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Virulence of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) Strain ESC-1 to the German Cockroach (Dictyoptera: Blattellidae) and Its Compatibility with Insecticides

PARI PACHAMUTHU,[1] SHRIPAT T. KAMBLE,[1, 2] AND GARY Y. YUEN[3]

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ABSTRACT Virulence of *Metarhizium anisopliae* (Metschnikoff) Sorokin strain ESC-1 against the German cockroach, *Blattella germanica* (L.), was determined using 5 concentrations ranging from 8×10^7 to 2×10^9 spores per milliliter. The calculated LD_{50} value was 4.18 by 10^8 spores per milliliter (4.18×10^5 spores per cockroach). In vitro study was conducted to determine the compatibility of *M. anisopliae* strain ESC-1 with chlorpyrifos, propetamphos, and cyfluthrin. Insecticides did not affect conidial germination but did adversely affect the growth and sporulation of *M. anisopliae* strain ESC-1. The growth of *M. anisopliae* colonies on media amended only with 50 and 500 ppm of chlorpyrifos and 500 ppm of propetamphos treatments at 3, 6, and 9 d was significantly inhibited compared with the control. Similarly, sporulation was significantly reduced in treated colonies exhibiting partial colony growth. The colonies cultured on SDAY media amended with 50 ppm of chlorpyrifos had significantly reduced sporulation compared with the control and no sporulation was observed in colonies cultured on media amended with 500 ppm of chlorpyrifos and propetamphos.

KEY WORDS *Metarhizium anisopliae*, *Blattella germanica*, virulence, insecticide compatibility, germination, sporulation

THE ENTOMOPATHOGENIC FUNGUS *Metarhizium anisopliae* (Metschnikoff) Sorokin has been isolated from at least 200 insect species and is a potential candidate for microbial control. Each of the isolates differs significantly in its pathogenicity against a target species; therefore, a potential pathogen must be evaluated individually against a target insect pest (Zimmermann 1993). Different strains of *M. anisopliae* have been used for controlling many agriculturally important insect pests such as beetles (Liu et al. 1989, Moorhouse et al. 1993), aphids (Milner and Soper 1981), planthoppers (Samuels et al. 1989), and pear psylla (Puterka et al. 1994). The use of this fungus as a potential control agent in the household environment also was evaluated against termites (Kramm et al. 1982) and ants (Kelley-Tunis et al. 1995).

Despite considerable research on the use of *Metarhizium* sp. for crop insect control, there are very few reports on the application of this fungus to control the German cockroach, *Blattella germanica* (L). *Metarhizium anisopliae* strain ESC-1 was formulated commercially as Bio-Path for controlling cockroaches by Ecoscience, but it was discontinued because of inconsistent mortality. Kaakeh et al. (1996, 1997) reported lethal time of *M. anisopliae* to German cockroaches but not the lethal concentrations. Moreover, Kaakeh et al. (1996) included mortality data based on contact assay and there was no documentation of total number of *M. anisopliae* spores required to cause mortality in German cockroaches. Bio-Path, the commercial product of *M. anisopliae* strain ESC-1, required 28 d for 85% mortality (Kaakeh et al. 1996; Kamble and Prabhakaran, unpublished data). Although *M. anisopliae* has the potential to be an effective agent, the extended time required to control cockroaches has been the most important factor in the failure of Bio-Path. Therefore, there is a critical need to enhance the biological activity of *M. anisopliae* by integrating it with sublethal doses of conventional insecticides leading to an integrated pest management (IPM) approach.

Efficacy of the fungus *Beauveria bassiana* (Balsamo) Vuillemin was improved by combining it with sublethal doses of insecticides (Ferron 1971, Anderson et al. 1989). *B. bassiana* was found to be compatible with abamectin and triflumuron (Anderson et al. 1989) but incompatible with diflubenzuron, carbaryl, methomyl, and methyl parathion (Gardner et al. 1979). The integration of any fungal isolate with an insecticide requires a thorough knowledge of the compatibility between the two agents (Gardner et al. 1979, Vanninen and Hokkanen 1988), particularly the percentage of spore germination, growth, and sporulation. Based on colony growth and percentage of germination, many entomopathogenic fungal strains were found to be compatible with insecticides, including phosphamidon (Urs et al. 1967), permethrin (Clark et al. 1982), diazinon, cypermethrin (Vanninen and Hokkanen 1988, Moorhouse et al. 1992), carbofuran (Li and Hol-

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dom 1994), aldicarb (Samuels et al. 1989, Li and Holdom 1994), and pirimicarb (Vanninen and Hokkanen 1988). In contrast, BHC (Urs et al. 1967), azinphos-methyl, carbofuran (Clark et al. 1882), chlorpyrifos (Samuels et al. 1989, Li and Holdom 1994), ethoprophos, and fenamiphos (Li and Holdom 1994) inhibited the growth of entomopathogenic fungal strains.

Another indicator of compatibility between a fungus and insecticide is fungal sporulation (Li and Holdom 1994). Sporulation can be affected by pesticides (Gardner and Storey 1985) and a potential inhibitory effect of pesticides can affect the epizootic condition of the disease or reduce the efficacy of the strains in the field. In most of the compatibility studies, sporulation of the fungus was affected by insecticide concentrations (Urs et al. 1967, Gardner et al. 1979, Samuels et al. 1989).

Although in vitro compatibility studies between entomopathogens and insecticides have been conducted for agricultural pests, such research concept has not been thoroughly evaluated to control German cockroaches in urban settings. This concept is not new, yet the data on lethal concentrations of *M. anisopliae* and the effect of insecticides on conidial germination, mycelial growth, and sporulation of *M. anisopliae* are essential components in developing an effective IPM program for controlling German cockroaches. In vitro research on combination of fungi and insecticides will provide viable baseline information on their compatibility before conducting in vivo studies. Furthermore, the integration of biological agents with sublethal concentrations of insecticides will reduce environmental contamination and improve human safety. We are hypothesizing that in vitro compatibility studies will enable us to select the insecticide doses that will enhance the effect of *M. anisopliae* and also will reduce the amount of insecticide used in the in vivo studies. This research was undertaken to evaluate the virulence of *M. anisopliae* strain ESC-1 against German cockroaches, to determine the lethal dose required to kill 50% of the target population, and to assess compatibility of *M. anisopliae* with commercially used insecticides at acceptable concentrations by the in vitro procedure.

Materials and Methods

Insects. The Chemical Specialities and Manufacture's Association (CSMA) strain of German cockroach is a insecticide-susceptible strain used as the target insect. The German cockroaches were reared on Purina dog chow (Ralston Purina, St. Louis, MO) and water, and maintained in Plexiglas containers (59 by 24 by 24 cm). The cockroaches were reared at 27 ± 2°C, 60 ± 10% RH, and a photoperiod of 12:12 (L:D) h.

Conidial Production. The *M. anisopliae* strain ESC-1 was obtained from EcoScience, East Brunswick, NJ. The conidia were produced on Sabouraud dextrose agar yeast (SDAY) media (Moorhouse et al. 1992). This medium was prepared by mixing 1% (wt:vol) peptone, 1% (wt:vol) yeast, 4% (wt:vol) glucose, and 1.6% (wt:vol) agar (Difco, Detroit, MI) in distilled water. After autoclaving at 121°C for 20 min, ≈16 ml of media was poured into sterile petri dishes (10 by 1.5 cm). Ten microliter of the spore solution, prepared in 0.05% Triton X-100, was placed in the center of each petri dish containing the media and sealed with parafilm. Spore cultures in sealed petri dishes were incubated at constant temperature (27 ± 2°C) in the dark for 21 d. Conidia were harvested in sterile water containing 0.05% Triton X-100. Conidia from each plate were scraped with a sterile spatula, and the spore solution was filtered through an 8-layered cheese cloth (Style 280, Chicopee Mills, NY), centrifuged (3,500 × *g* for 15 min at 4°C), and resuspended in sterile water containing 0.05% Triton X-100. The spore concentration was determined using a Neubauer hemocytometer. Spore concentration was determined by using the formula (total number of spores from both sides divided by $2 \times [0.1\ mm^3]) \times (1 \times 10^3\ mm^3$ per milliliter), where 0.1 mm^3 is the height and 1 by 10^3 mm^3 is the total area of the hemocytometer.

Topical Application with *M. anisopliae*. Based on the results of the preliminary experiment, 5 spore concentrations (8×10^7, 1.5×10^8, 5×10^8, 8.5×10^8, and 2×10^9 spores per milliliter) and a control (treated with 1 μl of 0.05% Triton X-100) were used for the subsequent bioassay. The required spore concentrations were measured from the stock solution and 1 μl of the spore suspension was applied topically on the 1st ventral abdominal segment of each adult male German cockroach by using a Hamilton microliter syringe (Reno, NV). The treated cockroaches were placed in the Plexiglas container (15 by 6 cm) (1 container per replication) and incubated at 27 ± 2°C and ≈85% RH. Relative humidity within each container was monitored by placing the probe of the digital hygrometer and thermometer (Fisher, St. Louis, MO) inside the container and sealing it with masking tape. Mortality was observed daily for 21 d, and dead cockroaches were removed daily. Moistened Whatman No. 1 filter paper (18.5 cm) was placed in each container to maintain high humidity (≈85%). Food, water, and filter paper were changed every 3 d. Each treatment was replicated 4 times with 10 adult male cockroaches per treatment, and the entire experiment was repeated twice. The mortality data were pooled and analyzed by probit analysis using the POLO program (Robertson and Preisler 1992), which provided both the chi-square and *t*-test values.

In Vitro Study on Compatibility of Insecticide with *M. anisopliae*. Commercial formulations of propetamphos (Safrotin, 18.9% [AI], Sandoz Agro, Des Plaines, IL), chlorpyrifos (Dursban Pro, 23.5% [AI], Dow Agroscience, Indianapolis, IN), and cyfluthrin (Tempo, 24.3% [AI], Bayer, KS, City, MO) were used in this study. The manufacturer's label recommended rates were 5,000 ppm (AI) for chlorpyrifos and propetamphos, and 500 ppm (AI) for cyfluthrin for German cockroach control. We used the concentrations (sublethal doses) of 0.5, 5, 50, and 500 ppm (AI) for chlorpyrifos and propetamphos, and 0.05, 0.5, 5, and 50 ppm (AI) for cyfluthrin. The desired insecticide concentrations were prepared by serial dilution of the com-

mercial formulation in distilled water. Because the insecticides used are emulsifiable concentrates (EC), the emulsifiers will enable the technical grade material to disperse in the water uniformly to form a suspension.

The SDAY medium was prepared as described under conidial production with slight modifications that consisted of adding dextrose solution (sterilized through a Nalgene reusable filter system by using a 0.22-μm filter membrane (Fisher, St. Louis, MO) to the autoclaved media containing peptone, yeast, and agar. When the media cooled sufficiently, different concentrations of propetamphos, chlorpyrifos, and cyfluthrin were added (60 ml of insecticide solution was added to 540 ml of media [1:9 ratio]). In the SDAY-amended insecticide media, dextrose, yeast, agar and peptone are in solution, whereas insecticides are in suspension. The bottles containing the insecticide-amended SDAY media were then hand-shaken and rolled on the clean bench for 3 min to ensure the uniform mixing of insecticide with the media. Approximately 16 ml of media amended with insecticides was poured into each petri dish and allowed to solidify at room temperature under the table top horizontal Laminar flow (Envirco, Jefferson, NM). Ten microliters of suspension of conidia in sterile distilled water containing 0.05% Triton X-100 was placed in the center of plate, which was then sealed with parafilm and incubated in the dark at 27 ± 2°C.

Conidial Germination, Colony Diameter, and Sporulation. Conidial germination was determined 12 h later by observing the spores for germ tube development. The diameter of each culture was measured on 3, 6, and 9 d after incubation. Length and width of colony growth were measured for each culture, and the averages of these values were used to express the colony growth per plate. After 14 d, the culture plates were stored at 4°C until conidial collection. Subsequently, conidia from each culture plate were collected by washing them in 50 ml of 0.05% Triton X-100. The colony was initially washed with 20 ml of 0.05% Triton X-100, and 15 ml of the solution was used in the ensuing 2 washes. The spore solutions were then centrifuged, resuspended in 0.05% Triton X-100, and the final concentration determined usingthe Neubauer hemocytometer.

Experimental Design. The experimental design for determining the effect of insecticide on germination, colony growth, and sporulation was a randomized complete block design with 8 replications per treatment. Conidial germination and sporulation data were transformed by arcsin and log transformation procedures, respectively. Data were analyzed by PROC GLM (SAS Institute 1990). The values were compared by least significant difference (LSD) tested by using PROC GLM.

Results

Virulence of *M. anisopliae* Strain ESC-1. The cockroach mortality observed in the initial experiment was 16, 20, and 90% when exposed to *M. anisopliae* spores

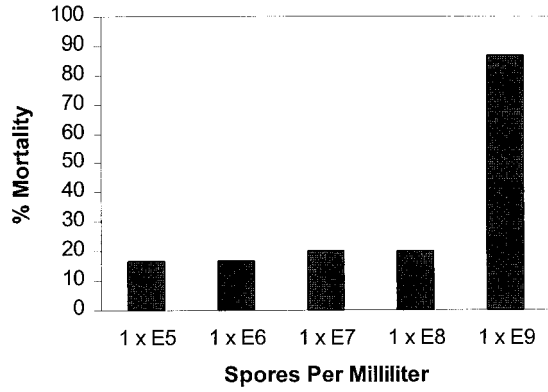

Fig. 1. Mortality caused by *M. anisopliae* strain ESC-1 to CSMA strain of German cockroach. E, exponential.

(Fig. 1). Based on initial mortality data, the concentrations from 8×10^7 to 2×10^9 spores per milliliter were selected for the subsequent 2 bioassays. The average cockroach mortality ranged from 31 to 86% in the treated populations, whereas 16% mortality was observed in the untreated cockroaches (Table 1). The LD_{50} value obtained was 4.18×10^8 spores per milliliter (4.18×10^5 spores per cockroach) with a upper limit of 6.34×10^8 spores per milliliter (6.34×10^5 spores per cockroach) and a lower limit of 2.73×10^8 spores per milliliter (2.73×10^5 spores per cockroach) (99% CI). The t-ratio was 6.85 and the χ^2 value was 1.68. Fig. 2a indicates the conidia at the time of incubation, whereas Fig. 2b illustrates the germinated conidia bearing the germ tube (if the length of the germ tube was at least half the length of the spore, the conidia was considered to have germinated). The percentage of germination of *M. anisopliae* spores used in the initial experiment was 91%, whereas the percentages of conidial germination used in 1st and 2nd bioassays were 92 and 93%, respectively.

Effect of Insecticides on Spore Germination. The *M. anisopliae* strain ESC-1 did not exhibit any significant difference ($P > 0.05$) in the conidial germination resulting from incorporation of insecticides into the SDAY media (Table 2). The conidial germination of *M. anisopliae* strain ESC-1 cultured on media incorporated with insecticides (chlorpyrifos, propetamphos, and cyfluthrin) was 96 or 97%.

Effect of Insecticides on Fungal Colony Growth. There were significant differences in growth of *M.*

Table 1. Mortality (mean ± SEM) in CSMA strain of German cockroach caused by *M. anisopliae* strain ESC-1

Spore concn/ml	n^a	% mortality
8.0×10^7	80	31.3 ± 1.8
1.5×10^8	80	32.5 ± 7.1
5.0×10^8	80	62.5 ± 10.6
8.5×10^8	80	76.5 ± 8.8
2.0×10^9	80	86.3 ± 1.8
Control	80	16.3 ± 1.8

[a] Number of male German cockroaches used for each concentration.

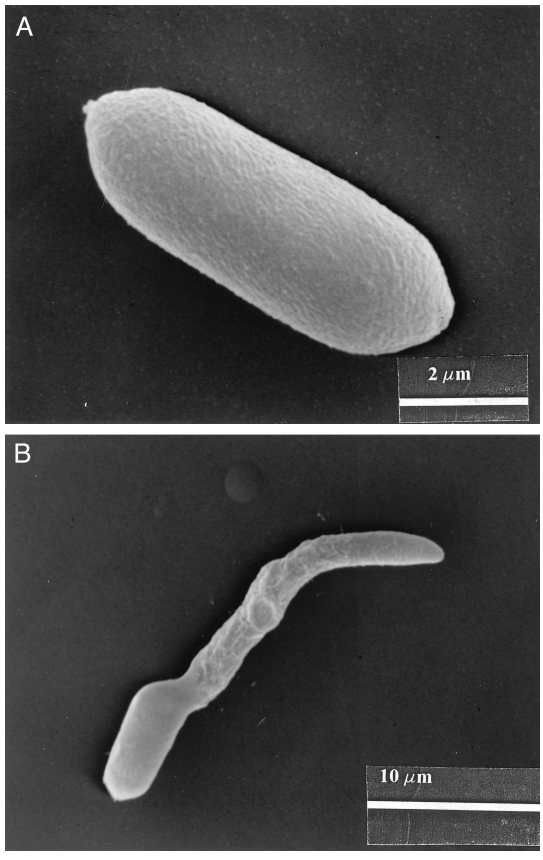

Fig. 2. (a) Scanning electron micrograph of *M. anisopliae* conidia strain (14,000×) cultured on SDAY media at time zero. (b) Germ tube development (3,800×) after 12 h of incubation at 27 ± 2°C.

anisopliae cultured on media amended with chlorpyrifos (50 and 500 ppm) and propetamphos (500 ppm) compared with the control (Table 3). The diameter of the colonies observed on 3, 6, and 9 d was as follows: 16.3, 28.6, and 39.7 mm for chlorpyrifos at 500 ppm; 17.1, 31.4, and 45.5 mm for chlorpyrifos at 50 ppm; and 16.1, 26.2, and 35.3 mm for propetamphos at 500 ppm, respectively. Growth of *M. anisopliae* cultured on media amended with 0.5 and 5 ppm of chlorpyrifos was higher than on the control. On day 3, the growth of *M. anisopliae* was statistically similar among colonies cultured on media amended with 0.5 ppm and 5 ppm of chlorpyrifos and the control. The colony diameters in these 2 treatments, however, were significantly larger than the control on day 6 and 9.

Table 2. Conidial germination (mean ± SEM) of *M. anisopliae* strain ESC-1 cultured on SDAY media amended with selected insecticides

SDAY media + insecticides (ppm)	% germination	Arcsin-transformed values[a]
Chlorpyrifos (0.5)	96.13 ± 0.99	1.37a
Chlorpyrifos (5)	96.50 ± 2.07	1.39a
Chlorpyrifos (50)	97.13 ± 1.46	1.41a
Chlorpyrifos (500)	96.75 ± 1.36	1.40a
Propetamphos (0.5)	95.87 ± 2.23	1.37a
Propetamphos (5)	96.50 ± 1.41	1.39a
Propetamphos (50)	97.00 ± 2.00	1.41a
Propetamphos (500)	96.00 ± 1.31	1.37a
Cyfluthrin (0.05)	97.13 ± 1.46	1.41a
Cyfluthrin (0.5)	97.13 ± 1.46	1.41a
Cyfluthrin (5)	97.00 ± 2.07	1.41a
Cyfluthrin (50)	96.63 ± 1.30	1.39a
Control	97.00 ± 1.41	1.41a

[a] Transformed values followed by same letter are not significantly different by Fisher LSD ($P > 0.05$). Values under each column are means of 8 replicate plates. LSD conidial germination, 0.05.

Table 3. Diameter (mean ± SEM) of *M. anisopliae* strain ESC-1 cultured on insecticide-amended SDAY media

SDAY media + insecticides (ppm)	Day 3	Day 6	Day 9
Chlorpyrifos (0.5)	21.8 ± 1.2a	39.0 ± 2.5a	54.9 ± 1.6ab
Chlorpyrifos (5)	21.5 ± 1.5abc	39.3 ± 2.6ab	55.5 ± 1.6a
Chlorpyrifos (50)	17.1 ± 0.4d	31.4 ± 0.7d	45.5 ± 1.9e
Chlorpyrifos (500)	16.3 ± 0.7e	28.6 ± 1.7e	39.7 ± 1.2f
Propetamphos (0.5)	21.5 ± 1.3abc	38.8 ± 2.6ab	54.7 ± 2.1ab
Propetamphos (5)	21.7 ± 1.4ab	38.9 ± 2.3ab	54.0 ± 1.8bcd
Propetamphos (50)	21.0 ± 1.1c	38.1[b] ± 2.3c	52.7 ± 1.3d
Propetamphos (500)	16.1 ± 1.5e	26.2 ± 1.5f	35.3 ± 1.9g
Cyfluthrin (0.05)	21.7 ± 1.5ab	38.6 ± 2.5ab	54.3 ± 1.9abc
Cyfluthrin (0.5)	21.5 ± 1.7abc	38.6 ± 3.1ab	54.0 ± 2.6bcd
Cyfluthrin (5)	21.5 ± 1.4abc	38.3 ± 3.1bc	54.1 ± 2.4bcd
Cyfluthrin (50)	21.2 ± 1.5abc	38.7 ± 2.5ab	54.3 ± 2.4abc
Control	21.3 ± 1.3bc	37.6 ± 2.7c	53.1 ± 2.1cd

Means followed by same letter are not significantly different by Fisher LSD test ($P > 0.05$). Values under each column are means of 8 replicate plates. LSD of 3-d growth, 0.62; LSD 6-d growth, 0.91; LSD 9-d growth, 1.46.

Metarhizium anisopliae cultured on propetamphos-amended SDAY media had the same growth patterns compared with the control colonies except for the colonies raised on propetamphos 500 ppm-amended media (Table 3). There was no significant difference in the growth of *M. anisopliae* cultured on media amended with 0.5, 5, and 50 ppm of propetamphos on day 3. *M. anisopliae* colonies cultured on media amended with 0.5 and 5 ppm of propetamphos, however, were significantly larger than the control colonies on day 6. There was no difference between the control and 50 ppm of propetamphos. On day 9, there was no significant difference between the colony diameter in the control compared with 5 and 50 ppm of propetamphos, but the colony diameter in media amended with 0.5 ppm of propetamphos was significantly larger than in the control.

Unlike the growth pattern observed on SDAY media amended with chlorpyrifos and propetamphos, cyfluthrin-amended media did not exhibit any inhibitory effect on the growth of *M. anisopliae* cultures (Table 3). On days 3 and 9, there was no significant difference in growth of the *M. anisopliae* cultured on SDAY media amended with 0.05, 0.5, 5, and 50 ppm of cyfluthrin and the control. The growth of *M. anisoplaie* cultured in media amended with 5 ppm of cyfluthrin was statistically similar to the control on day 6. *M. anisopliae* had better growth patterns on media amended with 0.05,

Table 4. Sporulation of *M. anisopliae* strain ESC-1 cultured on insecticide-amended SDAY media after 14 d

SDAY media + insecticides (ppm)	Spore concn ± SEM[a]	Log transformed values[b]
Chlorpyrifos (0.5)	1.17 ± 0.24	20.86bcde
Chlorpyrifos (5)	1.40 ± 0.29	21.04abc
Chlorpyrifos (50)	0.36 ± 0.14	19.62f
Chlorpyrifos (500)	0.00 ± 0.00	0.00g
Propetamphos (0.5)	1.21 ± 0.27	20.89bcde
Propetamphos (5)	1.38 ± 0.24	21.06ab
Propetamphos (50)	1.20 ± 0.14	21.01abc
Propetamphos (500)	0.00 ± 0.00	0.00g
Cyfluthrin (0.05)	1.07 ± 0.27	20.76e
Cyfluthrin (0.5)	1.09 ± 0.17	20.79de
Cyfluthrin (5)	1.16 ± 0.38	20.83cde
Cyfluthrin (50)	1.41 ± 0.49	20.99abcd
Control	1.60 ± 0.25	21.18a

[a] Spore concentration is expressed in 10^9 conidia per plate.
[b] Transformed values followed by same letter are not significantly different by Fisher LSD ($P > 0.05$). Values under each column are means of 8 replicate plates. LSD sporulation, 0.21.

0.5, and 50 ppm of cyfluthrin compared with the control.

Effect of Insecticides on Sporulation. There was a significant difference ($P < 0.05$) in the effect of sporulation in *M. anisoplaie* cultured on media amended with selected insecticides. The sporulation ranged from 0.00 to 1.6×10^9 spores per culture with the highest sporulation observed in the control, whereas the *M. anisopliae* cultured on 500 ppm of chlorpyrifos and propetamphos-amended media did not sporulate (Table 4). The spore concentration of *M. anisopliae* cultured on media amended with 50 ppm of chlorpyrifos was significantly lower than that of the other treatments. Only 4 treatments (5 ppm of chlorpyrifos, 5 and 50 ppm of propetamphos, and 50 ppm of cyfluthrin) had sporulation concentrations that were similar to that of the control.

In chlorpyrifos-amended media, no significant differences were observed in the spore concentrations between *M. anisoplaie* cultured on 0.5 and 5 ppm of chlorpyrifos, but the values were significantly different for colonies cultured on 50- and 500-ppm treatments. There were no significant differences in spore concentration of *M. anisopliae* cultured on 0.5, 5, and 50 ppm of propetamphos. In cyfluthrin-amended media, there were significant differences in spore concentration between 0.05 ppm and 50 ppm of cyfluthrin, whereas the values were similar for treatments 0.5, 5, and 50 ppm of cyfluthrin.

Discussion

We found that *M. anisopliae* strain ESC-1 caused appreciable high mortality in the susceptible CSMA strain of German cockroach at 21 d following treatment. Kamble and Prabhakaran (unpublished data) and Kaakeh et al. (1996) also reported a similar finding in the susceptible Orlando-N and JWax strains of German cockroach, where high mortality (85 and 100%, respectively) was observed by a contact method after 28 d. Trends observed in our study are similar to the results of Kaakeh et al. (1996), where mortality caused by *M. anisopliae* strain ESC-1 increased with increased contact time between infected and uninfected German cockroach nymphs as well as the number of infected nymphs used with uninfected nymphs. Although low mortality at spore concentrations of $\leq 1.5 \times 10^8$ spores per milliliter was observed in our study, there was a linear relationship between the spore doses used and the mortality observed. These results were further substantiated by the chi-square value, which clearly indicated that the model fits the probit analysis. According to Liu et al. (1989), Puterka et al. (1994), and Jones et al. (1996), the virulence of any fungal isolate to cause mortality in insects is directly related to the spore concentration. Our data follow a similar pattern, in which mortality increased from 31.3 to 76.5%, translating to a 40% increase in mortality for a 10-fold increase in spore concentration. Based on our results, *M. anisopliae* strain ESC-1 with high viability (>90% germination) and a high spore concentration (4.18×10^8 spores per milliliter [5×10^5 spores per cockroach]) is needed for obtaining high mortality in German cockroaches at optimum conditions of 27°C and ≈85% RH. Our data indicate that *M. anisopliae* strain ESC-1can be an effective biopesticide for cockroach control after 21 d; however, such a lengthy time becomes the most limiting factor. We agree with Kaakeh et al. (1996) that *M. anisopliae* can be used in an IPM system, further hypothesizing that the fungus can yield higher mortality if it is used in combination with sublethal doses of insecticides.

Growth and sporulation of *M. anisopliae* strain ESC-1 were more sensitive to insecticides than was the conidial germination. There was no significant difference in conidial germination of *M. anisopliae* strain ESC-1 on media amended with different concentrations of chlorpyrifos, propetamphos, and cyfluthrin. High germination of *M. anisopliae* strain observed after 12 h of incubation on media amended with insecticides could have been influenced by previous exposure of conidia to SDAY media, resulting in enough reserve nutrients (carbohydrates, lipids, and amino acids) (Hawker and Madelin 1976, Van Etten et al. 1983) to initiate the process of germ tube development when placed in a solid substrate containing exogenous nutrients (Dillon and Charnley 1990). St. Leger et al. (1989) also demonstrated that *M. anisopliae* conidia cultured in yeast extract medium (YEM) on polystyrene produced germ tubes within 7–12 h after incubation. Rapid germination of *M. anisopliae* could be attributed to metabolic activities that take place within a conidium by using reserve nutrients and a minimum amount of exogenous carbon source needed for germ tube formation after its placement on a substrate (Gottlieb 1976, Smith and Grula 1981, St. Leger et al. 1989). Al-Aidross and Seifert (1980) demonstrated that conidia of wild and mutant *Metarhizium* strains produced germ tubes in water agar and they attributed the early germination of some strains to carbohydrate metabolism. In contrast to the germ tube requirement, hyphae requires exogenous utilizable

carbon and nitrogen for its growth (Smith and Grula 1981, St. Leger et al. 1989). Moorhouse et al. (1992) reported a similar pattern in which the majority of the pesticides did not affect conidial germination, except for zineb and chlorothalonil. Moorhouse et al. (1992) also reported that colony growth was affected by all pesticides except propamocarb. Therefore, germination alone should not be used as an indicator for predicting the compatibility between insecticide and entomopathogens. But high germination of an isolate implies its potential to be an effective agent under optimal conditions of temperature, relative humidity, and the presence of exogenous nutrient resources.

According to our results, colony growth was observed on media amended with all concentrations of insecticides, and there also was a significant difference in the growth pattern among insecticides. Growth of *M. anisopliae* strain ESC-1 was determined to be more sensitive to chlorpyrifos followed by propetamphos. Lack of inhibitory effect on colony growth caused by incorporation of cyfluthrin into SDAY media could be attributed to the highest concentration used, which was 1/10 in comparison to chlorpyrifos and propetamphos treatments. Moorhouse et al. (1992) showed that partial inhibition of colony growth was caused by high concentrations of insecticides present in the SDAY media. Inhibition of *M. anisopliae* growth at high concentrations of chlorpyrifos also was documented by Samuels et al. (1989) and Li and Holdom (1994), but the growth and sporulation were compatible as insecticide concentration decreased. In our study, the growth and sporulation effect observed at 500 ppm of chlorpyrifos and propetamphos is because of the formulated product. Thus, it is difficult to contemplate whether the inhibitory effect is caused by active ingredients or other inert ingredients. Our results on growth and sporulation are contrary to those of Vanninen and Hokkanen (1988), who stated that inhibition of mycelial growth does not necessarily mean that sporulation will be affected. We found that a ≈20% reduction in colony growth cultured on SDAY media incorporated with 500 ppm of chlorpyrifos and propetamphos resulted in zero sporulation. The difference between these 2 studies could be the result of different compounds used in the study as well as to the method used for collecting spores. Vanninen and Hokkanen (1988) used a crude method of assessing the sporulation (i.e., spores were collected by placing a piece of tape on the colonies) instead of collecting all the spores from the plates.

There was no relationship between the insecticide concentration and sporulation as no increased sporulation due to decreased insecticide concentration was observed. Li and Holdom (1994) also reported a similar pattern where spore concentrations were not consistent between different treatments, and colonies cultured on high insecticide concentrations had better sporulation than the colonies cultured on low concentrations. The relationship between germination, growth, and sporulation indicates that factors affecting germination of *M. anisopliae* strain ESC-1 may be different from the ones affecting growth and sporulation in the presence of insecticides. Campbell et al. (1983) and Li and Holdom (1995) showed that uptake of carbohydrate and nitrogen from exogenous sources is essential for growth and sporulation of *M. anisopliae.* The SDAY-incorporated insecticide media used in our study had carbohydrate (dextrose) and nitrogen (peptone). Although the conidia are able to produce the germ tube, the presence of high concentrations of insecticide in the media seems to have an effect on growth and sporulation. The trend observed in our study also was reported by St. Leger et al. (1989), where germination was not affected but appressoria formation and hyphal differentiation were significantly affected by incorporation of certain macromolecule inhibitors into the nutrient media. Insecticide concentrations ranging from 0.1 to 10 times the recommended field rates have significant effects on growth and sporulation. Because there is a direct relationship between growth and insecticide concentration, this factor can impose a major limitation in the use of these 2 agents under laboratory or field conditions. If the concentration chosen is high (label recommended), the insecticide by itself can result in high mortality and lead to buildup of insecticide resistance faster or the insecticide present within the dead or infected insects might enhance or reduce the growth and sporulation of entomopathogens. Thus, in vitro studies can provide meaningful data that enable researchers to select the appropriate insecticide concentrations for conducting in vivo studies.

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