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Assessment of *Beauveria bassiana* Nov. EO-1 Strain, Which Produces a Red Pigment for Microbial Control

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ABSTRACT

A new strain of the fungus *Beauveria bassiana* Nov. EO-1 (ATCC 74037), which produces a red pigment in solid and liquid culture, has been isolated from an infected whitefly. The red pigment was extracted and has been identified by mass spectrometry as oosporein, a potent dibenzoquinone mycotoxin.

In order to assess the potential of this entomogenous fungi for microbial control purposes, a mycelium bead formulation was developed as a source for pathogenic conidial spores and oosporein production. The mycelium bead preparation was found to be a stable fungal carrier. Conidiation and germination studies have revealed the mycelium bead viability is 100% over a 1-yr period when stored at 4°C. Conidial spore production from the mycelium beads has been falling substantially per time from an initial value of 1.5×10^8 spores per bead to 3×10^5 spores per bead after a year storage at 4°C. However, the mycelium bead formulation continues to produce oosporein on agar media, at the same intensity throughout the 1 yr period.

In in vitro and in vivo small scale greenhouse experiments *Beauveria bassiana* Nov. EO-1 were compared with known entomogenous fungi, *Beauveria sp.* and *Paceilomyces sp. Beauveria bassiana* Nov. EO-1 was

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found to have a high pathogenicity against foliage insect pests (e.g., whiteflies and mealy bugs), and against soil insects (e.g., citrus root weevils). The utilization of a mycelium bead based on this strain, *Beauveria bassiana* Nov. EO-1, as a source of conidial spores and oosporein may have broad applications for the control of various insect pests.

Index Entries: Red pigment; oosporein; entomogenous fungi; microbial control; *Beauveria bassiana;* mass spectrometry.

INTRODUCTION

In the course of entomopathogenic fungi biocontrol screening, it was observed that one fungal culture, isolated from a dead whitefly, produced a strong red pigment on agar plates. The same red pigment was observed when the fungi was grown in liquid media. The red pigment was identified as oosporein (2,2′,5,5′-tetrahydroxy-4,4′-dimethyl-bi-1,4-cyclohexadiene-1-yl-3,3′, 6,6′-tetrone (Fig. 1)) and the fungus was identified as *Beauveria bassiana* Nov. EO-1 (ATCC 74037).

Beauveria bassiana is perhaps one of the most extensively studied entomopathogenic fungi with regard to controlling different insect pests (*1,2*). At present, there is a renewed interest in the use of entomopathogenic fungi as a microbial agent for insect pest control. Several companies in the United States and abroad (e.g., Phermon Corp., Ciba Geigy, Bayer AG, EcoScience, and so on) are currently exploring the use of *Beauveria bassiana* as a mycoinsecticide agent.

The genus *Beauveria* is known to produce different toxins and enzymes (*2–6*). Several researchers have examined cell-free filtrates of *Beauveria bassiana* cultures for microbial control purposes and found them toxic upon oral and topical applications (*7,8*). In contrast to those findings, other researchers have demonstrated the toxicity of culture filtrates, only when injected, no activity was found when the filtrates were applied topically (*5,9,10*). The structure of one toxin, beauvericin, has been reported (*6*). However, the complete structures, or the nature of the toxins, produced by the different *Beauveria bassiana* cultures in the above reports were not disclosed or identified.

Other reseachers have examined pigments obtained from different *Beauveria* species. Yellow *Beauveria bassiana* pigments were identified as tenellin and bassianin (*11*). A green pigmentation was reported to be a common characteristic of *Beauveria bassiana,* at the mycelium base when the strain was cultured on Sabourauds maltose agar (*12*). A red pigment was obtained from several mutant strains of *Beauveria bassiana* and *Beauveria brongniartii* (*3,12,13*). However, these specific mutant strains have lost their ability to produce the red pigment when the strains were maintained and transferred on agar media (*2,12,14*). These mutant *Beauveria* strains were not tested for microbial control purposes, as the main em-

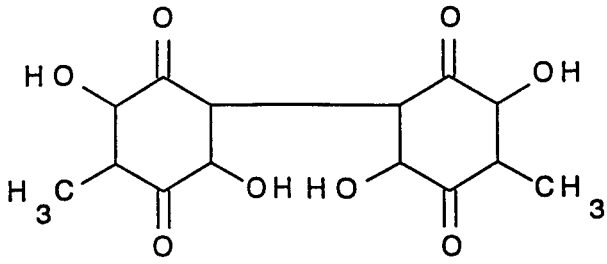

Fig. 1. Structure of oosporein.

phasis was for medical applications (*14*). The red pigment from the *Beauveria* mutant strains was identified as oosporein (*3*).

In general, it can be said that no literature information exists explaining oosporein's role or the relationship between the appearance of the red pigment oosporein in species of *Beauveria bassiana* and the virulence or pathogenicity of these *Beauveria bassiana* cultures against insects pests.

Oosporein has been primarily isolated from other different fungi such as *Chaetomium trilaterale* (*15*), *Verticillium psalliotae* (*16*), *Chaetomium aureum* (*17*), *Oospora colorans* (*18*), and *Acremonium sp* (*19*). Synthetic and natural oosporein derivatives have been studied extensively in the past, for their structure formation (*20*) and toxicological effects. These studies were a direct result of the contamination of different agricultural storage crops and poultry with oosporein producing fungi. Oosporein has been found to be toxic to day old cockerels (*21*). It has also been found to be the causal agent of avian gout (*17*). Oosporein exhibits inhibitory growth and phytotoxic effects on plants (*15,17*) and antibacterial activity (*16,22*). Toxicity studies of oosporein in mice and hamsters indicated a LD_{50} of 0.5 mg/kg body weight when injected intraperitoneally. However, oral administration of oosporein in mice, with concentrations as high as 7 mg/kg for 42 d, was found nonlethal (*16*). Oral oosporein administration in broiler chicks resulted in a LD_{50} of 5.77 mg/kg (*23*).

In order to define if *Beauveria bassiana* Nov. EO-1, can be a potential biocontrol candidate, we have incorporated *Beauveria bassiana* Nov. EO-1 mycelium, produced by submerged culture, into a formulated alginate bead. The mycelium beads were examined for viability, germination,and sporulation as well as for the bead's ability to produce oosporein in vitro. The pathogenicity and virulency of these mycelium beads were evaluated in vivo against a number of insect pests.

MATERIALS AND METHODS

Fungal Strains

Beauveria bassiana Nov. EO-1 ATCC 74037 was originally isolated in July 1989 by Lance Osborne from the University of Florida, in Apopka,

FL, from a dead sweet potato whitefly. A single spore isolate, exhibiting the highest reddish color when grown on agar media was selected for further studies. *Chaetomium trilaterale* ATCC 24912, a known oosporein producing strain in submerged culture (*15*), was chosen for the comparison and identification of oosporein from *Beauveria bassiana* Nov. EO-1.

Fungal Maintenance

For short storage periods all fungal strains were maintained on agar slants and plates composed of 0.5% peptone, 0.5% yeast extract, 0.5% malt extract (Difco Laboratories, Detroit, MI), 14% glucose,and 10% agar. After growth at 25°C for 7 d, the cultures were transferred and maintained at 4°C. For long storage periods *Beauveria bassiana* Nov. EO-1 was grown on agar plates as described above, after the plates were microscopically checked for culture characteristics and purity, the plates were harvested with sterile 0.05% Tween 80. The amount of spores per mL was determined by hemacytometer counts. The spore solution was cryoprotected by adding sterile 30% glycerol in a 1:1 ratio. The spore solution was mix thoroughly and dispensed aseptically into 2 mL cryovials. The vials were frozen and stored at −80°C.

Inoculum

Inoculum for submerged cultures was prepared by homogenizing the culture slant contents using a Tekmar tissumizer (Cincinnati, OH), and transferring the homogenate to a 1 L Erlenmeyer shake flask containing 500 mL, pH adjusted to 6.0, of the following media: YM broth (Bacto yeast extract 3 g, Bacto malt extract 3 g, Bacto peptone 5 g, Bacto dextrose 10 g, Difco Laboratories, Detroit, MI). The inoculum was shaken reciprocally at 120 rpm at 25°C. After 24 h of incubation, the inoculum was used as a seed culture for a 15 L fermentor.

Fermentation and Growth Conditions

In order to obtain a high dry cell weight of *Beauveria bassiana* Nov. EO-1 mycelium biomass, a semi-fed batch fermentation was run allowing for high sugar level maintenance. By maintaining high sugar concentrations, unstable blastospore formation was inhibited while mycelium production was favored. A 20 L fermentor (Chemap) was used with the following fermentation media: 30 g/L cotton seed flour (Pharmamedia), 17 g/L corn steep liquor, 60 g/L glucose and 100 ppm of Polyglycol P-2000 antifoam (Dow-Corning). Glucose levels were maintained at about 30 g/L for the fermentation duration. The fermentation was performed at an agitation rate of 300–400 rpm, aeration rate of 1 vvm, temperature of 27°C and pH of 6.0. Mycelium dry cell weight was determined at regular intervals by the standard method. Glucose consumption changes were monitored during the fermentation course by removing a 50 mL sample, at

various time intervals from the fermentor. The fermentation broth color change from yellow brown to deep red was observed in 24 h. After 7 d cultivation, the fermentation broth was harvested by centrifugation. The wet concentrated mycelium biomass was formulated and the supernatant was used for oosporein extraction.

Formulation

Mycelium biomass obtained as described above, was formulated by mixing with 20% vermiculite (W.R. Grace Co.-Conn.), 30% wheat bran (Canadian Harvest, Canada) and 1% aqueous sodium alginate (Sigma, St. Louis, MO). The mixture was mixed vigorously in a Waring blender for 1 min and then gently stirred until the solution was evenly dispersed. Beads were made by adding drops of the mycelium alginate mixture into 0.25*M* aqueous $CaCl_2$ stirred solution. The beads formed in the coagulation bath were removed after 20 min, rinsed with D.I. water, and then dried using a fluidized bed dryer (Niro-Aeromatic, Columbia, MD). Several batches of dried beads were prepared, each batch consisted of approx 2000 g of dried beads. Each batch was stored in covered plastic boxes at 4°C. Samples from each batch were also placed in different temperatures for shelf life and stability tests.

Mycelium Bead Viability Assays

A common viability evaluation method for spores is the Colony Forming Unit (CFU) technique. This technique measures the number of colony forming units per unit volume that form on an agar plate. However, because the bead formulation contains mostly mycelial hyphae and not spores, the CFU method can not be used to measure mycelium viability owing to a lack of sensitivity. Therefore, a different viability test was used that is based on the development of hyphae and spores on the surface of whole mycelium bead. Such a viability process was carried out by randomly selecting 36 beads. The beads were aseptically plated on a YM agar plate (15 × 100 mm square style grid Petri plate, Falcon 1012, Becton Dickinson), and incubated at 25°C for 3 d. The number of beads covered with fungal mycelium and conidial spores was recorded after 3 d and the percentage of viable beads was calculated.

Mycelium Bead Sporulation Assay

Randomly, 36 beads were rehydrated with sterile water for 30 min and placed aseptically in empty wells of 96 wells plate (Corning polystyrene #25860). The plate was incubated at 25°C for 7 d, after the first 24 h, sterile D.I. water was added to each well using an water atomizer. Then the plate was reincubated for the remaining time. At day 7 the spores were harvested from each well by adding 200 μL of sterile 0.05% Tween 80 to each well, from a test tube containing 10 mL. Then the wells contents

were mixed with a sterile inoculating needle (Fisher), and carefully pipeted back into the original test tube. The tube was vigorously mixed for 20 s and the spores from the resulting suspension were counted using a hemacytometer.

Spore Germination

Spores obtained from the mycelium beads as described above were diluted by serial dilution to obtain a 1×10^5 spores/mL suspension. A 100 μL of sterile Sabourauds dextrose broth (30 g/L Difco) was added to two columns of a 96 well polystyrene plate (Corning). 100 μl of spore suspension was added to each well for a total of 16 wells. The plate was incubated at 25°C for 24 h. Using an inverted phase microscope (Ziess 32x), the number of viable (germ tube present) and nonviable (no germ tube present) spores were counted.

BIOASSAYS

Pathogenicity of Mycelium Beads Against Soil Insects

This bioassay was based on the introduction of mycelium beads in sterile chandler soil. The beads were mixed with the soil at different rates of 5, 10, and 25 beads/10cc of soil and placed in plastic cups. Five third stage larvae of *Artipus floridanus* (citrus root weevil) were put into each soil cup (5 cups per each rate) and 350 mL of sterile distilled water was added to each cup for bead activation in the soil. As a negative control cups filled with 10cc of soil and larvae without the introduction of the mycelium beads were used.

In Vitro Pathogenicity of Spores Obtained from *Beauveria bassiana* Nov. EO-1 Beads Against Foliage Insects

A fungal spore solution of 1×10^6 spore/mL was prepared by harvesting spores from mycelium beads that were incubated in plastic wells as described in the sporulation assay section. The spores were harvested with 0.05% Tween 80 solution. Thirty-six early fourth instars of *Bemisia tabaci* (sweet potato whitefly—SPWF) were placed on a microscope slide. The slide containing the SPWF instars was dipped for 10 s in the conidia spore solution and then placed in a humidity chamber at 100% relative humidity and incubated at 25°C under constant light. (A detailed bioassay procedures is described in ref. *24*).

In order to compare the pathogenicity of *Beauveria bassiana* Nov. EO-1 with known entomogenous fungi, *Beauveria bassiana* ATCC 74040 and *Paecilomyces fumosoroseus* ATCC 20874, known whitefly mortality producers,

were used for these comparisons. Conidial spore solutions from each fungus were obtained by incubating all fungi on PDA (Potato Dextrose agar) agar plates. As described above in the pathogenicity tests, slides with 36 instars of SPWF were dipped for 10 s in each conidial solution containing 1×10^6 spores/mL. As negative control, 0.05% Tween solution without conidial spores were used. Before dipping the instars into the spore solution, all fungal solutions were tested for germination and viability. After seven days of incubation, SPWF mortality was counted for each fungal spore treatment.

In Vivo Pathogenicity of Fermentation Broth Containing the Red Pigment Against Foliage Insects

Beauveria bassiana Nov. EO-1 fementation broth, cultivated for 7 d and containing the red pigment as described in the fermentation section, was used to evaluate the in vivo toxicity against SPWF and mealy bugs. Hibiscus plants were infested with known amounts of whitefly and mealy bug scales. The fermentation broth solution was brushed on the four lower leaves and the plants were placed under greenhouse conditions. After 7 d, mortality against SPWF and mealy bugs was assessed.

Extraction of the Red Pigment

One liter fermentation broth samples, obtained after 7 d cultivation, were filtered to remove mycelium particles and pH adjusted to 2.0 using acetic acid. The broth was extracted with ethyl acetate at a 1:2 ratio. An orange-red pigment was obtained and the liquid was evaporated almost to dryness yielding a dense orange red extract.

Purification of the Red Pigment

The concentrated orange red pigment extract obtained was purified by dissolving the pigment extract in 100 mL of ethyl acetate and loaded on a Dowex ion exchange resin column (50w-x8, Dow Chemical, Midland, MI). The red pigment fraction obtained was then further evaporated to dryness and the resulting extract was used for analytical purposes.

Analytical Instrumentation and Procedures

For analyzing the red pigment extracts, low and high resolution mass spectrometric experiments were carried out by using a Finnigan TSQ-70 and a Finnigan MAT-90 BEQQ instruments, respectively. Samples were analyzed by probe-MS in both the electron impact ionization (EI) and chemical ionization (CI) modes. Chemical ionization was accomplished using methane with 1% ammonia as the reagent gas. During probe-MS analysis, the samples were heated from 50 to 350°C at a rate of 30°C/min.

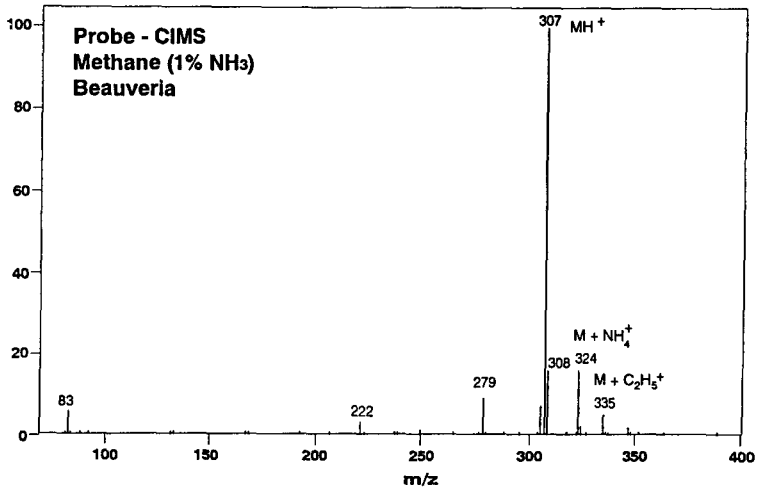

Fig. 2. Mass spectra of the red pigment produced by *Beauveria bassiana* Nov. EO-1 acquired by probe-MS in the chemical-ionization mode.

FTIR experiments were also carried out using a Nicolet 60 SX FTIR instrument. Samples were prepared by casting a film from methanol on a barium fluoride disk, and transmission FTIR spectra were acquired using infrared microspectroscopy.

RESULTS

Identification of the Red Pigment Mass Spectrum

The purified red pigment extract obtained from *Beauveria bassiana* Nov. EO-1, as described in the materials and methods section, was analyzed by probe-MS using both low and high resolution mass spectrometry. Figure 2 shows the mass spectrum of the red material produced by *Beauveria bassiana* Nov. EO-1 acquired by probe-MS in the chemical-ionization (CI) mode. The ion at *m/z* 307 is owing to the protonated molecule ion (MH^+) suggesting that the molecular weight of the major compound in the red material is 306. The other ions in the mass spectrum *m/z* 324, 335, and 347 are owing to M + $NH4^+$, M + $C_2H_5^+$ ions, respectively, which are very common for methane (with 1% NH_3) CI spectra. The presence of all these ions also confirmed the molecular weight (306) corresponding to oosporein.

A high resolution (at 7000 resolution) probe-MS analysis in the chemical ionization mode of the red material suggested the exact mass of the MH^+ ion at 307.0419 and an empirical formula $C_{14}H_{11}O_8$. This corresponds to the empirical formula of the protonated molecule ion of oosporein (*see* Fig. 1).

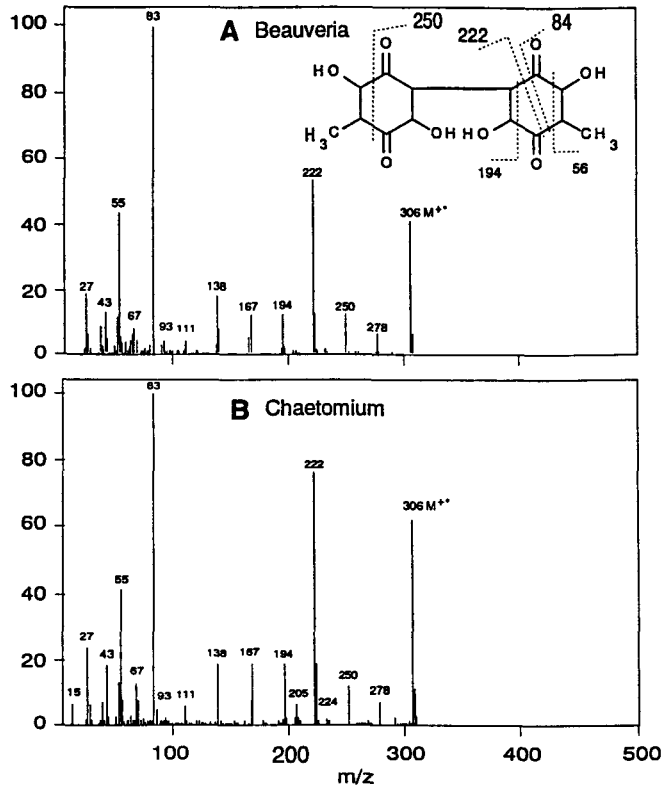

Fig. 3. Comparison of mass spectra of the red pigments obtained from *Beauveria bassiana* Nov. EO-1 vs *Chaetomium trilaterale.*

Figure 3A shows the electron impact ionization (EI) mass spectrum of the red material from *Beauveria bassiana* Nov. EO-1. The ion of *m/z* 306 is caused by the molecule ion (M^+). The fragmentation pattern in the EI spectrum is consistent with oosporein's structure. The probable fragmentation pathways can be rationalized as shown in Fig. 3A. In order to confirm our identification, we compared the red pigment obtained from *Chaetomium trilaterale,* a known oosporein producer, by using the same procedures for fermentation, extraction and purification as used for *Beauveria bassiana* Nov. EO-1. Figure 3B represents the electron impact (EI) mass spectrum of the red material from *Chaetomium trilaterle.* Comparison of the EI mass spectra of both pigments (Fig. 3A,B) showed that, in fact, the spectra are almost identical, suggesting that the red pigment obtained from *Beauveria bassiana* Nov. EO-1 is indeed oosporein.

FTIR

The isolated red fractions produced by *Beauveria bassiana* Nov. EO-1 and *Chaetomium trilaterale* were analyzed by FTIR (Fig. 4). The absorptions

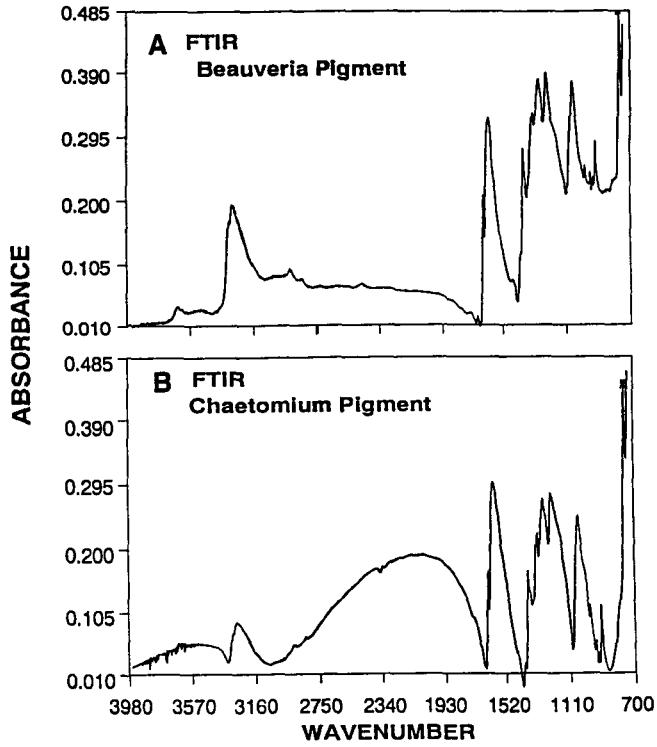

Fig. 4. Absorption spectrum of the red pigment produced by *Beauveria bassiana* Nov. EO-1 vs *Chaetomium trilaterale.*

in the FTIR spectrum can also be rationalized for that of oosporein structure. The absorptions at 1613 and 3292 cm^{-1} are representative of quinone and hydroxyl moieties of oosporein, respectively. The small signal at 1648 cm^{-1} is probably owing to the C = C stretch.

A comparison of the FTIR data of the red material from both fungi (Fig. 4) showed that they are almost identical, once again suggesting that the red material from *Beauveria bassiana* Nov. EO-1 is oosporein.

NMR

A proton NMR spectrum of the red extract showed only one strong peak at 1.8 ppm, which is consistent with that expected for the two equivalent 5,5-CH_3 group in oosporein. Surprisingly, the chemical shifts for the four OH groups were not observed. Similar observations for oosporein obtained from *Chaetomium* sp. were also made by Cole in 1981, and they attributed this lack of OH signals to the exchange in dimethyl D_6-sulfoxide, the solvent used for NMR study.

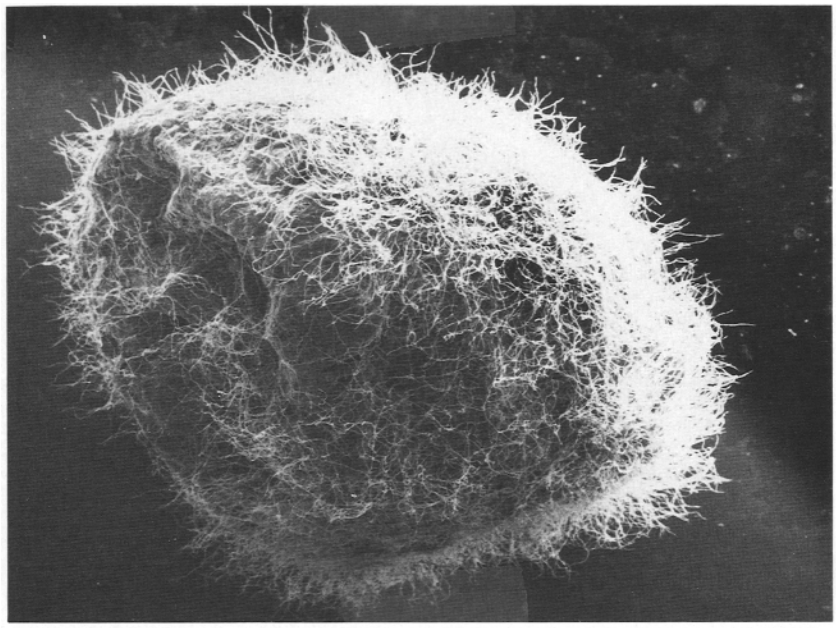

Fig. 5. Formation of hyphae of *Beauveria bassiana* Nov. EO-1 on the bead surface at 12 h after rehydration.

Sporulation, Germination, and Viability of Mycelium Beads of *Beauveria bassiana* Nov. EO-1

Figures 5 and 6 describe the process of aerial formation of hyphae and conidial spores on *Beauveria bassiana* Nov. EO-1 mycelium beads. As can be seen in Fig. 5, the bead is covered with mycelium after 24 h of incubation. Figure 6 illustrates the conidiation process from the mycelium. Figure 7 describes the percent of viability of *Beauveria bassiana* Nov. EO-1 mycelium beads when the mycelium beads were assayed on YM agar. As can be seen from Fig. 7, the half-life of the mycelium beads stored at 25°C is around 250 d, whereas the half-life of mycelium beads stored at 4°C is > 300 d. Figure 8 describes the amount of conidial spores obtained from mycelium beads under two different storage temperatures 4 and 25°C. The beads were assessed in plastic wells without agar nutrient support. As can be seen from Fig. 8, mycelium beads that have been stored at 25°C lost their ability to sporulate over 1×10^6 spores per bead after 2 mo. However, beads that were stored at 4°C maintained their capacity to sporulate and produced over $> 10^6$ spores per mycelium bead after 6 mo of storage.

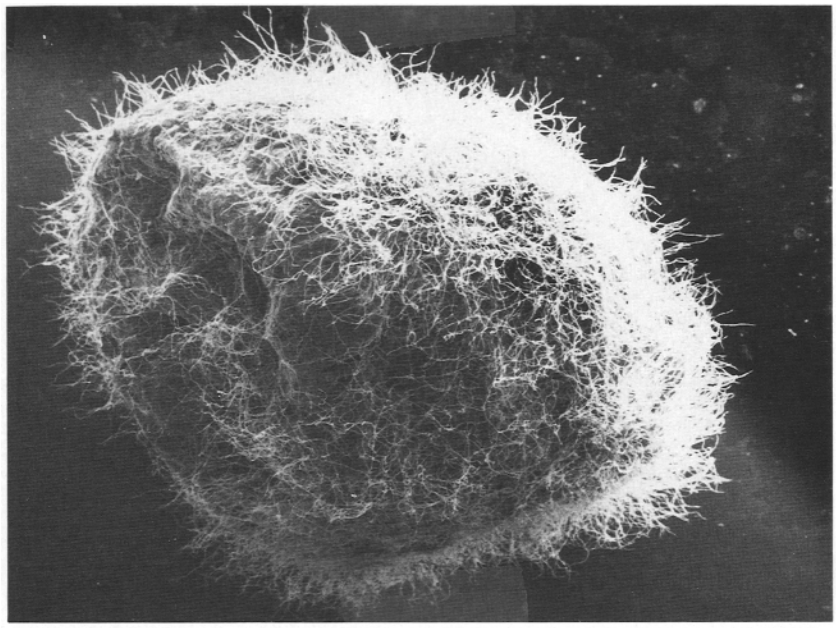

Fig. 6. Formation of conidia on the surface of *Beauveria bassiana* Nov. EO-1 bead.

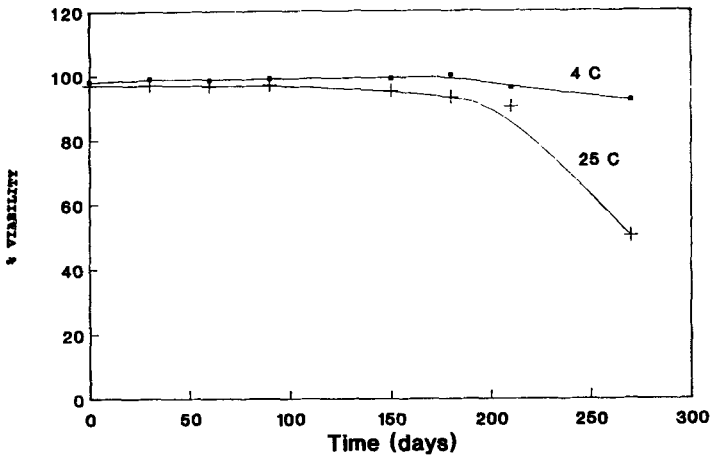

Fig. 7. Percentage of viability of *Beauveria bassina* Nov. EO-1 mycelium beads at 4 and 25°C on agar YM medium.

All conidial spore germination assays conducted on *Beauveria bassiana* Nov. EO-1 mycelium beads, stored at 4 and 25°C resulted in 100% conidial spore germination. All *Beauveria bassiana* Nov. EO-1 mycelium beads, regardless of storage temperature and agar media used (e.g., YM agar, water agar, and PDA agar), secreted the red pigment into the agar media at the same intensity level. It seems that the red pigment is predominantly produced during surface aerial mycelium growth and this red

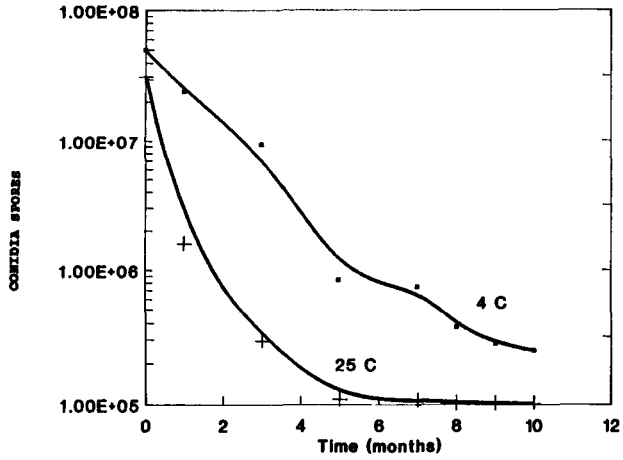

Fig. 8. Amount of conidia spores obtained from mycelium beads stored at 4°C.

Table 1
Pathogenicity of *Beauveria bassiana* Nov. EO-1 on *Aritpus floridanus* in Soil

Batch no.	Rate CC/soil	Spores/bead	Viability of beads, %	Percent average mortality	Percent infection
JE111	5g		100%	20.0 ± 8.0	0
	10g	1.17×10^5	100%	24.0 ± 10.4	0
	25g		100%	28.0 ± 9.1	0
JE112	5g		100%	36.0 ± 10.4	0
	10g	1.37×10^6	100%	44.9 ± 3.5	0
	25g		100%	36.0 ± 10.4	16.0 ± 6.7
JE120	5g		100%	24.0 ± 8.8	20.0 ± 5.7
	10g	4.59×10^5	100%	45.7 ± 8.5	43.2 ± 8.5
	25g		100%	64.0 ± 6.7	56.0 ± 8.8

pigment production is supported by the agar nutrients. Without agar nutrient support the red pigment will not appear on the agar plate. The red pigment is not part of the mycelium as it is, for example, with the yellow colored mycelium, which is characteristic of many *Beauveria bassiana* strains. The physiological nutrient and metabolic requirements for optimum red pigment production have yet to be characterized.

Pathogenicity of *Beauveria bassiana* Nov. EO-1

Table 1 presents the pathogenicity of different *Beauveria bassiana* Nov. EO-1 mycelium bead batches to *Artipus floridanus* (citrus root weevil) larvae. As can be seen from Table 1, sporulation levels were comparable between the batches. However, infectivity and mortality were different.

Table 2
Total Whitefly Mortality

Strain	Spore concentration	Percent germination	Percent mortality
Beauveria bassiana Nov. EO-1*	1.0×10^6	94.3	76.0
ATCC 74037	3.4×10^7	96.0	92.0
Beauveria bassiana ATCC 74040	1.6×10^6	88.5	52.0
Paecilomyces fumosoroseus[a] ATCC 20874	4.2×10^6	97.6	98.0
Paecilomyces fumosoroseus[a] Strain No. 109	2.9×10^6	98.3	92.0
Control	0	—	6.0

[a]Fungi were originally isolated from sweet potato whitefly.

Batch JE 120 had the highest mortality and infectivity rate among the three batches tested. A dose response (Table 1), based on the amount of mycelium bead in soil vs. mortality and infectivity of the insect, has been established.

Table 2 compares the SPWF mortality rate obtained from the different entomopathogenic fungi species tested. As can be seen from Table 2, *Beauveria bassiana* Nov. EO-1 has high virulency against whitefly. However, *Paecilomyces fumosoroseus* strain, ATCC 20374, which was also isolated from a whitefly, performed much better at the same spore concentration.

Table 3 presents the total mortality obtained from fermentation broth containing the red pigment on mealy bug scales placed on hibiscus leaves. As can be seen from Table 3, 49.8% of scales on the treated leaves were infected. These scales were colored by a dark pink or sienna color; however, the fungus was not visible. This could be owing to the low greenhouse humidity conditions. One of these leaves was removed from the plant and placed in a petri dish with wet filter paper. After 3 d all infected scales showed fungal growth.

DISCUSSION

Research has shown that pathogenicity varies greatly among different strains of *Beauveria bassiana*. The discovery of *Beauveria bassiana* Nov. EO-1, which consistently synthesizes the red pigment oosporein, and which has high pathogenicity against soil insects as well as foliage insects, may suggest that oosporein may play a role in the infection process of insects. From the experiments described in this paper, *Beauveria bassiana* Nov. EO-1 mycelium beads can produce oosporein on different nutrient media after a year of storage, without decreased oosporein production.

Table 3
Percent Infection of Mealy Bugs on Hibiscus Leaves
Treated with Fermentation Broth After Three Days in Greenhouse Conditions

Leaf	Number of live scales Pretreatment	Number of dead scales Posttreatment	Percent mortality
A	75.0	30.0	40.0
B	53.0	34.0	64.0
C	55.0	17.0	31.0
D	50.0	35.0	70.0
Total	233.0	116.0	49.8
Control (untreated leaf)	25.0	2.0	6.0

Our studies also showed that this fungus does not lose the ability to produce oosporein when transferred on agar nutrients.

The broad insect pest spectrum of *Beauveria bassiana* Nov. EO-1 indicates that this fungus may be used for biocontrol purposes. However several obstacles for commercialization need to be solved first. The formulation based on mycelium beads, as described in this paper, should be much improved before it is used commercially. The formulation should have a minimum of one year stability at 25°C storage, as well as a higher sporulation rate.

Because oosporein is a potent mycotoxin, the use of *Beauveria bassiana* Nov. EO-1 must be carefully evaluated in toxicological and environmental studies in order to avoid potential adverse environmental effects.

Although this study did not produce any information that would of itself suggest a plausible mode of oosporein's action on insect pests, it is postulated that oosporein plays a significant role in the infectivity process, especially at the stage where the fungus has already penetrated the host insect. Oosporein may act as an antagonist to the intestinal bacterial flora, allowing the fungal mycelium to develop faster inside the host insect as a result of the decreased competition levels.

It seems that using an oosporein producing fungi in microbial control may have also the following advantages: The red pigment appearance on the infected insect can be used as a fungal infectivity indicator, and the production of oosporein during the introduction of *Beauveria bassiana* Nov. EO-1 mycelium beads in soil may prevent the feeding of antagonistic fungi or soil bacteria on the bead, because of the antibacterial activity of oosporein, thus allowing the completion of the sporulation process of the fungi in soil.

Further investigation of the role of oosporein in the pathogenicity process of infecting soil or foilage insects is necessary, as well as studies on the phytotoxicity effects of oosporein on plants treated with the fungus.

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REFERENCES

1. Roberts, D. W. and Yendol, W. G. "Use of Fungi for Microbial Control of Insects, in Bargs, H. D. and Hussey, eds., *NIW Microbial Control of Insects and Mites,* London. 125–149.
2. Ferron, P. (1978), *Ann. Rev. Entomol.* **23,** 409–442.
3. Vining, L. C., Kelleher, W. J., and Schwartim, A. E. (1962), *Can. J. Microbiol.* **8,** 931–933.
4. Ferron, P. (1985), *Mycopathologia,* **91,** 109–116.
5. West, E. J. and Briggs, J. D. (1968), *J. Econ. Entomology* **61,** 684–687.
6. Hamill, R. L., Higgens, D. E., Boaz, H. E., and Gorman, M. (1969), *Tetrahedron Letts.* **49,** 4255.
7. Dresner, E. (1950), *J. NY Entomol. Soc.* **58,** 269–278.
8. Toumanoff, C. (1931), *Ann. Parasital. Humaine Comp.* **9,** 462–482.
9. Kodiara, Y. (1961), *J. Fac. Textile Sci. Tech.* **5,** 1–68.
10. Steinhaus, E. A. and Bell, C. R. (1953), *J. Econ. Entomol.* **46,** 582–598.
11. Wat, Chi-Kit, McInnes, A. G., Smith, D. G., Wright, J. C., and Vining, L. C. (1990), *Can. J. Chem.* **55,** 4090.
12. Sohair, H., Basyouni, E., Brewer, D., and Vining, L. C. (1968), *Can. J. Botany* **46,** 441.
13. Paris, S. and Ferron, P. (1979), *J. Invert. Pathol.* **34,** 71–77.
14. Brewer, D. (1993), Personal communication.
15. Cole, R. J., Kirksey, J. W., Cutler, H. G., and Davis, E. E. (1974), *J. Agric. Food. Chem.* **22,**
16. Wainwright, M., Betts, R. P., and Teale, D. M. (1986), *Trans. Br. Mycol. Soc.* **86(1).**
17. Pegram, R. A. and Wyatt, R. D. (1981), *Poultry Sci.* **60,** 2429–2440.
17a. Pegram, R. A., Wyatt, R. D., and Smith, T. L. (1981), *Avian Dis.* **26,**
18. Kogl, F. and Van Wessem, G. C. (1944), *Rec. Trav. Chim.* **63,** 5.
19. Divekar, P. V., Haskins, R. H., and Vining, L. C. (1959), *Can. J. Chem.* **37,** 2097.
20. Smith, J. and Thomson, R. H. (1960), *Tetrahedron* **10,** 148–152.
21. Brown, T. P., Fletcher, O. O., and Wyatt, R. D. (1987), *Avian Dis.* **31,** 868–877.
22. Taniguchi, M., Kawaguchi, T., Tanki, T., and Susumu, O. (1984), *Agric. Biol. Chem.* **48(4),** 1065–1067.
23. Manning, R. O. and Wyatt, R. D. (1984), *Poultry Sci.* **63,** 251–259.
24. Osborne, L. S., Eyal, J., et al. (1993), *Bioassay of Entomogenous Fungi on Whitefly.* "Biological control" submitted for publication 1993.