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# Effect of Ultraviolet Radiation on Fungi *Beauveria bassiana* and *Metarhizium anisopliae*, Pure and Encapsulated, and Bio-Insecticide Action on *Diatraea saccharalis*

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## Abstract

**The effect of ultraviolet radiation on entomopathogenic fungi can be very prejudicial for causing damage to the conidia. Formulations can help protecting these fungal structures against radiation. The objective of this study was to evaluate the effect of UV radiation on pure and encapsulated conidia *Beauveria bassiana* and *Metarhizium anisopliae sensu lato*, and to evaluate their pathogenicity on the sugarcane borer, *Diatraea saccharalis*. The pure conidia and the sodium alginate capsules containing the fungi were submitted to the ultraviolet radiation in different temperatures and exposure times. On the pure conidia, the radiation had a deleterious effect after 5 minutes of exposure, going from 94% to 52% germination for *B. bassiana* and from 96% to 54% for *M. anisopliae*. The alginate formulation protected the *B. bassiana* conidia against the radiation in all times they were evaluated (15 minutes to 48 hours), because, even after exposure, the fungi remained viable. The dry encapsulated conidia *B. bassiana* caused 79.6% mortality of the studied pest and the *M. anisopliae* caused only 10%.**

## Keywords

**Entomopathogenic Fungi, Microbial Control, Formulation, Lepidoptera, Ultraviolet Exposure**

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## 1. Introduction

Entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* have pathogenic action on various agricultural insect pests [1]-[9]. Its use is considered to be environmentally friendly, being an important tool in Integrated Control of Pests and Organic Agriculture used to control pests such as Hemiptera, Coleoptera, Lepidoptera and others that attack economically-important crops such as coffee, sugar cane, citrus fruit, vegetables, soy, etc. Among the advantages of the agricultural use of microbial products (bio pesticides), it is possible to underscore specificity and selectivity, low probability of target insects building up resistance, longer-lasting pest control, low toxicity to the environment and applicator, lower cost of development and registration, etc. [10].

However, most microbial products available in Brazil to control insects is not formulated, instead, they are produced and applied *in natura*. Ultraviolet radiation and intense heat reduce the viability of conidia in the field, being major obstacles to the successful use of entomopathogenic fungi in agriculture [11]. Temperature is important for entomopathogenic fungi because it affects their metabolism by altering processes for the production of enzymes, toxins, spore germination, development of the germinative tube, penetration, colonization and reproduction. On the other hand, radiation can affect conidia germination and the early stages of development of the germinative tube [12]. According to Bell [13], the fungus *B. bassiana* loses its infection ability when subjected to direct sunlight for three hours.

To maintain the conidia viability in the field, reduce costs and ensure biological control, one alternative is the use of formulated products that may provide benefits such as protecting conidia from radiation, increasing shelf live and facilitating storage, transport, etc. [14] [15].

Among commercial bio-pesticides currently available, approximately 25% of sales are of products formulated by oil dispersions and the remaining 75% is sold only as technical products, with no treatment or addition of substances that ensure improvements during the stages of transport, storage, handling practicality, field persistence, efficient pest control, etc. [16] [17]. Micro-encapsulated formulations (particles) contain the microorganisms encapsulated in materials such as gelatin, starch, cellulose, sodium alginate, etc. [18]. Each particle can contain hundreds of conidia, and, after the drying process and having an increased storage period, it is ready for agricultural use, and can be applied directly on the soil or on the target pest, and even on plants, without the need for dispersion in an aqueous medium, facilitating the application [18]. Such encapsulated particles may be used to control various pests, due to the wide spectrum of fungi used.

Among agricultural pests, the borer *Diatraea saccharalis* (Lepidoptera: Crambidae) is a highlighted concern on cane sugar plantations. It is an insect that easily scatters, has high intensity of attack and produces great financial losses [19]. The damage it causes may be direct, through opening galleries in the culm of the plant, reducing the flow of sap, and thus making it more susceptible to falling over due to the action of wind and rain, droughts, etc. However, the damage can also be indirect, as the holes favor the penetration of phytopathogenic microorganisms into the culm as well as red rot caused by *Colletotrichum falcatum* and *Fusarium moniliforme* fungi, which invert the sucrose, thereby decreasing the purity and quality of the sap, resulting in a lower yield of alcohol and sugar [20]. It is estimated that for each 1% of borer infestation rate, the industrial losses are around 20 to 30 kg of sugar per hectare [21].

Therefore, this study aimed to evaluate the effect of ultraviolet radiation on conidia of the formulated and unformulated entomopathogenic fungi *B. bassiana* and *M. anisopliae*, in order to verify the pathogenicity of both fungi on the insect *Diatraea saccharalis*.

## 2. Material and Methods

### 2.1. Obtaining the Fungi *B. bassiana* and *M. anisopliae* Sensu Lato

The *B. bassiana* (strain IBCB 66) and *M. anisopliae* (strain IBCB 425) fungi, both are stored in the Collection of Entomopathogenic Fungi Oldemar Cardim Abreu of Instituto Biológico/Campinas-SP/Brazil. The *B. bassiana* conidia were provided by Biocontrol Sistema de Controle Biológico Ltda. (Sertãozinho city, SP state, Brazil) and the *M. anisopliae* by Toyobo do Brasil Ltda. (Salto city, SP state, Brazil). Both companies follow the production methodology described in [22], which consists of the production method in solid fermentation. Initially, the rice is pre-cooked in boiling water, for about 15 minutes. The water is drained and the rice is placed in trays. After cooling, 100 g of rice is distributed into each polypropylene plastic bag (35 cm long × 22 cm wide). These bags are closed with metal clips, autoclaved for 30 minutes at 120°C and cooled in ambient

conditions. In each bag, 1.0 mL of a suspension containing $5.0 \times 10^7$ conidia·mL$^{-1}$ is inoculated. In laminar flow, the inoculation is done with the aid of a disposable syringe, then sealing the hole with a sticky label and agitating the recipient for a uniform distribution of the inoculation. After the inoculation, the bags are stored, for 10 days, in a controlled-climate room ($25°C \pm 1°C$ and 12 hours photoperiod) for germination of the conidia and growth of the fungus. After this period, the bags are opened and the content is transferred to a plastic tray. The trays are kept stacked in an antiseptic room at $25°C \pm 1°C$, $70\% \pm 10\%$ related humidity (RH) and 12 hours photoperiod, for 8 days. These periods, after several tests, were defined as the better time to the fungi growth and development. After 4 days, the trays are cross-stacked, thereby allowing air circulation between them and consequently a faster drying of the rice with fungus. After drying, the conidia are extracted from the rice by sieving.

## 2.2. Formulation of the Conidia in Sodium Alginate

In a 1.0 L Becker, a solution was prepared with distilled water and CaCl$_2$ at a concentration of 0.03 M. In a second Becker, the 1.0% (m/v) sodium alginate mixed with the fungi *M. anisopliae* or *B. bassiana* 0.5% (m/v), was solubilized, separately, and the Tween 80® 0.02% (m/v) was added. The material was homogenized by turbo extraction in an Ultra-turrax, IKA T25 equipment. Under vigorous agitation, the CaCl$_2$ solution was slowly added to the sodium alginate solution and the fungi, which were under mild agitation, forming the capsules. The phase transfer was performed with a peristaltic pump, model Pump Pro TPM *600 55RPM from the brand Watson-Marlow Inc., set to total 35 drops/minute. Once the capsules were formed, they were placed, washed in distilled water to remove excess CaCl$_2$. To obtain the dry capsules, drying was conducted in an oven at $24°C$ for 48 hours for both fungi (Patent number BR10 2015 016269 3).

## 2.3. UV Radiation Test

### 2.3.1. UV Radiation Chamber (Natural Products Laboratory/UFSCar)

In the first test, the pure conidia of the fungi *B. bassiana* and *M. anisopliae* were evaluated for resistence to UV radiation. Suspensions of $1.0 \times 10^5$ conidia.mL$^{-1}$ were standardized in a Neubauer chamber and to evaluate the germination of the conidia. A thin layer of PDA (Potato-Dextrose-Agar) was placed in Petri dishes with pentabiotic (0.5 g/L) to avoid bacterial contamination. After the solidification of the medium, each dish received 0.1 mL of the fungal suspensions, prepared in the determined concentration. The suspensions were spread with the help of a Drigalski spatula, then flamed and properly cooled.

The dishes were placed in a chamber radiated by G15T8E USHIO lamps of 15W ($l = 45.0$ cm, $w = 2.6$ cm, USHIO, Japan), the emission spectrum of which is concentrated in the UV-B region (306 mm). In this experiment, 4 chamber lamps were switched on, equivalent to an irradiance of 6153.3 mW·m$^{-2}$ and the energy inside the chamber of 22.15 kJ·m$^{-2}$·h$^{-1}$ and two central lamps (5653 mW·m$^{-2}$ or 20.35 kJ·m$^{-2}$·h$^{-1}$). Radiance measurement was performed using a spectroradiometer (Ocean Optics Model USB2000 + Rad) connected to a portable computer. The chamber used was built of wood completely lined with mirrors ($l = 60.0$ cm, $h = 40.0$ cm and $w = 60.0$ cm) absorbing no radiation. The lamps were positioned approximately 25 cm above the samples. Each lamp has a bi-volt reactor and individual drive system with on/off switch. On the lower part is an area of 250 mm height to store the samples. The right side has a $300 \times 150$ mm perforated grid and the left side has two mini-fans, "coolers" which when turned on, allow the circulation of air by the systmem, enabling its cooling. A thermostat monitors the internal temperature and controls the automatic activation of the mini-fans. The front part has a door for moving the samples, the thermostat and the buttons to switch the lamps on or off.

The dishes containing the fungi remained for times of 5, 10, 15, 20, 25 and 30 minutes. Upon removal from the chamber, the dishes were kept for 24 hours in B.O.D. (Biochemical Oxygen Demand) at $25.5°C \pm 0.5°C$ and a 12 hours photoperiod. After this time, the dishes were observed under a Leica DM 500 microscope, with a magnification of 400 times, to count the 100 conidia, germinated and not germinated, establishing a ratio.

In the second experiment, 15 capsules of the formulations were separated and placed in open Petri dishes, the control treatments remained with the dishes fully covered with aluminum foil.

Samples were under the same amount of radiation described previously for the 4 lamps switched on. The exposure to radiation and samples extraction occurred every 15 minutes over a period of 3:30 hours.

Twelve capsules, 4 per dish, were distributed in Petri dishes (9.0 cm in diameter) containing 20 mL of PDA and incubated in a BOD at 25.5°C ± 0.5°C and a 12 hours photoperiod and the growth of the colonies from each capsule was observed over 7 days. After this period, a percentage was calculated, evaluating the number of colonies formed, considering, that each capsule would originate one colony.

The third experiment was performed according to the methodology described in the previous section. However, the samples were removed at pre-determined periods of 2, 4, 6, 8, 10, 12, 24, 36 and 48 hours of exposure, and then analyzed for colony formation.

### 2.3.2. UV Radiation Chamber (Microbiology Laboratory/UNIVAP)

The samples were placed in a radiation chamber Model Qsun XE3HC, whose emission spectrum concentrated in the UV-B region (306 nm). In this experiment, an irradiance of 4.977 mW·m$^{-2}$ was used and the energy inside the chamber was 17.92 kJ·m$^{-2}$·h$^{-1}$. The irradiance was measured with the use of a spectroradiometer (Ocean Optics model USB2000 + Rad) connected to a portable computer. The front part has a door for moving the samples, the thermostat and the buttons for switching the lamps on or off.

The samples were taken every 15 minutes over a period of 3:30 hours. The inoculation of the fungus, incubation of the dishes, the visual analysis of the fungal growth and the percentage calculation were performed as previously described. The temperature during this period was 26°C ± 0.5°C controlled by a thermostat on the equipment. These tests were prepared with 3 repetitions.

## 2.4. Test of Pathogenicity and Virulence of Conidia Formulated and Not Formulated Applied in Dry and Wet Forms in Caterpillars of *D. saccharalis*

The caterpillars of *D. saccharalis*, rearing under an artificial diet, were provided by the Laboratory of Entomology, Bonfim Mill, Raizen Group, located in Guariba city, São Paulo state, Brazil. The pathogenicity was investigated through contact tests where caterpillars, 3$^{rd}$ to 4$^{th}$ instar (1.5 cm long), were placed in containers with 0.05 g of dry encapsulated conidia, wet encapsulated conidia (immediately after preparation) and not encapsulated conidia of fungi *B. bassiana* and *M. anisopliae*. The containers with the insects were kept in a room with a temperature of 25°C ± 1°C and 70% RH and a photoperiod of 12 hours [23]. Each treatment was prepared with 5 repetitions and 30 insects in total. The evaluations to verify insect mortality were performed daily until the 15$^{th}$ day, and the dead insects were placed in moist chambers to confirm pathogen mortality. The culms of sugar cane were changed when necessary.

## 2.5. Statistical Analysis

The SISVAR program (Version 5.3) was used for statistical analysis. The trials conducted were completely randomized. Data was subjected to variance analysis, and for significance, the Tukey test was performed at 5% for comparison of the averages.

## 3. Results and Discussion

## 3.1. Viability Test for Formulated and Non-Formulated Conidia Subjected to UV Radiation

### 3.1.1. UV Radiation Chamber (Laboratory of Natural Products-UFSCar)

For the test where only pure conidia were evaluated, the effect of the radiation was verified in the first 5 minutes of contact with the fungus (**Table 1**). With the 4 lamps switched on, for the fungus *B. bassiana*, in 5 minutes of exposure to radiation, germination reached 52% compared to 94% in the control, at 10 minutes this index fell to 11% and at 15 minutes it fell to 1.0% (**Table 1**). For the fungus *M. anisopliae* the fall was from 96% to 64% in the first 5 minutes and at 10 minutes the rate of germination fell to 0% (**Table 1**).

When evaluated with two lamps switched on there was a tolerance to the radiation for a little longer. However, with 20 minutes of exposure, the fungus *B. bassiana* presented 7.0% and *M. anisopliae* 2.0% germination (**Table 2**).

*Experiment* 2

The effect of the radiation on the conidia in the sodium alginate capsules, evaluated every 15 minutes over a period of 3:30 hours, was not harmful to the fungus *B. bassiana* (**Table 3**). The fungal colonies grew with no morphological change.

**Table 1.** Average germination percentage of the conidia after exposure to ultravioleta radiation with an irradiance of 6153.3 mW·m$^{-2}$ or of 22.15 kJ·m$^{-2}$·h$^{-1}$.

| Treatments | Beauveria bassiana[1] | Metarhizium anisopliae |
|---|---|---|
| Control (without radiation) | | |
| 5 minutes | 94.0 ± 1.15 a | 96.0 ± 0.88 a |
| 10 minutes | 93.0 ± 0.57 a | 93.0 ± 1.15 a |
| 15 minutes | 93.0 ± 0.33 a | 97.0 ± 0.88 a |
| 20 minutes | 95.0 ± 1.73 a | 93.0 ± 1.52 a |
| 25 minutes | 96.0 ± 0.57 a | 95.0 ± 0.57 a |
| 30 minutes | 96.0 ± 1.20 a | 96.0 ± 1.15 a |
| Pure conidia | | |
| 5 minutes | 52.0 ± 6.48 b | 64.0 ± 4.63 b |
| 10 minutes | 11.0 ± 2.18 c | 0.0 ± 0.00 c |
| 15 minutes | 1.0 ± 0.88 c | 1.0 ± 0.88 c |
| 20 minutes | 0.0 ± 0.00 c | 0.0 ± 0.00 c |
| 25 minutes | 0.0 ± 0.00 c | 0.0 ± 0.00 c |
| 30 minutes | 0.0 ± 0.00 c | 0.0 ± 0.00 c |
| Test F | 467.418* | 951.525* |

[1]Averages followed by the same letter in the column do not differ amongst themselves by the Tukey test at 5% probability. *Significant at 5% probability for Test F.

**Table 2.** Average germination percentage of the conidia after exposure to ultraviolet radiation with an irradiance of 5653 mW·m$^{-2}$ or 20.35 kJ·m$^{-2}$·h$^{-1}$.

| Treatments | Beauveria bassiana[1] | Metarhizium anisopliae |
|---|---|---|
| Control (without radiation) | | |
| 5 minutes | 97.0 ± 0.57 a | 96.0 ± 0.57 a |
| 10 minutes | 93.0 ± 1.20 a | 93.0 ± 1.20 a |
| 15 minutes | 97.0 ± 0.88 a | 97.0 ± 0.88 a |
| 20 minutes | 97.0 ± 1.20 a | 97.0 ± 1.20 a |
| 25 minutes | 96.0 ± 0.67 a | 96.0 ± 0.66 a |
| 30 minutes | 94.0 ± 0.88 a | 94.0 ± 0.88 a |
| Pure conidia | | |
| 5 minutes | 65.0 ± 7.57 b | 97.0 ± 0.88 a |
| 10 minutes | 57.0 ± 0.00 b | 61.0 ± 1.73 b |
| 15 minutes | 27.0 ± 1.15 c | 14.0 ± 2.64 c |
| 20 minutes | 7.0 ± 1.45 d | 2.0 ± 1.15 d |
| 25 minutes | 0.0 ± 0.00 d | 1.5 ± 0,88 d |
| 30 minutes | 0.0 ± 0.00 d | 0.0 ± 0.00 d |
| Test F | 309.22* | 381.01* |

[1]Averages followed by the same letter in the column do not differ amongst themselves by the Tukey test at 5% probability. *Significant at 5% probability for Test F.

**Table 3.** Percentage of colonies formed from the IBCB 66 isolate of *Beauveria bassiana* formulated exposed to ultraviolet radiation, with an irradiance of 6153.3 mW·m$^{-2}$ or of 22.15 kJ·m$^{-2}$·h$^{-1}$, at different times (T = 26°C ± 0.5°C).

| Exposure times | Formulated | |
|:---:|:---:|:---:|
| | Control (without radiation)[1] | Radiation Exposure |
| 0 | 100 ± 0.00a | 100 ± 0.00 a |
| 15 minutes | 100 ± 0.00 a | 100 ± 0.00 a |
| 30 minutes | 100 ± 0.00 a | 100 ± 0.00 a |
| 45 minutes | 100 ± 0.00a | 100 ± 0.00 a |
| 1:00 hour | 100 ± 0.00 a | 100 ± 0.00 a |
| 1:15 hours | 100 ± 0.00 a | 100 ± 0.00 a |
| 1:30 hours | 100 ± 0.00 a | 100 ± 0.00 a |
| 1:45 hours | 100 ± 0.00 a | 100 ± 0.00 a |
| 2:00 hours | 100 ± 0.00 a | 100 ± 0.00 a |
| 2:15 hours | 100 ± 0.00 a | 100 ± 0.00 a |
| 2:30 hours | 100 ± 0.00 a | 100 ± 0.00 a |
| 2:45 hours | 100 ± 0.00 a | 100 ± 0.00 a |
| 3:00 hours | 100 ± 0.00a | 100 ± 0.00 a |
| 3:15 hours | 100 ± 0.00 a | 100 ± 0.00 a |
| 3:30 hours | 100 ± 0.00 a | 100 ± 0.00 a |
| Test F | 1.0E = 0.009 n.s | |

[1]Averages followed by the same letter in the column do not differ amongst themselves by the Tukey test at 5% probability. n.s: non-significant at 5% probability for Test F.

*Experiment* 3

This test aimed to expose the sodium alginate capsules to UV radiation for the maximum amount of time. The results of the effect of this exposure at the different times at which the capsules were exposed to the radiation are described in **Table 4**.

It can be verified that exposure to radiation at these times did not affect the germination capacity of the conidia, because each irradiated capsule maintained the ability to form a colony.

### 3.1.2. UV Radiation Chamber (Laboratory of Microbiology-UNIVAP)

This test, conducted at UNIVAP aimed to verify the effect of other radiation intensities on the encapsulated formulations of the fungi *B. bassiana* and *M. anisopliae*. For the fungus *B. bassiana*, it was observed that growth occurred normally and 100% of the capsules placed in BDA gave rise to fungal colonies (**Table 5**) with no apparent morphological changes.

For filamentous fungi, most studies present reduction in germination and/or growth when these have been exposed to UV radiation, which is more significant and evident when the exposure time was increased [24] [25]. This same trend was observed in this study for unformulated conidia.

[26] evaluated sodium alginate formulations containing the fungi *B. bassiana* and *M. anisopliae* regarding exposure to simulated sunlight for up to 48 hours with radiation equivalent to 1.3 kJ·m$^2$ and they verified that the percentage of sporulated particles in the alginate formulation of both fungi was not affected. For the pure fungus, the sporulation decreased linearly with the time of exposure. For *M. anisopliae*, close to 100% of alginate particles could still sporulate after 48 hours of constant treatment with sunlight, which was not observed for pure conidia. For *B. bassiana*, after 48 hours of exposure, it was observed that 80% of the alginate particles containing the fungus could still germinate.

[24] evaluated the effect of UV radiation on fungi strains from *B. bassiana*, *M. anisopliae*, *M. flavoviride* and *Paecilomyces fumosoroseus*. The dried fungal inoculum were exposed to UV irradiation for 1, 2, 4 and 8 hours,

**Table 4.** Percentage of colonies formed from the IBCB 66 isolate of *Beauveria bassiana* exposed to ultraviolet (UV) radiation, with an irradiance of 6153.3 mW·m$^{-2}$ or of 22.15 kJ·m$^{-2}$·h$^{-1}$, between 0 to 48 hours (T = 26˚C ± 0.5˚C)—UFSCar chamber.

| Exposure Times (h) | Formulated | |
| --- | --- | --- |
| | Control (without radiation)[1] | Radiation Exposure |
| 0 | 100 ± 0.00a | 100 ± 0.00a |
| 2 | 100 ± 0.00 a | 100 ± 0.00 a |
| 4 | 100 ± 0.00 a | 100 ± 0.00 a |
| 6 | 100 ± 0.00 a | 100 ± 0.00 a |
| 8 | 100 ± 0.00 a | 100 ± 0.00 a |
| 10 | 100 ± 0.00 a | 100 ± 0.00 a |
| 12 | 100 ± 0.00 a | 100 ± 0.00 a |
| 24 | 100 ± 0.00 a | 100 ± 0.00 a |
| 36 | 100 ± 0.00 a | 100 ± 0.00 a |
| 48 | 100 ± 0.00 a | 100 ± 0.00 a |
| Test F | 1.0E = 0.009 n.s | |

[1]Averages followed by the same letter in the column do not differ amongst themselves by the Tukey test at 5% probability. n.s: non-significant at 5% probability for Test F.

**Table 5.** Percentage of colonies formed from the IBCB 66 isolate of *Beauveria bassiana* exposed to ultraviolet radiation, with an irradiance of 4.977 mW·m$^{-2}$ and energy of 17.92 kJ·m$^{-2}$·h$^{-1}$, at different time (T = 26˚C ± 0.5˚C)—UNIVAP chamber.

| Exposure times | Formulated | |
| --- | --- | --- |
| | Control (without radiation)[1] | Radiation Exposure |
| 0 | 100 ± 0.00 a | 100 ± 0.00 a |
| 15 minutes | 100 ± 0.00 a | 100 ± 0.00 a |
| 30 minutes | 100 ± 0.00 a | 100 ± 0.00 a |
| 45 minutes | 100 ± 0.00 a | 100 ± 0.00 a |
| 1:00 hour | 100 ± 0.00 a | 100 ± 0.00 a |
| 1:15 hours | 100 ± 0.00 a | 100 ± 0.00 a |
| 1:30 hours | 100 ± 0.00 a | 100 ± 0.00 a |
| 1:45 hours | 100 ± 0.00 a | 100 ± 0.00 a |
| 2:00 hours | 100 ± 0.00 a | 100 ± 0.00 a |
| 2:15 hours | 100 ± 0.00 a | 100 ± 0.00 a |
| 2:30 hours | 100 ± 0.00 a | 100 ± 0.00 a |
| 2:45 hours | 100 ± 0.00 a | 100 ± 0.00 a |
| 3:00 hours | 100 ± 0.00 a | 100 ± 0.00 a |
| 3:15 hours | 100 ± 0.00 a | 100 ± 0.00 a |
| 3:30 hours | 100 ± 0.00 a | 100 ± 0.00 a |
| Test F | 1.0E = 0.009 n.s | |

[1]Averages followed by the same letter in the column do not differ amongst themselves by the Tukey test at 5% probability. n.s: non-significant at 5% probability for Test F.

corresponding to irradiances of 1.08, 2.16, 4.32 and 8.46 kJ·m$^2$, respectively. The authors verified that 92% of the strains of *M. flavoviride*, 61% of *B. bassiana*, 26% of *M. anisopliae* and only 3.0% of *P. fumosoroseus* showed more than 50% survival after 1 hour of irradiation. In addition, all of the strains of *M. flavoviride*, 54% of the strains of *M. anisopliae*, 42% of *B. bassiana* and only 21% of *P. fumosoroseus* showed more than 0.1% survival after 4 hours of irradiation. These results show that with the exposure time of 1 hour, the fungus *B. bassiana* was more resistant compared to the fungus *M. anisopliae*. But with the exposure time of 4 hours, *M. anisopliae*, was more tolerant. In this study, both fungi when in non-formulated conidia had their germination impaired in relation to the increase in radiation. In the case of the encapsulated fungus, the radiation had no effect at the different evaluation times, tolerating this exposure for a period of 48 hours without compromising the growth of the fungus.

[25] studied the effect of radiation on the fungus *Clonostachys rosea*, and found that conidia germination was inversely proportional to irradiance. The highest germination rates were found in lower doses of radiation and *vice versa*. Sensitivity to variations in UV radiation between different strains of *C. rosea* were observed. After 2 hours exposure (irradiation of 4.2 kJ·m$^{-2}$), two strains germinated in approximately 40%, and five presented viability below 10%. The LQC 62 was the most tolerant strain, with germination of more than 60%, significantly greater than the other strains. The use of the formulation in this case was of extreme necessity for protection against radiation, since the actual outcomes were more efficient than the results found by other authors.

The damaging effects of sunlight imply the short persistence of microbial control agents in the field, after application. As a result, farmers need to consume more material and work more hours, with successive reapplications and an increase in final production cost. This is one of the challenges that must be overcome for the increase in the use of bio-pesticides as well as greater acceptance by the farmer. With this in mind, large companies have been investing in the development of formulations and incorporation of various UV radiation protectors to produce bio-insecticide [27]-[29]. The results in this study showed that in addition to assisting in the photochemical stability of conidia, alginate capsules are a low-cost product.

[27] found that a rapid inactivation of conidia of *B. bassiana* occurred after exposure to UV light or natural sunlight. However, the inactivation rate was reduced by the addition of protective substances. Regardless, various substances have proven to be ineffective, with regard to their photo-protective properties when tested in studies with baculovirus, such as, ascorbic acid, zinc oxide, folic acid and molasses [30]-[32]. Among the substances evaluated by the authors, eggwhite and milk powder were most effective in reducing the conidia inactivation rate. The probable reason for this is that its protein content absorbs UV-B radiation [33] and acts as a protective barrier on the surface of the conidia. In this study, the product that acted as protection was the sodium alginate polymer.

[34] studied the influence of radiation on encapsulated *B. bassiana* formulations prepared with lignin polymer by the Spray-drying technique. The authors subjected the formulations to exposure times of 4, 8, 5, 12, 17, 20, 24, 36 and 48 hours, and found that the polymer protected the conidia, as the formulations produced a conidia mortality rate ten times lower. Although they are different polymers, it appears that in both studies, there was fungi protection.

This way, the studies that have been conducted with the addition of protective substances or even developing more advanced formulations, such as those using polymer in their composition, with the function of protection, are justified. In this study, it was observed that the presence of the sodium alginate protection in the capsule had a decisive action in the maintenance of conidia germination. In the final formulation, a capsule, has various conidia aggregated and it is believed that the innermost conidia of the capsule are better protected, justifying the stability gain to the increased persistence to radiation. Despite the UV radiation being non-ionizing, which acts on the surface and is not capable of penetrating the capsule, it is understood that the objective of the capsule was reached, because by protecting the inside of this capsule from the deleterious effect of the solar radiation in the field, there are chances for the viable conidia inside to be sources of inoculum, initiating the epizootics causing the mortality of pests.

## 3.2. Pathogenicity Test and Virulence of Formulated and Non-Formulated Conidia Applied in Dry and Wet Form in *Diatraea saccharalis* Caterpillars

The non-formulated fungus *B. bassiana* was more aggressive than the formulated fungus, because on the 7$^{th}$ day the mortality rate of the caterpillars had already reached 100% (**Table 6**). In the treatments with the formulations,

the mortality rates at the same time were 40.2% and 29.6%, respectively, for the dry and wet formulations. This was already expected; because the non-formulated conidia have easier germination readily available compared to the formulated that have to beat the polymer barrier. On the 15th day of evaluation, the mortality rates reached 79.6% and 66.8%, in the treatments with the dry and wet capsules, respectively, with no statistical difference in mortality rates neither in the treatment with pure conidia and dry formulation (**Table 6**).

The same result was observed for the fungus *M. anisopliae*, where the effects of the formulation were more enhanced, with respect to the release kinetics of the conidia (**Table 7**).

For this fungus, the mortality rates in the formulations did not exceed 17% and this may imply a barrier exerted by the polymer. This fact can be explained by the fungal activity, which, in the case of formulations, can take longer because the germ tube may break through the capsule barrier, thus having a slower action.

[26] evaluated formulations containing dry mycelium of the fungi *B. bassiana* and *M. anisopliae* in alginate matrices, corn starch and corn oil. The authors verified that the fungus *B. bassiana* was faster compared to *M. anisopliae*, corroborating the data observed in this study. However, formulations and dry mycelia caused similar mortality rates as pests, where the formulation was not a barrier to the fungal activity.

The slowest mortality rate can be explained by the reduction in stability that is related to the encapsulated material load used. The technique of using sodium alginate is based on the formulation of a gel from the reaction that occurs between certain metal cations such as calcium ($Ca^{2+}$), and an aqueous solution of sodium alginate [35]. The gel formed is biochemically inert and has a porosity that is dependent on the type of alginate and metal ion used. [36] observed that these sodium alginate gels generally present a high porosity, resulting in elevated levels of diffusion of the active ingredient it contains. This result could be due to the high concentration of conidia used or even the fragility of the structure of the capsules caused by the high fungal load to which the material was subjected. Being a polymer derived from alginic acid, it has the ability form gel, retaining a large quantity of water inside, without dissolution. The authors also found that when the capacity of the encapsulation of the material was extrapolated, it resulted in a reduction of the stability of the pellets and in lower swelling rate, which could explain the rapid release of the fungal conidia. In this study, there seems to have been an inverse effect, with the germination of the conidia being slower.

**Table 6.** Average mortality rates confirmed in *Diatraea saccharalis* caterpillars after application of encapsulated formulation of the IBCB 66 isolate of *Beauveria bassiana* to 7 and 15 days of evaluation (T = 25.5˚C ± 0.5˚C).

| Treatments[1] | Mortality confirmed by day 7 (%)[2] | Mortality confirmed by day 15 (%) |
|---|---|---|
| Absolute control | 0.0 ± 0.00 c | 0.0 ± 0.00 c |
| Non-formulated conidia | 100 ± 0.00 a | 100 ± 0.00 a |
| BVR dry capsule | 40.2 ± 12.2 b | 79.6 ± 5.21 ab |
| BVR wet capsule | 29.6 ± 8.59 bc | 66.8 ± 9.75 b |
| Test F | 31.56[*] | 60.48[*] |

[1]BVR (*Beauveria bassiana*); [2]Averages followed by the same letter in the column do not differ amongst themselves by the Tukey test at 5% probability. [*]Significant at 5% probability for Test F.

**Table 7.** Average mortality rates confirmed in *Diatraea saccharalis* caterpillars after application of encapsulated formulations of the IBCB 425 isolate of *Metarhizium anisopliae* to 7 and 15 days of evaluation (T = 25.5˚C ± 0.5˚C).

| Treatments[1] | Mortality confirmed by day 7 (%)[2] | Mortality confirmed by day 15 (%) |
|---|---|---|
| Absolute control | 0.0 ± 0.00 b | 0.0 ± 0.00 c |
| Non-formulated conidia | 86.8 ± 6.62 a | 100 ± 0.00 a |
| MET dry capsule | 3.4 ± 3.40 b | 10.2 ± 4.16 bc |
| MET wet capsule | 0.0 ± 0.00 b | 17.0 ± 3.40 b |
| Test F | 95.56[*] | 183.04[*] |

[1]MET (*Metarhizium anisopliae*); [2]Averages followed by the same letter in the column do not differ amongst themselves by the Tukey test at 5% probability. [*]Significant at 5% probability for Test F.

Thus, different fungal concentrations should be evaluated to achieve a balance between the stability of the capsules and the release of the fungus. Another important factor that could be considered is how the fungal propagules in the formulation will interact on the surface of the insect. These specific interactions are still little known and may be influenced according to the type of formulation [37].

## 4. Conclusions

The UV-B radiation was deleterious to the pure conidia of the fungi *M. anisopliae* and *B. bassiana* in the first 5 minutes of exposure.

The formulated *B. bassiana* and *M. anisopliae* fungi in sodium alginate capsules were tolerant to photochemical degradation even after exposure to 48 hours of UV radiation, where the conidia remained viable.

*B. bassiana* and *M. anisopliae* fungi, non-formulated and formulated in sodium alginate capsules, were pathogenic to the studied insect *D. saccharalis.*

The process developed and applied for the preparation of the formulation was efficient for covering the conidia of fungi, attributing characteristics such as resistance to UV radiation.

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## References

[1] Marques, E.J., Alves, S.B. and Marques, I.M.R. (2000) Virulence of *Beauveria bassiana* (Bals.) Vuill. to *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae) after Conidia Storage in Low Temperature. *Anais da Sociedade Entomológica do Brasil*, **29**, 303-307. http://dx.doi.org/10.1590/S0301-80592000000200012

[2] Wenzel, I.M., Giometti, F.H.C. and Almeida, J.E.M. (2006) Pathogenicity of IBCB 66 Isolate of *Beauveria bassiana* in Sugarcane Borer *D. saccharalis* in Laboratory Conditions. *Arquivos do Instituto Biológico*, **73**, 259-261.

[3] Almeida, J.E.M. and Alves, S.B. (2010) Recommendations for Use of Micoinsecticides Designed to Leafhopper Control Sugarcane Spitlebug. In: Alves, L.F.A., Neves, P.M.J.O. and Farias, M.R. (Orgs.), *Recommendations for Use of Entomopathogenic Fungi to Pests Control*, CP2, Piracicaba, 33-36.

[4] Ahmed, S., Ashraf, M.R., Hussain, A. and Riaz, M.A. (2009) Pathogenicity of Isolates of *Metarhizium anisopliae* from Gujranwala (Pakistan) against *Coptotermes heimi* (wasmann) (Isoptera: Rhinotermitidae). *International Journal of Agriculture and Biology*, **11**, 707-711.

[5] Batista Filho, A., Prando, H., Leite, L.G. and Neves., P.M.J.O. (2010) Recommendations for Use of Micoinsecticides Designed to Plantain Borer. In: Alves, L.F.A., Neves, P.M.J.O. and Faria, M.R. (Orgs.), *Recommendations for Use of Entomopathogenic Fungi to Pests Control*, CP2, Piracicaba, 21-24.

[6] Giometti, F.H.C., Wenzel, I.M., Almeida, J.E.M., Leite, L.G. and Zappelini, L.O. (2010) Selection of Isolates of *Beauveria bassiana* for Control of Adult Cotton Boll Weevil *Anthonomus grandis* (Coleoptera: Curculionidae). *Arquivos do Instituto Biológico*, **77**, 167-169.

[7] Faria, M.R., Moino Jr., A., Sosa-Gomez, D.R. and Neves, P.M.J.O. (2010) Recommendations for Use of Micoinsecticides Designed to Whitefly Control in Greenhouse. In: Alves, L.F.A., Neves, P.M.J.O. and Faria, M.R. (Orgs.), *Recommendations for Use of Entomopathogenic Fungi to Pests Control*, CP2, Piracicaba, 49-52.

[8] Neves, P.M.J.O., Moino Jr., A. and Alves, S.B. (2010) Recommendations for Use of Micoinsecticides Designed to Coffee Borer Control. In: Alves, L.F.A., Neves, P.M.J.O. and Faria, M.R. (Orgs.), *Recommendations for Use of Entomopathogenic Fungi to Pests Control*, CP2, Piracicaba, 25-28.

[9] Zappelini, L.O., Almeida, J.E.M., Batista Filho, A. and Giometti, F.H.C. (2010) Isolates Selection of Entomopathogenic Fungus *Metarhizium anisopliae* (Metsch.) Sorok. Aiming Control of Sugarcane Borer *Diatraea saccharalis* (Fabr., 1794). *Arquivos do Instituto Biológico*, **77**, 75-82.

[10] Alves, S.B. (1998) Pathology and Microbial Control: Advantages and Disadvantages. In: Alves, S.B., Ed., *Controle Microbiano de Insetos*, 2nd Edition, Fealq, Piracicaba, 21-38.

[11] Rangel, D.E.N., Anderson, A.J. and Roberts, D.W. (2008) Evaluating Physical and Nutritional Stress during Mycelial Growth as Inducers of Tolerance to Heat and UV-B Radiation in *Metarhizium anisopliae* Conidia. *Mycological Research*, **112**, 1362-1372. http://dx.doi.org/10.1016/j.mycres.2008.04.013

[12] Alves, S.B. and Lecuona, R.E. (1998) Epizootiology Applied to the Microbial Control of Insects. In: Alves, S.B., Ed.,

*Controle Microbiano de Insetos*, 2nd Edition, Fealq, Piracicaba, 97-170.

[13]  Bell, J.V. (1974) Mycoses. In: Cantwell, G.E., Ed., *Insect Diseases*, Marcel Dekker Inc., New York, 185-236.

[14]  Jones, K.A. and Burges, H.D. (1998) Technology of Formulation and Application. In: Burges, H.D., Ed., *Formulation of Microbial Pesticides—Beneficial Microorganisms*, *Nematodes and Seed Treatments*, Kluwer Academic, Dordrecht, 7-30. http://dx.doi.org/10.1007/978-94-011-4926-6_2

[15]  Batista Filho, A., Alves, S.B., Alves, L.F.A., Pereira, R.M. and Augusto, N.T. (1998) Formulation of Entomopathogens. In: Alves, S.B., Ed., *Controle Microbiano de Insetos*, 2nd Edition, Fealq, Piracicaba, 917-965.

[16]  Faria, M.R. and Wraight, S.P. (2007) Mycoinsecticides and Mycoacaricides: A Comprehensive List with Worldwide Coverage and International Classification of Formulation Types. *Biological Control*, **43**, 237-256. http://dx.doi.org/10.1016/j.biocontrol.2007.08.001

[17]  Michereff Filho, M., Faria, M., Wraight, S.P. and Silva, K.F.A.S. (2009) Micoinsecticides and Micoacaricides in Brasil: As We Are after Four Decades? *Arquivos do Instituto Biológico*, **76**, 769-779.

[18]  Burges, H.D. (1998) Formulation of Microbial Pesticides. Springer, Dordrecht. http://dx.doi.org/10.1007/978-94-011-4926-6

[19]  Campos, M.B.S. and Macedo, N. (2004) Sugarcane Expanding the Field of Attack. *Cultivar Grandes Culturas*, **6**, 23-26.

[20]  Gallo, D., Nakano, O., Neto, S.S., Carvalho, R.P.L., Batista, G.C., Filho, E.B., Parra, J.R.P., Zucchi, R.A., Alves, S.B., Vendramim, J.D., Marchini, L.C., Lopes, J.R.S. and Omoto, C. (2002) Entomologia agrícola. Fealq, Piracicaba.

[21]  Demetrio, P.A., Zonetti, P.P. and Munhoz, R.E.F. (2008) Clones Evaluation of Sugarcane Promising RBs for Resistance to Sugarcane Borer (*D. saccharalis*) in the Northwest of Paraná. *Iniciação Científica Cesumar*, **10**, 13-16.

[22]  Leite, L.G., Batista Filho, A., Almeida, J.E.M. and Alves, S.B. (2003) Produção de fungos entomopatogênicos. AS, Ribeirão Preto.

[23]  Simi, L.D. (2010) Susceptibility of *D. saccharalis* Lifecycle (Lepidoptera: Crambidae) to the Action of Entomopathogenic Fungi. MSc. Dissertation, Faculdade de Ciências Agrárias e Veterinárias, UNESP, Jaboticabal.

[24]  Fargues, J., Goettel, M.S., Smits, N., Ouedraogo, A., Vidal, C., Lacey, L.A., Lomer, C.J. and Rougier, M. (1996) Variability in Susceptibility to Simulated Sunlight of Conidia among Isolates of Entomopathogenic Hyphomycetes. *Mycopathologia*, **135**, 171-181. http://dx.doi.org/10.1007/BF00632339

[25]  Costa, L.B., Rangel, D.E.N., Morandi, M.A.B. and Bettiol, W. (2012) Impact of UV-B Radiation on *Clonostachys rosea* Germination and Growth. *World Journal of Microbiology and Biotechnology*, **28**, 2497-2504. http://dx.doi.org/10.1007/s11274-012-1057-7

[26]  Pereira, R. and Roberts, D.W. (1991) Alginate and Cornstarch Mycelial Formulations of Entomopathogenic Fungi *Beauveria bassiana* and *Metarhizium anisopliae*. *Journal of Economic Entomology*, **84**, 1655-1661. http://dx.doi.org/10.1093/jee/84.6.1657

[27]  Edgington, S., Segura, H., De La Rosa, W. and Williams, T. (2000) Photoprotection of *Beauveria bassiana*: Testing Simple Formulations for Control of the Coffee Berry Borer. *Integrated Journal of Pest Management*, **46**, 169-176. http://dx.doi.org/10.1080/096708700415490

[28]  Cohen, E., Joseph, T. and Wassermann-Golan, M. (2001) Photostabilization of Biocontrol Agents by Berberine. *Integrated Journal of Pest Management*, **47**, 63-67. http://dx.doi.org/10.1080/09670870150215612

[29]  Leland, J.E. and Behle, R.W. (2005) Coating *Beauveria bassiana* with Lignin for Protection from Solar Radiation and Effects on Pathogenicity to *Lygus lineolaris* (Heteroptera: Miridae). *Biocontrol Science and Technology*, **15**, 309-320. http://dx.doi.org/10.1080/09583150400016936

[30]  Shapiro, M., Poh Agin, P. and Bell, R. (1983) Ultraviolet Protectants of the Gypsy Moth (Lepidoptera: Lymantriidae) Nuclearpolyhedrosis Virus. *Environmental Entomology*, **12**, 982-985. http://dx.doi.org/10.1093/ee/12.3.982

[31]  Shapiro, M. (1985) Effectiveness of B Vitamins as UV Screens for the Gypsy Moth (Lepidoptera: Lymantriidae) Nucleopolyhedrosis Virus. *Environmental Entomology*, **14**, 705-708. http://dx.doi.org/10.1093/ee/14.6.705

[32]  Ignoffo, C.M. and Garcia, C. (1994) Antioxidant and Oxidative Enzyme Effects on the Inactivation of Inclusion Bodies of the *Heliothis* Baculovirus by Simulated Sunlight-UV. *Environmental Entomology*, **23**, 1025-1029. http://dx.doi.org/10.1093/ee/23.4.1025

[33]  Jaques, R.P. (1970) Tests on Protectants for Foliar Deposits of a Polyhedrosis Virus. *Journal of Invertebrate Pathology*, **17**, 9-16. http://dx.doi.org/10.1016/0022-2011(71)90119-4

[34]  Leland, J.E., Stoneville, M.S., Behle, R.W. and Peoria, I.L. (2004) Formulation of Entomopathogenic Fungus, *Beauveria bassiana*, with Resistance to UV Degradation for Control of Tarnished Plant Bug, *Lygus lineolaris*. *Beltwilde Cotton Conference*, San Antonio, 5-9 January 2004, 1800-1808.

[35]  Moretini, A. and Melo, I.S. (2007) Formulation of Fungus *Coniothyrium minitans* for White Mold Control Caused by

*Sclerotinia sclerotiorum. Pesquisa Agropecuária Brasileira*, **42**, 155-161.
http://dx.doi.org/10.1590/S0100-204X2007000200003

[36] Batista, D.P.C., Souza, R.S., Santos-Magalhães, N.S., Sena-Filho, J.G., Teodoro, A.V., Grilo, L.A.M. and Dornelas, C.B. (2013) Formulações bioinseticidas de *Beauveria bassiana* a base de nanocompósito polimérico visando o controle de praga do coqueiro. 12° Congresso Brasileiro de Polímeros, 12, Florianópolis 2013.

[37] Jackson, M.A., Dunlap, C.A. and Jaronski, S.T. (2010) Ecological Considerations in Producing and Formulating Fungal Entomopathogens for Use in Insect Biocontrol. *BioControl*, **55**, 129-145.
http://dx.doi.org/10.1007/s10526-009-9240-y