beauveria bassiana* on some insect pest species. However, little is known about its effectiveness on cotton Lepidopteran pests. This review is done to learn more about *B. bassiana* for its application in controlling cotton insect pests, especially Lepidopteran species.

Main body: Different sections of the current review deal with the related description and action modes of *B. bassiana* against insects, multi-trophic interactions between *B. bassiana* and plants, arthropods, soil and other microbes, and biological control programs including *B. bassiana* during last decade. Advantages and constraints in applying *B. bassiana* and challenges in commercialization of *B. bassiana*-based biopesticide have been addressed. In this review, emphasis is put on the application methods and targeted insects in various studies with regard to their applicability in cotton.

Conclusion: This review helps us to identify the knowledge gaps related to application of *B. bassiana* on cotton pest in general and especially in Lepidopteran species in Benin. This work should be supported by complementary laboratory bioassays, station and/or fields experiments for effective management of cotton Lepidopteran pests in Benin.

Keywords: Biopesticide, Entomopathogenic fungus, *Beauveria bassiana*, Action modes, Pest management, Cotton

Background

The hopeful perspective projected for crop protection and public health as a result of the introduction of chemical pesticides is now open to serious question because of an alarming increase of resistance occurring in insects, pathogens, vertebrates, and a lesser extent in

weeds (National Research Council [NRC] 1986). In the case of insect pests, one of the practices inducing selection of resistant crop populations is due to an overuse of same or closely related chemical insecticides (Food and Agriculture Organization [FAO] 2012). Facing this challenge, integrated pest management (IPM) options become attractive and environmentally friendly for crops protection against insect pests. Of these, biological control focusing on the use of natural enemies of insects, especially on entomopathogenic fungus, remains an interesting alternative tactic in the framework of pest status changing (Sewify et al. 2009). Of particular

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interest is the ability displayed by various genera of entomopathogenic fungi to colonize wide plant species in different families, both naturally and artificially following inoculation, ensuring protection against not only insect pests but also plant pathogens (Jaber and Ownley 2018).

Several studies suggest that fungus *Beauveria bassiana* is a promising agent for use as bio-insecticide to control various pest targets. This article reviews the currently available literatures on the description of *B. bassiana* and its modes of action against insects, describes the multi-trophic interactions between *B. bassiana* and plants, arthropods, soil, and other microbes. It also reviews biological control programs including *B. bassiana* for crop protection from 2009 to 2019.

This review analytically synthesizes the studies on crop pest management using *B. Bassiana* for its possible application in cotton insect pest management.

Synthesis methodology

The literatures searching strategy adopted in the current review was mainly based on the use of databases engines Google Scholar and CABI Direct. Various key words were used, namely “*Beauveria*” and “pest” and “biological control”. There were about 17 000 results on Google Scholar including all languages. The 30 most relevant articles were retained for review. With CABI Direct search, there were 1994 results (approx.) for all fields over the period of 2009 to 2019. The 22 most relevant articles were retained for review. In sum, 110 documents including articles (99), book chapters (4), theses (3), research memory (1) and research reports (3) were used to carry out the present bibliographic synthesis.

B. bassiana (Balsamo) Vuillemin: a biological control agent

Description of the fungus *B. bassiana*: history, taxonomy and morphology

History

In 1835, the entomologist BASSI Agostino of Lodi, discovered the causal agent of pebrine disease that turned legions of Italy’s silkworms into white mummies (Lord 2005). The characteristic appearance of cadavers covered with a white powdery layer gave rise to the descriptor, “white muscardine disease”. Later, BEAUVÉRIE Jean described this pathogen as *Botrytis bassiana* (De Kouassi 2001). The genus *Beauveria* was formal only around the twentieth century when Vuillemin (1912) claimed that *Botrytis bassiana* (Bals.-Criv.) was a species that belongs to the genus *Beauveria* (Halouane 2008).

The classification of hyphomycetes and especial species of the genus *Beauveria* has been subjected to several revisions because of the increasing taxonomic complexity of *Beauveria*. The main characteristic of hyphomycetes (Deuteromycotina) is that they lack

sexual reproduction, making the taxonomists consider only morpho-ontogenic characteristics to discriminate between species (De Kouassi 2001). Moreover, there was an increasing in taxonomic complexity of *Beauveria*. Subsequently, the entomopathogenic hyphomycetes consisted of more than twenty genera including *Beauveria* (Sung et al. 2001) which were thought to belong to Clavicipitaceae family and were potentially related to the genus *Cordyceps* (Humber 2000). Despite its cosmopolitan distribution and long history in mycology, the teleomorphic state of *B. bassiana* was not clear. However, Schaerffenberg (1955) cited by Sung et al. (2006) reported the teleomorphic Clavicipitaceae of *B. bassiana*, but with little evidence. A new species *Cordyceps bassiana* has been described from China on a carpenterworm larva, *Prionoxystus robiniae* (Lepidoptera: Cossidae), and probably related to *B. bassiana* (Li et al. 2001). Later, Sung et al. (2006) not only provided an additional insight into the systematics of *Cordyceps*, but also confirmed the anamorph feature of *B. bassiana* when culturing the fungus with artificial diet.

Taxonomy of *B. bassiana*

The complete systematic position of *B. bassiana* according to Sung et al. (2006) and Halouane (2008) is as follows:

- Kingdom: Fungi,
- Phylum: Ascomycota,
- Class: Sordariomycetes,
- Order: Hypocreales,
- Family: Clavicipitaceae / Cordicipitaceae or ophiocordicipitaceae
- Genus: *Beauveria*,
- Species: *B. bassiana* (Bals.-Criv.) (Vuil., 1912).

In some documents, the pathogen was considered to belong to Cordycipitaceae (Vigneshwaran 2015) or Ophiocordicipitaceae (Sensagent 2000–2016).

Morphology of *B. bassiana*

The fungus *B. bassiana* (Bals.) Vuill. was of terrigenous origin, saprophyte ubiquitous and pathogen for many insect orders such as: Lepidoptera, Hemiptera, Coleoptera, Hymenoptera, Homoptera, Hemiptera and Orthoptera (Sabbahi 2008; Li et al. 2001). The asexual spores of *B. bassiana* are conidia of white to yellowish color bearing by long zigzag transparent and septal filaments. Hyphae diameter varies between 2.5 µm and 25 µm.

Different types of conidia can be produced by *B. bassiana* depending on the environment. In the presence of air (aerobic environment), the fungus produces spherical

(1–4 μm in diameter) or oval (1.55–5.5 μm \times 1–3 μm in size) conidia but in an anaerobic condition, it produces oval shape blastospores (2–3 μm in diameter and 7 μm in length). Blastospores and conidia are all infectious organs (Weiser 1972; Lipa 1975; Sabbahi 2008).

Mode of action of *B. bassiana* against insects

The infection cycle of *B. bassiana* in invertebrates bodies has been depicted by Mascarin and Jaronski (2016).

Asexual spores (conidia) are dispersed by wind, rain splashing or even by arthropod vectors facilitating the fungus to establish infection on susceptible hosts (Ortiz-Urquiza and Keyhani 2013).

The host infection by the fungus occurs in four steps: adhesion, germination and differentiation, penetration, and dissemination.

1st step: adhesion.

It is characterized by recognition and compatibility mechanisms of conidia of the host cuticle cells (Vey *et al.* 1982 reported by De Kouassi 2001). Conidia (or in some cases blastospores) were attached to insect's cuticle by electrostatic and chemical forces (Mascarin and Jaronski 2016). Then, through the production of mucilage, they induced epicuticular modification (Wraight and Roberts 1987) leading to conidia germination.

2nd step: germination-differentiation.

Germination is a process that depends on environmental conditions, host physiology (biochemical composition of the host cuticle) as well. Such conditions can stimulate or inhibit it (Butt *et al.* 1995; Butt and Beckett 1994; Smith and Grula 1982; St Leger *et al.* 1989b). When conditions are suitable, conidia or blastospores germination leads to germ tubes formation. In fact, conidia germinate and form a germ tub with rehydration and chemical stimuli (Mascarin and Jaronski 2016).

Differentiation is characterized by the appressoria or penetration peg establishment, which serves as inking point, softening the cuticle and promoting penetration. For this purpose, the germ tub may form a specialized structure, namely appressorium (i.e., an enlarged cell expression bearing key hydrolytic cuticle-degrading enzymes) or penetration peg enabling hyphae growth to breach the host integument (De Kouassi 2001; Mascarin and Jaronski 2016).

However, appressoria production is highly dependent on nutritional value of the host cuticle (Magalhaes *et al.* 1988; St Leger *et al.* 1989a). A nutritious cuticle may stimulate mycelial growth rather than penetration (St Leger *et al.* 1989a).

3rd step: penetration.

From the appressorium or penetration peg and with the hydrolytic action of enzymes (proteases, chitinases, lipases: the most important being proteases), mechanical pressure, and other factors (such as oxalate), the fungus is able to penetrate all cuticle layers until reaching a nutrient-rich environment, i.e. the insect hemolymph.

4th step: dissemination within the host and to another host.

In the hemolymph, the fungus undergoes a morphogenetic differentiation from filamentous growth to single-celled, yeast-like hyphal bodies or blastospores that strategically exploit nutrients, colonize internal tissues, and disturb the host immune system. During this stage of the infection, the fungus can also secrete toxic metabolites that help to overcome the insect's immune defense mechanisms for successfully colonization. Some strains produce non-enzymatic toxins such as beauvericin, beauverolides, bassianolides, and isarolides increasing the speed of the infection process (Hajeczek and St-Leger 1994; Roberts 1981). These events eventually lead to the death of host that became mummified.

When the infected insect dies, the fungus produces an antibiotic called "Oosporin" that is used to overcome bacteria competition in insect gut (De Kouassi 2001). Then, *B. bassiana* hyphae cross the insect integument preferentially at the inter-segmental level and then become cottony white. Finally, conidiophores appear on the mummified cadavers after a few days and bear newly infection conidia (sporulation) for dispersal (passive dissemination).

Multi-trophic interactions between *B. Bassiana* and plants, arthropods, soil and other microbes

Mascarin and Jaronski (2016) depicted a conceptual summary of the multi-trophic interactions between *B. bassiana* and plants, arthropods, soils and other microbes in a landscape community scenario.

According to these scientists, a saprophytic life story occurs in the soil with conidia shifting in mycelium, whereas the ability of this fungus to form sclerotium remains unknown, as this propagule has been observed neither under *in vitro* nor under natural conditions; the fungus can also infect soil-inhabiting insects and may transfer nitrogen from the insect to the plant through establishment of root endophyte colonization.

Endophytism of *B. Bassiana* is the fungus life mode within the plant. For this purpose, *B. Bassiana* colonizes the roots and aerial organs such as: stems, leaves and seeds without causing damage to the plant. Then, insects feeding on these plant organs may get contaminated and then infected by fungal spores. Dead insects may sporulate, becoming a new contamination source through their sporulated cadavers, aerogenic spores or perhaps endophytic colonization for other organisms including

predators and parasitoids. Such organisms may interact with the fungus in transmitting its spores (vectors). Interactions should be more complex. Therefore, damage from insect pests would be controlled in plant with the endophytic colonization by the fungus that triggers systemic resistance defenses or by direct antagonism through antibiosis or nutrient competition.

The teleomorph trait of the fungus has been reported to be related to *Cordyceps* sp. (Li *et al.* 2001) and appears to be only found in Asia where it is commonly used in Chinese medicine (Mascarin and Jaronski 2016).

Success history of *B. bassiana* application in insect pests' management

Synthesis of control programs including *B. bassiana* application for crop protection

Several studies revealed the insecticidal potential of *B. bassiana* as mycopesticides and commercial endophytic fungi (Jaber and Ownley 2018). All the studies involved direct application of the entomopathogen to target pests or indirect application by inoculation of the pest host plant. In the last case, *B. bassiana* is considered as an endophyte of the host plant. In the endophytic colonization strategy, methods of inoculating plant species consists of seed coating and seedlings injection (Brownbridge *et al.* 2012), immersion of radicles, roots or rhizomes (Posada and Vega 2005, 2006), stem injection (Posada *et al.* 2007; Tefera and Vidal 2009), soil drenching (Posada *et al.* 2007) and foliar spraying (Gurulingappa *et al.* 2010; Posada *et al.* 2007). In this section, we summarized the control programs that successfully used *B. bassiana* application as crop protection from 2009 to 2019.

Against thrips in *Thysanoptera*

The fungus *B. bassiana* strain RSB showed its effectiveness against western flower thrips, *Frankliniella occidentalis* (*Thysanoptera*: *Thripidae*) causing 69%–96% mortality at concentrations of 1×10^4 – 1×10^7 conidia·mL⁻¹, 10 days after inoculation of first instars. In laboratory and in greenhouse trials, RSB applied to broccoli foliage significantly reduced adult and larval populations (Gao *et al.* 2012). The second instar larvae and pupae of the thrips which had been attacked by predatory mites were markedly more susceptible to *B. bassiana* infection than non-attacked thrips (Wu *et al.* 2015). On cucumber grown in greenhouse, single application of either fungus *B. bassiana* or the predatory mite *Neoseiulus barkeri* significantly reduced both larval and adult *F. occidentalis* populations (Wu *et al.* 2016).

Wu *et al.* (2013) performed laboratory and greenhouse evaluation of a new entomopathogenic strain of *B. bassiana* for control of the onion thrips *Thrips tabaci*. Among 20 isolates of *B. bassiana* tested for their

virulence against *T. tabaci* in laboratory bioassays, strain SZ-26 was found to be the most virulent, causing 83%–100% mortality in adults at 1×10^7 conidia·mL⁻¹ after its application for 4–7 days. The following greenhouse experiments revealed that the strain SZ-26 significantly reduced adult and larval numbers.

Furthermore, using sub-lethal doses of neem tree extract in combination with *B. bassiana* improved the effectiveness of the control strategy against *T. tabaci* (*Thysanoptera*: *Thripidae*) while reduced the amount of insecticide used (Al-mazra'awi *et al.* 2009).

Against *Coleoptera* in palm tree and stored grains

The entomopathogenic fungus *B. bassiana* was reported to be effective against the palm weevil *Rhynchophorus ferrugineus* (Oliv.) (*Coleoptera*: *Curculionidae*) when applied three methods (Injection of *B. bassiana* in naturally infested palm trees, periodical dusting application of fungal spores on palm trees, release of contaminated males of red palm weevil with fungal spores). Injection of naturally infested palm trees using *B. bassiana* reduced by up 90% of the weevil population (Sewify *et al.* 2009). In laboratory studies, Dembilio *et al.* (2010) showed that an indigenous strain CECT-20752 of *B. bassiana* obtained from a naturally infected *R. ferrugineus* pupa was able to infect eggs, larvae and adults of *R. ferrugineus* with median lethal concentration (LC_{50}) ranging from 6.3×10^7 to 3.0×10^9 conidia·mL⁻¹.

Efficacy of *B. bassiana* against *Cosmopolites sordidus* (Germar, 1824) (*Coleoptera*: *Curculionidae*) was observed when the beetles were immersed in a fungal suspension at a concentration of 1.12×10^9 conidia·mL⁻¹ with 54% to 66% mortality for strains (IBCB 74, IBCB 87 and IBCB 146), and the same as sporulation rate for the three strains (Almeida *et al.* 2009). Also, infection after *B. bassiana* application against *Ips typographus* (*Coleoptera*: *Curculionidae*) caused up to 92% mortality in the weevil (Mudrončková *et al.* 2013).

The *B. bassiana* strain PPRI5339 [BroadBand, an emulsifiable spore concentrate (EC) formulation] and *Metarhizium anisoplae* have been suggested for management of *Polyphylla fullo* (L.) (*Coleoptera*: *Scarabaeidae*) (Erler and Ates 2015). Young larvae (1st and 2nd instars) were more susceptible to infection than the older ones (3rd instar). The fungal formulation when applied caused up to 79.8% and 71.6% mortality in young and older larvae, respectively.

Essays in low-land farm using two flea beetle susceptible okra varieties (NH99/DA and LD88/1-8-5-2) with isolate of Botanigard 22WP-*B. bassiana* strain GHA mixed with water in a knapsack sprayer and applied to okra leaves from 2 weeks after planting plants at 3–57 g per 1.5 L of water and repeated at weekly interval for 6 weeks revealed that entomopathogenic fungus, *B.*

bassiana reduced the number of *Podagrica* spp. (Coleoptera: Chrysomelidae) in treated okra plants and enhanced yield (Kudemepo et al. 2018). Moreover, Kaiser et al. (2016) reported a synergistic interaction between *B. bassiana* spores and oil formulation to control the Pollen beetles *Meligethes* spp.

The endophytic colonization of radiata pine (*Pinus radiata* De Don) was found to be effective in controlling the bark beetle pest in forest trees plantation (Brownbridge et al. 2012). Likewise, *B. bassiana* was effective against *Ips avulsus* Eichhoff (Coleoptera: Scolytidae) by inducing approximately 84% adult mortality compared with untreated control (14% mortality) (Olatinwo et al. 2018).

The formulated product BbWeevil™ made of *B. bassiana* conidia was found to efficiently control the storage grain beetles *Oryzaephilus surinamensis*, *Sitophilus granarius*, *Tribolium castaneum* (Khashaveh et al. 2011). Likewise this product was effective against adults of *Callosobruchus maculatus* (F.) and *S. granarius* (L.) on stored grains in darkness (27 ± 2)°C and (65 ± 5)% relative humidity (RH) (Shams et al. 2011).

It has been proved that the lower dose of the *B. bassiana* product can be used in storage systems where the predator *Teretrius nigrescens* (Lewis) was already established to control the larger grain borer (LGB), *Prostephanus truncatus* (Horn), a cosmopolitan and major storage pest of maize causing up to 48% dry weight loss (Nboyine et al. 2015).

Against crop pests in Diptera, Lepidoptera and Hemiptera

B. bassiana proved its effectiveness against *Thaumastocoris peregrinus* Carpintero & Dellapé (Hemiptera: Thaumastocoridae), one pest of *Eucalyptus camaldulensis*, under laboratory conditions with mortality rate ranging from 37 to 80.1% after 10 days of application (Lorencetti et al. 2018).

The fungi *B. bassiana*, *Metarhizium anisopliae* (Metsch) Sor. and *M. flavoviride* (Gams & Rozsypal) applied at a concentration of 1×10^7 conidia·mL⁻¹ against the citrus pests (*Ceratitis rosa* Karsch, *C. capitata* Wiedemann (Diptera: Tephritidae) and *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae)), had significantly reduced adult populations in both insect pests compared with their effect on pupae in laboratory conditions. The estimated LC₅₀ values of the three fungal species ranged from 6.8×10^5 to 2.1×10^6 conidia·mL⁻¹ (Goble et al. 2011).

Wang et al. (2016) screened different *B. bassiana* strains against *Dendrolimus punctatus* (Lepidoptera: Lasiocampinae) and found that strains B-2, B-14 and B-19 were more virulent (lethal time 50 (LT₅₀) of B-2, B-14 and B-19 were 7.63, 7.62 and 7.88 d, respectively, and their LC₅₀ were 0.63×10^6 , 0.96×10^6 and 0.78×10^6

conidia·mL⁻¹, respectively) on *D. punctatus*, suggesting their high potential in biological control. According to Vijayavani et al. (2009), dry conidia of two *B. bassiana* strains SBT#11 and SBT#16 affected chrysalids of *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) under laboratory conditions. Both strains of *B. bassiana* were highly pathogenic causing 100% mortality in *S. litura*. Their effect was conidial concentration dependent. SBT#11 was more virulent with a lethal time 50 (LT₅₀) of 5.1 days in laboratory compared with SBT#16 with a LT₅₀ of 6 days. Fungal sporulation was observed in 87% of insect cadavers in the treated group. Hasyim et al. (2017) found that 2 g·L⁻¹ water concentration of the entomopathogen fungi of *B. bassiana* and *Verticillium lecanii* induced the annihilation in *S. exigua* larvae up to 90%.

Pathogenicity of the entomopathogenic fungi *M. anisopliae* and *B. bassiana* on larvae *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) has been assessed by Douro Kpindou et al. (2012a, b) who observed that the isolate (Bb11, known as Bba5653) of *B. bassiana* was more efficient compared with *Metarhizium* (Met 31). These entomopathogenic fungi may be promising biological control agents against the cotton bollworm *H. armigera*. Likewise, Toffa-Mehinto et al. (2014) showed that *B. bassiana* isolate Bb115 (mortality rates ranged from (65.8 ± 3.5) % (fifth instar) to (79.0 ± 3.0) % (first instar), respectively) was a promising biological control agent against the legume pod borer *Maruca vitrata* (Lepidoptera: Crambidae).

Moreover, Karthikeyan and Selvanarayanan (2011) studied *in vitro* bioefficacy of *B. bassiana* (Bals.) Vuill against selected insect pests of cotton *H. armigera*, *S. litura*, *Earias vittella* Fabricius. Among the three concentrations, 0.25% recorded the highest mortality of *H. armigera* (86.67%), *S. litura* (86.67%) and *E. vittella* (73.33%), respectively. When different concentrations of *B. bassiana* after 2nd spray against diamondback moth (*Plutella xylostella* Linn.) on cabbage (*Brassica oleracea* var. *capitata*) were tested in field and green house experiments, Kamal et al. (2018) reported that applying 2.4×10^8 conidia·mL⁻¹ recorded the highest reduction (72.64%) of larval population and the most effective treatment with the highest cost-benefit ratio (1:6.04) as compared with other *B. bassiana* concentrations.

Likewise, Xu et al. (2011) used 7 strains of *B. bassiana* (D1worm-5, D1 worm-9, L2 worm-8, L1-1-1, D6 worm-2, D10 worm-2, S9 worm-X-1) high virulent against the white worms of *Ostrinia furnacalis* Guenée (Lepidoptera: Crambidae) in China. The muscardine (sporulated dead worm) lethal rate has been 93%–100% at 1.06×10^{10} mL⁻¹ spores density.

Jaber and Ownley (2018) have reported that entomopathogenic fungi, often solely considered as insect

pathogens, play additional roles in nature, including endophytism, plant disease antagonism, plant growth promotion, and rhizosphere colonization. The fungus *B. bassiana* is a fungal entomopathogen that can colonize plants endophytically and plays a role in protecting plants from herbivory attack and disease. A concentration of 10^8 conidia·mL⁻¹ (in water) of *B. bassiana* applied either as a foliar spray or a soil drench reduced pests and disease attack in a common bean (*Phaseolus vulgaris*) (Parsa et al. 2013). Wraight et al. (2010) reported a high susceptibility of the second-instar larvae of several Lepidopteran pests including: diamondback moth (*P. xylostella* L. (DBM), European corn borer (ECB) (*O. nubilalis*), corn earworm (CEW) (*H. zea*), and fall armyworm (FAW) (*S. frugiperda*); beet armyworm (BAW) (*S. exigua*), black cutworm (BCW) (*Agrotis ipsilon*), cabbage worm (ICW) (*Pieris rapae*) and cabbage looper (CL) (*Trichoplusia ni*). Indeed, *B. bassiana* isolate 1200 exhibited higher virulence against all these pest species compared with the commercial *B. bassiana* strain GHA currently registered in the USA as BotaniGard®. On the other hand, the isolate Bba5653 was found to cause 94% mortality of DBM (*P. xylostella*) larvae with a cabbage yield of 44.1 t·hm⁻² in plots treated with water formulation at 1 kg conidia powder (CP) per hectare (Vodouhe et al. 2009). Likewise, colonization of cotton plants by the endophytes *B. bassiana* or *Lecanicillium lecanii* slowed down the reproduction and feeding in *Aphis gossypii* Glover (Hemiptera: Aphididae). Moreover, the consumption of wheat leaves colonized by either *B. bassiana* or *Aspergillus parasiticus* slowed down the growth of *Chortoicetes terminifera* (Walker, 1870) nymphs suggesting a possible role for endophytic entomopathogens in the regulation of insect populations (Gurulingappa et al. 2010). Also, neem oil formulation (Neemseto) at a concentration less than 0.25%, in combination with *B. bassiana* CG001 isolate could be applied to control the aphid *Lipaphis erysimi* (Kalt.) (Hemiptera: Aphididae) in kale (*Brassica oleracea* var. *acephala*) (Araujo et al. 2009).

In greenhouse and field experiments, commercialized *B. bassiana* strains ATCC74040 and GHA were applied on grapevine (*Vitis vinifera* (L.)) leaves either as conidial suspensions or as a formulated product (Naturalis®, strain ATCC74040) to control piercing-sucking insects (Rondot and Reineke 2018). Endophytic survival of *B. bassiana* inside leaf tissues of seven-week-old potted plants was evident for at least 21 days after inoculation, irrespective of the inoculum used. The endophytic colonization of grapevine plants by *B. bassiana* reduced infestation rate and growth of vine mealybug *Planococcus ficus* (Signoret, 1875) (Hemiptera: Pseudococcidae). In the vineyard *B. bassiana* has been detected as an endophyte in mature grapevine plants, 5 weeks after last

application with significant reduction in grape leafhopper, *Empoasca vitis* (Gothe, 1875) (Hemiptera: Cicadellidae) infestation.

Against Acarians

The application of *B. bassiana* isolates 444Bb and 445Bb with conidial suspensions at a concentration of 10^6 conidia·mL⁻¹ against *Tetranychus urticae* Koch. (Acari: Tetranychidae) induced drastic reduction in mycosis caused by this acarina (Draganova and Simova 2010). The mean mortality values of host individuals were $(83.78 \pm 3.62)\%$ and $(68.49 \pm 4.28)\%$ respectively on the first day for isolates 444Bb and 445Bb, but up to 100% for both isolates on the fourth day.

A single application of *B. bassiana* (1×10^8 spores·mL⁻¹) suspension after the release at low rate of the phytoseiid predatory mite, *Phytoseiulus persimilis* Athias-Henriot (10 prey:1 predator) successfully controlled *T. urticae* Koch population ($P < 0.001$), with the lowest corrected leaf damage (1.5%) on bean plants (Ullah and Lim 2017). The effectiveness of *B. bassiana* combined with *Purpureocillium lilacinum* Thom (1910) (Hypocreales, Ophiocordycipitaceae) TR1 was reported on *T. urticae* by Yeşilayer (2018) who recorded a mortality rate of 66.6% at the highest doses (10^8 conidia·mL⁻¹). In the Table 1, more information is depicted about the control programs that successfully used *B. bassiana* for crop protection from 2009 to 2019.

Benefits of using *B. bassiana*

The entomopathogenic fungus *B. bassiana* is one of the most effective agents in biological control widely described in the literature. It's found in all soil types (Jamal 2008; Lambert 2010). Different isolates were identified to attack a wide range of insects (707 species belong to 15 orders) and mites (13 species) (Lambert 2010; Zimmermann 2007). The use of *B. bassiana* is an environmentally friendly control mean compared to chemical pesticides. In addition to being more environmentally sound control method, *B. bassiana* is harmless to human health (Althouse et al. 1997; Faria and Wraight 2001). For some authors, the consumption of *B. bassiana* extracts would have positive effects on the immune system (Id. 2007 cited by Lambert 2010). Allergic reactions in people manipulating the fungus were scarcely reported (Lambert 2010). Furthermore, it is easy and relatively cheap to culture and maintain several *B. bassiana* strains in laboratory conditions compared with the production costs of chemical pesticides. In addition, solutions containing *B. bassiana* conidia can easily be applied in field using equipment and application method like those of synthetic insecticides (Fréchette et al. 2009).

Compared with other biocontrol agents acting by ingestion, a simple contact of *B. bassiana* with the insect is sufficient to trigger infection of susceptible host in suitable environmental conditions and insect physiology. Thus, this entomopathogenic fungus kills both adult and immature stages (eggs, larvae) causing the so-called “white muscardine” disease (Barron 2001, Groden 1999). The spores’ lifespan, once in the field was found to vary greatly according to the environment conditions, between 24 h to 26 days (Jamal 2008). However, some forms of resistance can survive longer, to ensure a long-term control (Lambert 2010).

According to Sabbahi (2008), the insecticidal activity of *B. bassiana* is faster than other entomopathogenic agents with a longer lifespan; conidia can persist in the environment through the spread of enzootic or epizootic diseases. Moreover, its effect on beneficial insects and other non-target organisms was limited. Finally, it is not theoretically possible for insects to develop resistance to *B. bassiana* because the fungus simultaneously uses several modes of action and as a living organism, it can adapt to various host changes.

For all these reasons, the use of *B. bassiana* as a biological insecticide is seen as a potential alternative to chemical insecticides.

Constraints related to the use of *B. bassiana*

Environmental constraints

The use of entomopathogenic fungi such as *B. bassiana* in pest control is not without constraints. In laboratory conditions, the fungus can kill insects 3 to 6 days after infection (Inglis *et al.* 2001). Field conditions may be suboptimal, leading to prolonged disease initiation and progression in the host; the expected lethal effect may take longer (Sabbahi 2008).

The efficacy of *B. bassiana* depends mainly on environmental abiotic factors, including moisture, temperature, precipitation, and ultraviolet (UV) radiation (Jaronski 2010; Fernandes *et al.* 2015; McCoy *et al.* 2002) for inoculum buildup and storage. Thus, according to the results from the studies carried out by Teng (1962) on the biology of *B. bassiana*, the favourable temperature for mycelial growth ranged between 13 °C and 36 °C. Mycelium development ceased at 8 °C and 40 °C. The optimal temperature for spore germination and mycelial growth was 24 °C, which, according to laboratory tests with *Dendrolimus punctatus* Walker (1855), was also favourable to infection. The upper general temperature limit for growth was 34 °C to 36 °C. Higher temperatures can significantly reduce the fungal production efficiency (Noma and Strickler 1999; Ugine 2011). Similarly, the optimal temperature (30 °C) for spore production was reported by 30 °C according to Teng (1962). Although the relative humidity most suitable for mycelial growth and spore germination is 100%, spores of

some strains of *B. bassiana* can germinate at a low relative humidity (56.8%). On the other hand, lower percentages of relative humidity (25%–50%) favored sporulation. However, *B. bassiana* can tolerate a relatively low humidity according to other authors. Fargues *et al.* (1997), showed that *B. bassiana* requires for its development the existing moisture in host micro-habitat or in the microenvironment of the host body surfaces (Faria and Wraight 2001), which would allow it to evolve in more severe environmental conditions. Conidia of hyphomycetes (including formerly *B. bassiana*) fungi strongly adhere to insect cuticle (Boucias *et al.* 1991), so that precipitation does not induce higher conidia loss (Burgess 1998). However, other studies showed that precipitation was responsible for significant loss of *B. bassiana* conidia adhered to the leaves of some monocotyledonous and dicotyledonous plants (Inglis *et al.* 2000; Inyang *et al.* 2000). Solar radiation (ultraviolet type A and B) (Fernandes *et al.* 2015; Jaronski 2010; Inglis *et al.* 1993) can rapidly inactivate *B. bassiana* conidia. This phenomenon reduces the effectiveness and persistence of *B. bassiana* on treated leaves. Cagañ and Švercel (2001) observed that an increased UV radiation induced a decline in the efficacy of *B. bassiana* against *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae). Thus, climatic conditions could influence the physiology of the fungus, its ability to infect the host, the infection progression within the living or dead host, cadaver sporulation, the dissemination ability and survival of infectious conidia, also the host’s susceptibility or resistance to infection (Sabbahi 2008).

Compatibility of *B. bassiana* with chemical pesticides

The effectiveness of a microbial control agent could be improved by combining it with low rates of pesticides (Islam and Omar 2012). When the interaction becomes synergistic, the combination would improve the effectiveness of the biological control agent while reducing the side effects of pesticides. However, achieving the synergistic interaction is not always easy so that in the case of *B. bassiana*, it is possible to get an antagonistic effect. And several pesticides can affect survival of *B. bassiana* depending on the strains. For instance, lufenuron even at low doses was found to be incompatible with *B. bassiana* (strain MTCC-984) (Purwar and Sachan 2005). Some chemical pesticides namely imidacloprid, flufenoxuron, teflubenzuron + phuzalon, endosulfan and amitraz have been used to assess their effect on conidial germination, vegetative growth and sporulation. Of all these chemical pesticides, only Imidacloprid was compatible with *B. bassiana* isolate DEBI008. On the other hand, flufenoxuron was incompatible with *B. bassiana* due to complete inhibition of its development (Alizadeh *et al.* 2007). In other studies, Amutha *et al.* (2010) investigated the compatibility of *B. bassiana* with twelve (12) insecticides for control of cotton pests by the

technique of poisoned feeds and found only chlorpyrifos 20% (mass fraction, the same as below) emulsifiable concentrate (EC) as less toxic to *B. bassiana*, while spinosad (45% suspension concentrate (SC)), econeem (1%), quinalphos (25% EC), acetampride (20%), endosulfan (35% EC) and thiodicarb (75 wettable powder (WP)) were slightly toxic. Imidacloprid (17.80% solution for seed treatment (LS)) and triazophos (40% EC) were moderately toxic and profenofos (50% EC), indoxacarb (14.5% EC) and methyldemeton were highly toxic. Furthermore after assessing the compatibility of five pesticides (phoxim, thiamethoxam, clothianidin, λ -cyhalothrin and β -cypermethrin) commonly used against *Bradysia odoriphaga* (Diptera: Sciaridae) with a high virulence strain of *B. bassiana* YB8, Fan et al. (2017) recommended the application of thiamethoxam and clothianidin in combination with *B. bassiana* for control of *B. odoriphaga*, while phoxim, λ -cyhalothrin and β -cypermethrin could be used only at low concentration. In addition, some acaricides belonging to the organophosphorus and organostanic chemical groups were reported to significantly affect conidia germination and the vegetative growth and sporulation of *B. bassiana* (de Oliveira and Neves 2004).

Another constraint is related to the timing and synchronization of *B. bassiana* applications with some chemical pesticides. For example, the efficacy of *B. bassiana* against adults of *Lygus lineolaris* Palisot de Beauvois (1818) (Heteroptera: Miridae) was not altered within four days delay before the application of fungicides. But when fungicides were applied before the use of *B. bassiana*, this resulted in an antagonistic effect with lower mortality of *L. lineolaris* adult population (Kouassi et al. 2003c reported by Sabbahi 2008). Herbicides and plant growth regulators have been extensively used in most agrosystems, and their compatibility with entomopathogenic fungi was not often established (Sabbahi 2008). But, glufosinate ammonium was not compatible with *B. bassiana* applied to control the potato beetle (Todorova et al. 1998). Indeed, this herbicide had an inhibitory effect on mycelial growth and sporulation of *B. bassiana*. However, diquat had a stimulating effect on the insecticidal activity of *B. bassiana* causing 50%–76.6% mortality of adult Colorado potato beetle *Leptinotarsa decemlineata* (Say).

Constraints related to the compatibility of *B. bassiana* with biological control agents

Few studies have focused on the possibility of combining an entomopathogen such as viruses, protozoa and bacteria with *B. bassiana* in order to increase control efficiency. In addition, the antagonism phenomenon between microorganisms constitutes a major limit to the combination of biological control agents. A biopesticide

used to control a pest may become ineffective in the presence of an antagonist agent in the field. For instance, *B. bassiana* is susceptible to some pathogenic fungi including *Penicillium urticae* which inhibits conidial germination and mycelial growth through a metabolite, patulin (Shields et al. 1981). The application method can affect the interactions between *B. bassiana* and other biocontrol agents. Al-mazra'awi et al. (2009) showed that, in the mixture application, onion thrips adults treated with *B. bassiana* and sub-lethal doses of neem tree extracts exhibited higher mortalities than insects treated with the fungus alone but without synergistic interactions. However, the two control agents interacted antagonistically when neem tree extract was used at full field application rate. But, in the topical application of *B. bassiana* and neem tree extract drenching, the treated insects exhibited mortalities higher than when each control agent was used alone and the two control agents interacted synergistically at sub-lethal doses of the neem tree extract. Togbé et al. (2014) evaluated the synergistic effects of *B. bassiana* (Bals.-Criv. Vuill.) (isolate Bb11) and *Bacillus thuringiensis* var. *kurstaki* (Berliner) (*Bt*) with neem oil in three agroecological zones in Benin and reported an absence of synergy between neem oil and *B. bassiana*, neem oil and *Bt* against cotton pests.

Nevertheless, Lewis et al. (1996) have shown that the combination of *Bt* and *B. bassiana* in maize field increased mortality of corn borer larvae, *Ostrinia nubilalis* (Hübner). Also, *B. bassiana* offers a way forward for the biological control of greenhouse crops. According to Jacobson et al. (2010), *B. bassiana*-based mycopesticide could be used as a second line of defense to support the preventive control of *Amblyseius cucumeris* (Acarina: Phytoseiidae) against berry thrips populations, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) feeding on cucumbers in a greenhouse. Combined application of *B. bassiana* (Balsamo) Vuillemin and predatory mite *Neoseiulus barkeri* Hughes were effective against *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) (Wu et al. 2015).

Barbercheck and Kaya (1991) reported that coinfections by nematodes (*Steinernema carpocapsae* (Weiser) or *Heterorhabditis bacteriophora* Poinar) and *B. bassiana* (Bals.) Vuill could speed the rate of lethal infection with high mortality in treated *S. exigua* (Hübner) populations.

Regarding the registration of a microbiological control agent, it's necessary to ensure the safety for non-target organisms, especially predatory insects and parasitoids (Inglis et al. 2001). The entomopathogen *B. bassiana* has a large spectrum of host insect, and its pathogenicity and virulence vary with isolates and host insects (Goettel et al. 1990). It should be important that new research activities focus on parasitoids, generalist predators,

Table 1 Summary of the control programs that successfully used *Beauveria bassiana* for crop protection from 2009 to 2019

| Continent | Country or region | Application strategy (alone or combined with other biocontrol agents) | Target pest | Crops / bio-assay condition | Inoculation Method | Authors |
|-----------|-------------------|--|---|---|---|-----------------------------|
| Africa | Benin | Alone | Diamondback moth (<i>Plutella xylostella</i> L.) (Lepidoptera: Plutellidae) | Cabbage leaves | Each disinfected leaf was dipped individually into 10^8 mL ⁻¹ conidia suspension (inoculum) of tested fungal specie for 1 min and introduced into a 2.5 cm × 7 cm plastic tube | Vodouhe et al. (2009) |
| Africa | Benin | Alone | Cotton bollworm <i>H. armigera</i> . (Hübner) (Lepidoptera: Noctuidae) | Laboratory experimentation | Applying topically to inoculate larvae by two <i>B. bassiana</i> isolates (Bb11 and Bb12) at 10^8 conidia·mL ⁻¹ on the third, fourth, fifth and the sixth instars of <i>H. armigera</i> | Douro et al. (2012b) |
| Africa | Benin | Alone | Cotton bollworm <i>H. armigera</i> . (Hübner) (Lepidoptera: Noctuidae) | Laboratory experimentation | Applying topically to inoculate larvae by <i>B. bassiana</i> (isolate Bb11) at 0, 10^4 , 10^5 , 10^6 , 10^7 conidia·mL ⁻¹ on fourth instars of <i>H. armigera</i> | Douro et al. (2012a) |
| Africa | Benin | Combined with Neem (<i>Azadirachta indica</i>) oil | <i>Earias</i> spp., <i>Diparopsis watersi</i> Rothschild (Lepidoptera: Noctuidae), <i>H. armigera</i> , <i>P. gossypiella</i> , <i>C. leucotreta</i> and <i>Dysdercus voelkeri</i> Schmidt (Hemiptera: Pyrrhocoridae) | Cotton (Field evaluation) | Foliar sprays | Togbé et al. (2014) |
| Africa | Benin | Alone | <i>Maruca vitrata</i> (Lepidoptera: Crambidae) | Experiments under laboratory conditions | Applying 2 µL of conidia suspension (10^7 , 10^8 , 10^9 and 10^{10} conidia·mL ⁻¹) formulated in peanut oil on different larval stages (first, second, third, fourth and the fifth instars) | Toffa-Mehinto et al. (2014) |
| Africa | Egypt | Alone | Red Palm weevil (<i>R. ferrugineus</i> (Oliv.)) (Coleoptera: Curculionidae) | Red Palm trees | 1-injection of <i>B. bassiana</i> in naturally infested palm trees 2-periodical dusting application of fungal spores on palm trees 3- release of contaminated males of red palm weevil with fungal spores | Sewify et al. (2009) |
| Africa | Egypt | Alone | Red Palm weevil (<i>R. ferrugineus</i> (Oliv.)) (Coleoptera: Curculionidae) | Red Palm trees under Laboratory and Field Conditions. | – | El-Sufty et al. (2009) |
| Africa | Kenya | Alone or Combined with The predator <i>Teretrius nigrescens</i> Lewis (Col.: Histeridae) | Larger grain borer (<i>Prostephanus truncatus</i> (Horn)) | stored maize | Two doses (1×10^9 CFU·kg ⁻¹ maize and 1×10^{10} CFU·kg ⁻¹ maize) of <i>B. bassiana</i> , with and without the predator, <i>T. nigrescens</i> , were applied to maize infested with <i>P. truncatus</i> | Nboyine et al. (2015) |
| – | Mediterranean | Alone | The Red Palm weevil (| Red Palm, bioassay in | - immersion of eggs, | Dembilio et al. |

Table 1 Summary of the control programs that successfully used *Beauveria bassiana* for crop protection from 2009 to 2019 (Continued)

| Continent | Country or region | Application strategy (alone or combined with other biocontrol agents) | Target pest | Crops / bio-assay condition | Inoculation Method | Authors |
|-----------|-------------------|---|--|---|--|--------------------------|
| | Basin | | <i>Rhynchophorus ferrugineus</i> (Olivier 1790)) (Coleoptera: Curculionidae) | laboratory conditions | larvae and adults in conidial aqueous suspension at different concentrations | (2010) |
| Africa | Nigeria | Alone | Flea beetle <i>Podagrica</i> spp. (Coleoptera: Chrysomelidae) | Okra (<i>Abelmoschus esculentus</i>) (L.) Moench | Botanigard 22WP (<i>Beauveria bassiana</i> strain GHA) was mixed with water in a knapsack sprayer and applied to okra leaves from 2 weeks after planting plants at 3–57 g per 1.5 L of water and repeated at weekly interval for 6 weeks. | Kudemepo et al. (2018) |
| America | Brazil | Alone and Combined with neem oil formulation (Neemseto®). | The aphid <i>Lipaphis erysimi</i> (Kalt.) (Hemiptera: Aphididae) | Kale, <i>Brassica oleracea</i> var. <i>acephala</i> D.C | Leaf discs dipping or spraying the aphids | Araujo et al. (2009) |
| America | Brazil | Alone | <i>Cosmopolites sordidus</i> adults (Germar, 1824) (Coleoptera: Curculionidae). | Bioassay in Laboratory | The beetles were immersed in a fungal suspension in the concentration of 1.12×10^9 conidia·mL ⁻¹ | Almeida et al. (2009) |
| America | Brazil | Alone | <i>Thaumastocoris peregrinus</i> (Hemiptera: Thaumastocoridae) | <i>Eucalyptus camaldulensis</i> , | At 1.0×10^8 conidia·mL ⁻¹ , solution was applied on leaves of <i>Eucalyptus camaldulensis</i> , provided as food for the insect substrates | Lorencetti et al. (2018) |
| America | Colombia | Alone | Various pests species and diseases | <i>Phaseolus vulgaris</i> | 10^8 conidia·mL ⁻¹ (or water) applied either as a foliar spray or a soil drench | Parsa et al. (2013) |
| America | USA | Alone | The diamondback moth (<i>Plutella xylostella</i>), European corn borer (<i>Ostrinia nubilalis</i>), corn earworm (<i>Helicoverpa zea</i>), and fall armyworm (<i>Spodoptera frugiperda</i>); beet armyworm (<i>Spodoptera exigua</i>), black cutworm (<i>Agrotis ipsilon</i>), cabbage worm (<i>Pieris rapae</i>) and cabbage looper (<i>Trichoplusia ni</i>). | Bio-assay in Laboratory | Larvae were topically sprayed and maintained on the treated substrate for 24 h at 100% relative humidity | Wraight et al. (2010) |
| America | USA | Alone | The small southern pine engraver, <i>Ips avulsus</i> Eichhoff (Coleoptera: Scolytidae) | Pine bolt | Field treatment by a commercial preparation of <i>B. bassiana</i> in protecting pine host plant tissue from colonization | Olatinwo et al. (2018) |
| – | – | Alone | Citrus insect pests, <i>Ceratitis rosa</i> Karsch, <i>C. capitata</i> Wiedemann (Diptera: Tephritidae) and <i>Thaumatotibia</i> | In laboratory conditions | – | Goble et al. (2011) |

Table 1 Summary of the control programs that successfully used *Beauveria bassiana* for crop protection from 2009 to 2019 (Continued)

| Continent | Country or region | Application strategy (alone or combined with other biocontrol agents) | Target pest | Crops / bio-assay condition | Inoculation Method | Authors |
|-----------|---------------------|---|---|--|--|---------------------------------------|
| Asia | China | Alone | <i>leucotreta</i> Meyrick (Lepidoptera: Tortricidae) The white worms of <i>Ostrinia furnacalis</i> Guenée (Lepidoptera: Crambidae) in China | In laboratory conditions | – | Xu et al. (2011) |
| Asia | China | Alone | The onion thrips <i>Thrips tabaci</i> 'adult and larval stages' | Broccoli, onion: in laboratory and greenhouse evaluation | Apply at 1×10^7 mL ⁻¹ conidia | Wu et al. (2013) |
| Asia | China | Alone and Combined with predatory mite <i>Neoseiulus barkeri</i> Hughes (Acarina: Phytoseiidae) | The western flower thrips (<i>Frankliniella occidentalis</i>) | Greenhouse cucumber | – | Wu et al. (2016) |
| Asia | China | Alone | <i>Dendrolimus punctatus</i> | Bioassay in laboratory conditions | By dipping larvae with spore suspensions | Wang et al. (2016) |
| Asia | India | Alone | The diamondback Moth, <i>Plutella xylostella</i> Linn.) | Cabbage <i>Brassica oleracea</i> var. <i>capitata</i> | Spraying of different concentrations of <i>B. bassiana</i> | Kamal et al. (2018). |
| Asia | India | Alone | <i>H. armigera</i> , <i>Spodoptera litura</i> Fabricius, <i>Earias vittella</i> Fabricius, <i>Aphis gossypii</i> Glover, <i>Bemisia tabaci</i> Gennadius and <i>Amrasca devastans</i> Distant | Bioassay in laboratory conditions | The liquid formulations tested were Beevicide and sprayed | Karthikeyan and Selvanarayanan (2011) |
| Asia | Indian subcontinent | Alone | <i>Spodoptera litura</i> (Fab.), (Lepidoptera: Noctuidae) | Bio-assay in laboratory using fresh cotton leaves | The fungal inoculum (10 mL per insect individual of aqueous suspension of 10^9 conidia·mL ⁻¹ with 0.02% Tween 80) was sprayed on the larvae | Vijayavani et al. (2009). |
| – | – | Combined with the predator <i>Neoseiulus barkeri</i> (Acari: Phytoseiidae) | <i>Frankliniella occidentalis</i> Pergande (Thysanoptera: Thripidae) | Bioassay in laboratory conditions | 1.9×10^3 CFU per insect | Wu et al. (2015) |
| Asia | Iran | Alone | <i>Oryzaephilus surinamensis</i> , <i>Sitophilus granarius</i> , and <i>Tribolium castaneum</i> | Bioassay in laboratory conditions | Commercially produced, formulated conidia of <i>B. bassiana</i> strain PPRI 5339 (BbWeevil™, Biological Control Products, South Africa) containing 2.9×10^9 conidia per gram of powder was used, Formulation was applied at five rates of: 0, 250, 500, 750 and 1 000 mg·kg ⁻¹ . | Khashaveh et al. (2011) |

Table 1 Summary of the control programs that successfully used *Beauveria bassiana* for crop protection from 2009 to 2019 (Continued)

| Continent | Country or region | Application strategy (alone or combined with other biocontrol agents) | Target pest | Crops / bio-assay condition | Inoculation Method | Authors |
|-----------------|-------------------|---|---|--|---|-----------------------------|
| | | | | | Fifteen lots of 1 kg of wheat grain (one lot for each exposure time-rate) were prepared and placed in separate cylindrical jars (2l capacity with screwed lids) and treated with the appropriate dose | |
| Asia | Iran | Alone | Adults of <i>Callosobruchus maculatus</i> (F.) and <i>Sitophilus granarius</i> (L.) | Bioassay in laboratory using stored grain of wheat and cowpea | Adults were tested with five 0, 250, 500, 750 and 1 000 mg·kg ⁻¹ and exposure intervals of 5, 10 and 15 days, in (24 ± 2) °C and (50 ± 5)% relative humidity | Khashaveh et al. (2011) |
| Asia | Jordan | Combined with neem (<i>Azadirachta indica</i>) tree extract | <i>Thrips tabaci</i> Lindeman (1889) (Thysanoptera: Thripidae) | Potted tomato plants in a greenhouse | -Topical application of a mixture of the two control agents -Topical application of <i>B. bassiana</i> and a drenching application of neem tree extract | Al-mazra'awi et al. (2009) |
| Europe and Asia | Turkey | Alone | June beetle, <i>Polyphylla fullo</i> (L.) (Coleoptera: Scarabaeidae) | Bioassay in laboratory conditions | - <i>B. bassiana</i> strain PPRI 5339, product (min. 4 × 10 ⁹ conidia·mL ⁻¹) was applied at 100, 150, and 200 mL per 100 L water | Erler and Ates (2015) |
| Europe | Bulgaria | Alone | <i>Tetranychus urticae</i> Koch. (Acari: Tetranychidae) | Laboratory conditions | treated by spraying conidial suspensions of isolates at concentrations of 10 ⁶ , 10 ⁷ and 10 ⁸ conidia/ml. Lethal | Draganova and Simova (2010) |
| Europe | Central Europe | Alone | <i>Ips typographus</i> , spruce bark beetle | Spruce | Tested by direct contact | Mudrončeková et al. (2013) |
| Europe | France | Alone | The pollen beetles (<i>Meligethes</i> spp.) | Bioassay in laboratory conditions | Spraying of an oil formulation of <i>Beauveria bassiana</i> | Kaiser et al. (2016) |
| Europe | Germany | Alone | -vine mealybug (<i>Planococcus ficus</i>) - grape leafhopper (<i>Empoasca vitis</i>) | Bioassay in greenhouse and field experiments with grapevine <i>Vitis vinifera</i> (L.) | Applied either as conidial suspensions (ATCC 74040 and GHA) or as a formulated product (Naturalis®, strain ATCC 74040) on grapevine leaves. | Rondot and Reineke (2018) |
| Europe | Hungary | Alone | <i>Tetranychus urticae</i> (Koch) (Acarina: Tetranychidae). | Bioassay in laboratory conditions | Applying at 10 ⁴ , 10 ⁵ , 10 ⁶ , 10 ⁷ , 10 ⁸ , 10 ⁹ and 3.7 × 10 ⁹ conidia mL ⁻¹ . | Yeşilayer (2018) |
| Europe | Romania | Alone | Beet armyworm (<i>Spodoptera exigua</i>) (Lepidoptera: Noctuidae) | Bioassay in laboratory conditions | 2 g/L water concentration of <i>B. bassiana</i> | Hasyim et al. (2017) |
| Europe | UK | Combined with predator <i>Phytoseiulus</i> | <i>Tetranychus urticae</i> Koch (Acari: Tetranychidae) | Bean plants | single application of <i>B. bassiana</i> (1 × 10 ⁸ spore per mL) after | Ullah and Lim (2017) |

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| Continent | Country or region | Application strategy (alone or combined with other biocontrol agents) | Target pest | Crops / bio-assay condition | Inoculation Method | Authors |
|-----------|-------------------|---|--|--|---|-----------------------------|
| | | <i>persimilis</i> (Acari: Phytoseidae) | | | application of the low release rate of <i>P. persimilis</i> (5 prey:1 predator) | |
| Oceania | Australia | Alone | <i>Aphis gossypii</i> and <i>Chortocetes terminifera</i> | Cotton, wheat, corn, pumpkin, bean, tomato | Feeding larvae using leaves colonized by the entomopathogens <i>B. bassiana</i> | Gurulingappa et al. (2010). |
| Oceania | New Zealand | Alone | Bark beetle pests of plantation forest trees | Radiata pine | Inoculation of seed and seedlings | Brownbridge et al. (2012) |

entomopathogenic nematodes and microorganisms in order to identify the factors that determine compatibility and synergy between the various biocontrol agents for their use in the biological control programs including *B. bassiana*.

Difficulties related to the formulation of biopesticide based on *B. bassiana*

Formulation is the conditioning of the biopesticide in a commercial form (mixture useable in the dry state or dispersed in water for spraying). According to Jones & Burges (1998) reported by Leggett et al. (2011), formulation of biocontrol agents can be used to: stabilize the organisms during production, distribution and storage; facilitate the handling and application of the product; protect the agent from harmful environmental factors; and enhance the activity of the organisms. From this definition, it appears that the goal of formulating biopesticides is to make these technologies more predictable and competitive comparable to synthetic pesticides:

- Facilitate dispersion, manipulation;
- Make the dosage more precise (measurement);
- Add interesting properties (dispersion, adhesion, protection against UV, against desiccation, penetration ...);
- Improve storage stability (keep these properties at best for 24 months);
- Finally, a biopesticide protects better the environment than chemical pesticide.

The difficulties related to the formulation of the biopesticide based on *B. bassiana* include finding materials to be combined, to get not only the viability of conidia but also their stability (eg. desiccation tolerance, UV protection, shelf life, etc.) and their effectiveness.

The granulated formulations can be obtained either by coating previously harvested spores (Leland and Behle 2005; Sabbahi 2008) or by growth and sporulation of the fungus on the surface of a granular nutrient carrier. The first method, already applied to many auxiliary microorganisms, easily industrialized, is still insufficiently tested in the field for entomopathogenic fungi and prospects for agronomical use cannot be accurately estimated (Sabbahi 2008). Oily formulations containing UV, humectant and nutrient protectors for spore germination and growth have great potential for better results (Bateman et al. 1994; Burges 1998; Goettel et al. 1990). In this point of view, Todorova and Weill (2006) showed in a field trial against the Colorado potato beetles that the oil had been added to prevent desiccation of conidia and milk for protection of very harmful UV rays for *B. bassiana*. Oils also provide better adhesion and adequate spore application to the hydrophobic cuticle and insect (Douro et al. 2012a, b). For example, Croda's technical expertise covers a range of technologies and processes for advanced additives and adjuvants. Through Croda's approach, the best approach to choose a formulation adjuvant is to use mild and biocompatible surfactants such as: low risk nonionic polymers, sorbitan esters and polysorbates.

Biopesticides market in Africa

Companies that obtained patents

The biopesticide market is still in its early stage of development because the rate of biopesticides sold versus chemical pesticides is only 0.25% (van Lenteren 2000 cited by Caron (2006)). Several reasons can explain the fragility of this market:

- Product approval procedures may take time.
- Lack of study on assessing or establishing evident profitability for the producer and the consumer in

comparison to chemicals. For example, the treatment cost of *B. bassiana* is not yet established. The treatment cost depends on the product price, the treatment frequency and labour for treatment (Sabbahi 2008).

- Most biopesticides consisted of living organisms often with relatively narrow spectrum of target pests and their effectiveness depends on environmental factors (Caron 2006).

Some of the companies that have obtained patents include: Biological Control Products SA (Pty) Ltd., BASF South Africa (Pty) Ltd., Plant Health Products (Pty) Ltd., Dudutech (Pty) Ltd. involved in the production and sale of biologically based products for agriculture in Africa and other part of the world.

Commercialized products

Information are given in Table 2 on commercialized products of manufacturers in Africa.

Attempting on the use of *B. bassiana* in cotton Lepidopteran pest management

Cotton as the first cash crop in Benin is income-generating for all links in the value chain. It contributes 80% to official export earnings and 13% to GDP (Afouda *et al.* 2013). In 2018–2019 season, the recorded production was 700 000 t, ranking Benin as the largest producer in West Africa (Tonavoh 2019). The increase in cotton production is related to the expansion of cultivated areas, the use of new cotton varieties (CRA-CF 2018). Expansion of cultivated area led to higher consumption of chemical pesticides in order to overcome insect pests damage (Westerberg 2017). Indeed, cotton is the most damaged crop in Benin. Over than 500 species have been reported attacking cotton plants (Celini 2001). In Benin, almost 90% of all imported pesticides are applied on cotton to control the various insect pests. This led to an overuse of chemicals with many side effects such as human hazards (frequent pesticide poisoning, skin and stomach irritation), insect pest resurgence and resistance, environmental pollution (Lawani *et al.* 2017). Insect resistance mainly of Lepidopteran species is major issue to be solved for boosting cotton production in Benin. The major Lepidopteran insect pests of cotton in Benin are carpophagous and phyllophagous caterpillars namely *H. armigera*, *Pectinophora gossypiella*, *Syllepte derogata* and *Diparopsis watersii*. Many studies have reported the resistance of *H. armigera* to synthetic pyrethroids as worldwide problem in Australia, China, India, Spain, Ivory-Coast and Benin (Martin *et al.* 2000; Djihinto *et al.* 2009; Brun-Barale *et al.* 2010). Managing these pests is a big challenge in the context of resistance (Djihinto *et al.* 2016). The most frequently used

active substances were insecticides such as acetamiprid, lambda-cyhalothrin, chlorpyrifos-ethyl, emamectin benzoate, profenofos or cypermethrin; all are known to be more or less toxic and may have detrimental effects on health after exposure (Gouda *et al.* 2018). Current management strategies to deal with insect resistance consisting of combining molecules having different modes of action or alternating insecticidal compounds with sequential applications do not always give expected level of control. Therefore, alternative environmental sound methods namely biological control using entomopathogenic organisms become an attractive option to manage especially resistance in Lepidopteran species. In this perspective, the entomopathogen *B. bassiana* is a promising candidate.

The current review addresses the great potential of *B. bassiana* for the control of several insect pest species. In Benin, more than 20 isolates of *B. bassiana* were stored at the entomopathogen bank of the International Institute of Tropical Agriculture (IITA) Benin station. Of these, only two isolates were tested on cotton Lepidopteran species namely *H. armigera* (Douro *et al.* 2012a, b). This study demonstrated that direct spray of the fungal suspension was effective against *H. armigera* inducing high mortality in larvae. There is a great need of information on the potential of *B. bassiana* to control cotton Lepidopteran species in the context of their resistance against the currently applied synthetic chemicals. Assessing the susceptibility of the main caterpillars destroying cotton plants to the different isolates, determining the lethal doses of virulent isolates are important steps in developing effective strategy of *B. bassiana* application in cotton production. Besides the effect of direct application of *B. bassiana* suspension on cotton Lepidopteran caterpillars, the endophytic colonization of cotton plant by *B. bassiana* may influence the survival of caterpillars feeding on different plant organs. Indeed, cotton is one of the plant species that can be colonized by the endophyte *B. bassiana* (Griffin 2007; Gurulingappa *et al.* 2010). This fungus species is known to colonize plant tissues giving a natural defense against different pests through various synthesized metabolites without being harmful to colonized plants. Assessing the effect of such colonization on cotton Lepidopteran species would be useful in defining effective control strategy in the context of insect resistance to chemicals. Laboratory studies should be supported by field experiments to find optimal use of *B. bassiana* in cotton production. The optimization of the use of *B. bassiana* through direct application or endophytic colonization of cotton plant would contribute to reducing control cost for a sustainable cotton production. Increase in cotton production would improve farmers' income and thereby food security in rural areas of Benin.

Table 2 Presentation of manufacturers in Africa and commercialized products

| Manufacturer | Registration | Products | Name of products | Brief description of products | Role/Disease or target pest controlling |
|--|--------------|--|-------------------------------------|--|---|
| Plant Health Products (Pty) Ltd. | South Africa | Biofungicide & Biostimulant | Eco-T | Contains <i>Trichoderma asperellum</i> (previously known as <i>T. harzianum</i>) | - Management of common root diseases including <i>Fusarium</i> , <i>Rhizoctonia</i> , <i>Pythium</i> and <i>Phytophthora</i> -Eco-T stimulates plant root growth, improves germination offering faster seedling development and promotes healthier, more productive plants |
| Plant Health Products (Pty) Ltd. | South Africa | Biofungicide & Biostimulant | Eco-T Ezi-Flo | Eco-T in a graphite/talc lubricant formulation, suitable for use with mechanical planters (Eco-T Ezi-Flo) | Management of common root diseases including <i>Fusarium</i> , <i>Rhizoctonia</i> , <i>Pythium</i> and <i>Phytophthora</i> -Eco-T stimulates plant root growth, improves germination offering faster seedling development and promotes healthier, more productive plants |
| Plant Health Products (Pty) Ltd. | South Africa | Aerial Biofungicide | Eco-77 (T-77) | Aerial biofungicide Eco-77 is a wettable powder (WP) concentrate of a beneficial aerial <i>Trichoderma atroviride</i> (previously known as <i>T. harzianum</i>) strain | -Effective against <i>Botrytis</i> on leaves, flowers and fruit for cucumbers and tomatoes and grapevine - Eco-77 colonizes pruning wounds and prevents pathogens like <i>Botrytis</i> from gaining entrance to the vineyard |
| Plant Health Products (Pty) Ltd. | South Africa | Bio-insecticide | Eco-Bb (Bb-Protec) | A concentrated wettable powder formulation of the entomopathogenic fungus, <i>Beauveria bassiana</i> . It is non-toxic, residue free and may be used right up to harvest | Eco-Bb infects and kills whitefly, spider mite, Fall armyworm (<i>Spodoptera frugiperda</i>), False codling moth <i>Thaumatotibia</i> (<i>Cryptophlebia leucotreta</i>), <i>Tuta absoluta</i> and various other agricultural insect pests |
| Plant Health Products (Pty) Ltd. | South Africa | Bacterial inoculant | -Eco-Rhiz Soya -Eco-Rhiz Lucerne | Eco-Rhiz Soya and Eco-Rhiz Lucerne are both biofertilizer, their bacterial inoculant containing <i>Bradyrhizobium japonicum</i> strain WB74 and <i>Sinorhizobium meliloti</i> strain RF14 respectively | Their bacterial inoculant improve fixing nitrogen in the root nodules of soya bean (soybean) and Lucerne (Alfalfa) |
| Biological Control Products SA (Pty) Ltd | South Africa | -Bio fertilizers - Soil inoculants -Plant stimulants - Bio-pesticides | Not specified | Not specified | -bio fertilizers that help in keeping and sustaining the biological population in soil, as well as aid in the nutrient management of plants - soil inoculants, which are a range of micro-organisms that build and replenish the biological population in soil -plant stimulants, which are a range of products that assist plants through periods of stress, as well as optimize plant development during the critical growth stages |

Table 2 Presentation of manufacturers in Africa and commercialized products (*Continued*)

| Manufacturer | Registration | Products | Name of products | Brief description of products | Role/Disease or target pest controlling |
|--|--------------|---|-------------------|---|---|
| | | | | | -and bio-pesticides that control plant pests and diseases |
| Biological Control Products SA (Pty) Ltd | South Africa | Bio-pesticide | Bb Weevil | CP formulation of <i>Beauveria bassiana</i> conidia | Used for the control of Coleoptera (Curculionidae) |
| Biological Control Products SA (Pty) Ltd | South Africa | Bio-pesticide | Bb Plus | WP formulation of <i>Beauveria bassiana</i> conidia | Used for the control of Hemiptera (Aphididae), Acari (Tetranychidae) |
| BASF South Africa (Pty) Ltd | South Africa | Less bio-pesticide and more chemicals, plastics and agricultural products | BroadBand® | Emulsifiable spore concentrate (EC) of <i>Beauveria bassiana</i> strain PPRI5339, BroadBand® contains 4×10^9 CFU·mL ⁻¹ . It a fungal contact insecticide, derived from a hardy African strain, isolated from the tortoise beetle and active at all stages of the life cycle of insects – eggs, larvae, juveniles and adults. | BroadBand® – a fungal contact insecticide for the effective reduction of target insects on crops including <i>Plutella xylostella</i> , <i>Thaumatotibia leucotret</i> , <i>Aonidiella aurantii</i> , <i>Tetranychus urticae</i> , <i>Phthorimaea operculella</i> , stinkbug, thrips, whiteflies. Its effectively controls the potato tuber moth (<i>Phthorimaea operculella</i>) on potatoes, red spider mite (<i>Tetranychus urticae</i>) and whitefly on tomatoes and thrips on onions |
| Dudutech Ltd. | Kenya | Beneficial fungi | Beauvitech®WP | A wettable powder (WP) formulation containing spores of <i>Beauveria bassiana</i> strain J25 | Target challenge: aphids, coffee berry borer, leafminers, mealybugs, scale insects, thrips, whiteflies Crop: coffee, flowers, fruits, vegetable |
| Dudutech Ltd. | Kenya | Beneficial fungi | Lecatech®WP | A biological insecticide containing <i>Lecanicillium lecanii</i> , a naturally occurring entomopathogenic fungus that is effective against whiteflies | Target challenge: aphids, leafminers, mealybugs, scale insects, thrips, whiteflies Crop: coffee, Flowers, Fruits, Vegetables |
| Dudutech Ltd. | Kenya | Beneficial fungi | Mytech®WP | A biological nematicide based on <i>Paecilomyces lilacinus</i> a naturally occurring nematophagous fungus for control of plant parasitic nematodes | Target challenge: enhanced root development, plant parasitic nematodes Crop: cereals, coffee, flowers, fruits, ea, vegetables |
| Dudutech Ltd. | Kenya | Beneficial fungi | Trichotech®WP | Contains spores of <i>Trichoderma asperellum</i> an antagonistic fungus that is used globally for control of soil borne fungal diseases | Target challenge: <i>Armillaria</i> , <i>Botrytis</i> Blight, Crown Gall, <i>Fusarium</i> , <i>Pythium</i> , <i>Rhizoctonia</i> , Root Rots, <i>Sclerotinia</i> , Stem Cankers Crop: cereals, flowers, fruits, tea, vegetables |
| Dudutech Ltd. | Kenya | Beneficial insects and predators | Amblytech C® | Contains <i>Amblyseius cucumeris</i> a predatory mite used for the management of Thrips | Target challenge: broad mite, cyclamen mite, red spider mite, thrips larvae Crop: flowers, fruits, tea, vegetables |

Table 2 Presentation of manufacturers in Africa and commercialized products (*Continued*)

| Manufacturer | Registration | Products | Name of products | Brief description of products | Role/Disease or target pest controlling |
|---------------|--------------|----------------------------------|---------------------------------------|---|--|
| Dudutech Ltd. | Kenya | Beneficial insects and predators | Amblytech® | Contains <i>Amblyseius californicus</i> , a predatory mite used for the management of red spider mites | Target challenge: red spider mite Crop: flowers, tea, vegetables |
| Dudutech Ltd. | Kenya | Beneficial insects and predators | Diglytech® | Contains living <i>Diglyphus isae</i> , a parasitic wasp that is ectoparasitic i.e. develops nearby, but outside the leaf miner larvae | Target challenge: leafminers Crop: flowers, vegetables |
| Dudutech Ltd. | Kenya | Beneficial insects and predators | Hypotech® | Contains the predatory mite <i>Hypoaspis miles</i> used for the management of thrips, fungus gnats, leaf miner, sciarid flies and shore flies | Target challenge: bulb mites, leafminers, sciarid flies, thrips Crop: flowers, vegetables |
| Dudutech Ltd. | Kenya | Beneficial insects and predators | Phytotech® | Contains a predatory mite <i>Phytoseiulus persimilis</i> marketed for management of the two-spotted mite (<i>Tetranychus urticae</i>) | Target challenge: red spider mite, two spotted mite Crop: flowers, fruits, vegetables |
| Dudutech Ltd. | Kenya | Beneficial nematodes | Nematech S® SP | A biological insecticide containing infective juveniles of <i>Steinernema feltiae</i> (isolate DDT-D2) in an inert carrier | Target challenge: leafminers, sciarid flies, thrips Crop: flowers, vegetables |
| Dudutech Ltd. | Kenya | Beneficial nematodes | Nematech H® | A biological insecticide containing infective juveniles of <i>Heterorhabditis bacteriophora</i> (isolate DDT- F27) in an inert carrier | Target challenge: caterpillars, cutworms, leafminers Crop: flowers, vegetables |
| Dudutech Ltd. | Kenya | Beneficial nematodes | Slugtech® SP | A biological molluscicide containing infective juveniles of <i>Phasmarhabditis hermaphrodita</i> (isolate DDT M1) in an inert carrier | Target challenge: slugs, snails Crop: flowers, vegetables |
| Dudutech Ltd. | Kenya | Beneficial virus | Helitech | <i>Helicoverpa armigera</i> nucleopolyhedrovirus (HearSNPV) isolated from nature in Kenya and has not been genetically altered | Target challenge: <i>Helicoverpa armigera</i> Crop: vegetables |
| Dudutech Ltd. | Kenya | Botanics | NEMguard® | A powerful polysulphide solution for the management of plant parasitic nematodes | Target challenge: plant parasitic nematodes Crop: lowers, fruits, vegetables |
| Dudutech Ltd. | Kenya | Botanics | NEMROC® | Azadirachtin | Target challenge: plant parasitic nematodes Crop: flowers, egetables |
| Dudutech Ltd. | Kenya | Traps and pheromones | Delta Trap® | Traps for control of flying insect pests. These traps are cost-effective, readily assembled traps, are easy to use enabling fast implementation for a quick infestation count | Target challenge: diamonback moth, <i>Duponchelia</i> , false coddling moth, <i>Tuta absoluta</i> Crop: flowers, vegetables |
| Dudutech Ltd. | Kenya | Traps and pheromones | Rolltech Trap® | Traps for control of flying insect pests. This product comes in Blue or Yellow depending on the target pests | Target challenge: aphids, leafminers, sciarid flies, thrips, whiteflies Crop: flowers, egetables |
| Dudutech Ltd. | Kenya | Traps and pheromones | -Blue STICKTECH® Yellow STICKTECH® | Blue STICKTECH® and Yellow STICKTECH® cards are used as the "attract and trap" technique for | Target challenge for Blue sticktech: thrips Target challenge for Yellow |

Table 2 Presentation of manufacturers in Africa and commercialized products (*Continued*)

| Manufacturer | Registration | Products | Name of products | Brief description of products | Role/Disease or target pest controlling |
|---------------|--------------|----------------------|----------------------------|---|--|
| | | | | control of flying insect pests | sticktech: aphids, false codling moth, leafminers, mealybugs, sciarid flies, whiteflies Crop: flowers, vegetables |
| Dudutech Ltd. | Kenya | Traps and pheromones | Duponchelia® (Pheromone) | These lures are rubber strips impregnated with a synthetic replica of the <i>Duponchelia</i> female pheromone | Target challenge: <i>Duponchelia</i> Crop: flowers, vegetables |
| Dudutech Ltd. | Kenya | Traps and pheromones | Tuta Absoluta® (Pheromone) | Rubber strip lures, impregnated with a synthetic replica of the <i>Tuta absoluta</i> female pheromone | Target challenge: <i>Tuta absoluta</i> Crop: vegetables |
| Dudutech Ltd. | Kenya | Traps and pheromones | Planococus® (Pheromone) | Mealybug lures are rubber strips impregnated with a synthetic replica of the mealy bug female pheromone | Target challenge: mealybugs Crop: flowers, vegetables |

Conclusions

Crop pests' management by using natural enemies becomes possible on a large scale within a reasonable time due to advances in research, combining laboratory and field studies in an operational perspective. Integrated pest management strategies including the use of *B. bassiana* currently available and reviewed here, offer better alternative to control insect pests especially cotton insect pests.

The major challenge in cotton production is to biologically control the key Lepidopteran species in the context of their resistance to the chemicals currently applied. Facing this challenge, the use of the entomopathogenic *B. bassiana* as a biopesticide could be a good alternative for insect pest control, one of the main constraints of cotton production in Benin. It can be used to minimize the frequency with chemical pesticides in conventional cotton and provides a non-existent solution in organic cotton.

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