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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, SHRIPAT T. KAMBLE, AND GARY Y. YUEN


**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 x 10^3 to 2 x 10^4 spores per milliliter. The calculated LD_{50} value was 4.18 x 10^4 spores per milliliter (4.18 x 10^4 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
dom 1994), aldicarb (Samuels et al. 1989, Li and Hold-
dom 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), ethoprop-
phos, and fenamiphos (Li and Holdom 1994) inhibited
the growth of entomopathogenic fungal strains.

Another indicator of compatibility between a fun-
gus and insecticide is fungal sporulation (Li and Hold-
dom 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, sporu-
lation of the fungus was affected by insecticide con-
centrations (Urs et al. 1967, Gardner et al. 1979, Sam-
uels et al. 1989).

Although in vitro compatibility studies between en-
tomopathogens and insecticides have been conducted
for agricultural pests, such research concept has not
been thoroughly evaluated to control German cock-
roaches 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 compat-
ibility before conducting in vivo studies. Furthermore,
the integration of biological agents with sublethal con-
centrations 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 en-
hance 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 viru-
ulence 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 com-
patibility of M. anisopliae with commercially used in-
secticides at acceptable concentrations by the in vitro
procedure.

Materials and Methods

Insects. The Chemical Specialties and Manufactu-
re’s Association (CSMA) strain of German cock-
roach is a insecticide-susceptible strain used as the
target insect. The German cockroaches were reared on
Purina dog chow (Rulston 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
1.8% (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.
Sporce 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
clot (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 he-
mcytometer. Spore concentration was determined by
using the formula (total number of spores from
both sides divided by 2 × (0.1 mm3)) × (1 × 108
mm3 per milliliter), where 0.1 mm3 is the height and 1 by
103 mm3 is the total area of the hemocytometer.

Topical Application with M. anisopliae. Based on
the results of the preliminary experiment, 5 spore
concentrations (8 × 107, 1.5 × 108, 5 × 108, 8.5 × 108,
and 2 × 109 spores per milliliter) and a control (treat-
ed with 1 μl of 0.05% Triton X-100) were used for the
subsequent bioassay. The required spore concentra-
tions 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 con-
tainer 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 propet-
aminphos (Safrotin, 18.9% [AI], Sandoz Agro, Des Plaines,
IL), chlorpyrifos (Dursban Pro, 23.5% [AI], Dow
Agroscience, Indianapolis, IN), and cyfluthrin (Tem-
po, 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 propet-
amphos, and 500 ppm (AI) for cyfluthrin for German
cockroach control. We used the concentrations (sub-
lethal doses) of 0.5, 5, 50, and 500 ppm (AI) for chlor-
pyrifos and propetamphos, and 0.05, 0.5, 5, and 50 ppm
(AI) for cyfluthrin. The desired insecticide concen-
trations were prepared by serial dilution of the com-

mmercial 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 15 ml of the solution was placed in the center of plate, which was then sealed with parafilm and incubated in the dark at 27 ± 2°C.

Conidial Germination and Sporulation Data. 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). Based on initial mortality data, the concentrations from 8 × 10^6 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 × 10^8 spores per cockroach) with a upper limit of 6.34 × 10^8 spores per milliliter (6.34 × 10^8 spores per cockroach) and a lower limit of 2.73 × 10^8 spores per milliliter (2.73 × 10^8 spores per cockroach) (95% 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 percentage 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>
<thead>
<tr>
<th>Table 1. Mortality (mean ± SEM) in CSMA strain of German cockroach caused by M. anisopliae strain ESC-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spore concn/ml</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>8.0 × 10^7</td>
</tr>
<tr>
<td>1.5 × 10^9</td>
</tr>
<tr>
<td>5.0 × 10^8</td>
</tr>
<tr>
<td>8.5 × 10^8</td>
</tr>
<tr>
<td>2.0 × 10^8</td>
</tr>
<tr>
<td>Control</td>
</tr>
</tbody>
</table>

* Number of male German cockroaches used for each concentration.
Table 2. Conidial germination (mean ± SEM) of M. anisopliae strain ESC-1 cultured on insecticide-amended SDAY media

<table>
<thead>
<tr>
<th>SDAY media + insecticides (ppm)</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpyrifos (0.5)</td>
<td>21.8 ± 1.2a</td>
<td>39.0 ± 2.5a</td>
<td>54.9 ± 1.6ab</td>
</tr>
<tr>
<td>Chlorpyrifos (5)</td>
<td>21.5 ± 1.5abc</td>
<td>39.3 ± 2.6ab</td>
<td>55.5 ± 1.6a</td>
</tr>
<tr>
<td>Chlorpyrifos (50)</td>
<td>17.1 ± 0.4a</td>
<td>31.4 ± 0.7a</td>
<td>45.5 ± 1.8a</td>
</tr>
<tr>
<td>Chlorpyrifos (500)</td>
<td>16.3 ± 0.7e</td>
<td>28.6 ± 1.7e</td>
<td>39.7 ± 1.2f</td>
</tr>
<tr>
<td>Propetamphos (0.5)</td>
<td>21.5 ± 1.3abc</td>
<td>38.5 ± 2.6abc</td>
<td>54.7 ± 2.1abc</td>
</tr>
<tr>
<td>Propetamphos (5)</td>
<td>21.7 ± 1.4ab</td>
<td>39.9 ± 2.3ab</td>
<td>54.0 ± 1.8bcd</td>
</tr>
<tr>
<td>Propetamphos (50)</td>
<td>21.0 ± 1.1c</td>
<td>38.1 ± 2.3c</td>
<td>52.7 ± 1.3d</td>
</tr>
<tr>
<td>Propetamphos (500)</td>
<td>16.1 ± 1.5e</td>
<td>26.2 ± 1.5f</td>
<td>35.3 ± 1.9g</td>
</tr>
<tr>
<td>Cyfluthrin (0.05)</td>
<td>21.7 ± 1.5abc</td>
<td>38.5 ± 2.5abc</td>
<td>54.3 ± 1.9abc</td>
</tr>
<tr>
<td>Cyfluthrin (0.5)</td>
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<td>38.6 ± 3.1ab</td>
<td>54.0 ± 2.0bed</td>
</tr>
<tr>
<td>Cyfluthrin (5)</td>
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</tr>
<tr>
<td>Cyfluthrin (50)</td>
<td>21.2 ± 1.5abc</td>
<td>38.5 ± 2.5abc</td>
<td>54.3 ± 2.4abc</td>
</tr>
<tr>
<td>Control</td>
<td>21.3 ± 1.3bc</td>
<td>37.6 ± 2.7c</td>
<td>53.1 ± 2.1cd</td>
</tr>
</tbody>
</table>

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.

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

<table>
<thead>
<tr>
<th>SDAY media + insecticides (ppm)</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
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<td>54.3 ± 1.9abc</td>
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<td>16.5 ± 1.7abc</td>
<td>38.6 ± 3.1ab</td>
<td>54.0 ± 2.0bed</td>
</tr>
<tr>
<td>Chlorpyrifos (500)</td>
<td>16.1 ± 1.5e</td>
<td>26.2 ± 1.5f</td>
<td>35.3 ± 1.9g</td>
</tr>
<tr>
<td>Propetamphos (0.5)</td>
<td>21.5 ± 1.4abc</td>
<td>38.3 ± 3.1bc</td>
<td>54.1 ± 2.4bed</td>
</tr>
<tr>
<td>Propetamphos (5)</td>
<td>21.5 ± 1.4abc</td>
<td>38.3 ± 3.1bc</td>
<td>54.1 ± 2.4bed</td>
</tr>
<tr>
<td>Propetamphos (50)</td>
<td>21.2 ± 1.5abc</td>
<td>38.5 ± 2.5abc</td>
<td>54.3 ± 2.4abc</td>
</tr>
<tr>
<td>Control</td>
<td>21.3 ± 1.3bc</td>
<td>37.6 ± 2.7c</td>
<td>53.1 ± 2.1cd</td>
</tr>
</tbody>
</table>

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.

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 was statistically similar among colonies 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.

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. There was no significant difference in the growth of M. anisopliae cultured on media amended with 0.5, 5, and 50 ppm of propetamphos. However, 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 treatments, 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 diameters 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 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. anisopliae 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,
respectively) was observed by a contact method after man cockroach, where high mortality (85 and 100%, and Kaakeh et al. (1996) also reported a similar finding strain of German cockroach at 21 d following treat-

appreciable high mortality in the susceptible CSMA different by Fisher LSD (\( P > 0.05 \)) in the effect of sporu-

Effect of Insecticides on Sporulation. There was a significant difference (\( P < 0.05 \)) in the effect of spora-

in M. anisoplate 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. anisoplaie cultured on 300 ppm of chlorpyrifos and propetamphos-amended media did not sporulate (Table 4). The spore concentration of M. anisoplaie cultured on media amended with 50 ppm of chlorpyr-

able high mortality in the susceptible CSMA strain of German cockroach at 21 d following treat-

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

<table>
<thead>
<tr>
<th>SDAY media + insecticides (ppm)</th>
<th>Spore concn. ( \times 10^9 ) SEM^a</th>
<th>Log transformed values^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpyrifos (0.5)</td>
<td>1.17 ± 0.24</td>
<td>20.96ecef</td>
</tr>
<tr>
<td>Chlorpyrifos (5)</td>
<td>1.40 ± 0.29</td>
<td>21.04abc</td>
</tr>
<tr>
<td>Chlorpyrifos (50)</td>
<td>0.36 ± 0.14</td>
<td>19.62f</td>
</tr>
<tr>
<td>Chlorpyrifos (500)</td>
<td>0.00 ± 0.00</td>
<td>0.00g</td>
</tr>
<tr>
<td>Propetamphos (0.5)</td>
<td>1.21 ± 0.27</td>
<td>20.98efcd</td>
</tr>
<tr>
<td>Propetamphos (5)</td>
<td>1.38 ± 0.24</td>
<td>21.06ab</td>
</tr>
<tr>
<td>Propetamphos (50)</td>
<td>1.20 ± 0.14</td>
<td>21.01abc</td>
</tr>
<tr>
<td>Propetamphos (500)</td>
<td>0.00 ± 0.00</td>
<td>0.00g</td>
</tr>
<tr>
<td>Cyfluthrin (0.05)</td>
<td>1.07 ± 0.27</td>
<td>20.75e</td>
</tr>
<tr>
<td>Cyfluthrin (0.5)</td>
<td>1.09 ± 0.17</td>
<td>20.79de</td>
</tr>
<tr>
<td>Cyfluthrin (5)</td>
<td>1.16 ± 0.38</td>
<td>20.83cde</td>
</tr>
<tr>
<td>Cyfluthrin (50)</td>
<td>1.41 ± 0.49</td>
<td>20.9abde</td>
</tr>
<tr>
<td>Control</td>
<td>1.60 ± 0.25</td>
<td>21.18a</td>
</tr>
</tbody>
</table>

^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 6 replicate plates. LSD sporulation, 0.21.

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

Discussion

We found that M. anisoplaie strain ESC-1 caused appreciable high mortality in the susceptible CSMA strain of German cockroach at 21 d following treat-

ment. Kamble and Prabhakaran (unpublished data) and Kaakeh et al. (1996) also reported a similar finding in the susceptible Orlando-N and JWax strains of Ger-

man cockroach, where high mortality (85 and 100%, respectively) was observed by a contact method after
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 two 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 appressorium 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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