

Original Article

Biocontrol of the Brown-banded Cockroach, *Supella longipalpa* F. (Blattaria: Blattellidae), with Entomopathogenic Fungus, *Metarhizium anisopliae*

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(Received 13 May 2013; accepted 17 Jan 2015)

Abstract

Background: Considering to the high distribution of cockroaches as urban pests, the efficacy of different formulations of *Metarhizium anisopliae* strain Iran 437C were assessed against the brown-banded cockroach, *Supella longipalpa* F. under laboratory and field conditions.

Methods: *Metarhizium anisopliae* isolates were screened with immersing adults of the brown-banded cockroaches in aqueous suspension of 10^8 conidia ml^{-1} followed by surface or bait treated with different doses of the most virulent isolate against the nymphs. Then formulations of conidia oil-in-water were examined versus cockroach nymphs using different plant oils and paraffin. Then they were evaluated and compared with aqueous suspension and control group. On a large-scale, the sunflower oil-in-water formulation of conidia was sprayed at houses using a hand sprayer.

Results: *Metarhizium anisopliae* IRAN 437C was the most virulent isolate against the brown-banded cockroach, causing 100% mortality in adults at seven days post-exposure. Inoculated bait with this isolate was not enough pathogenic against the cockroach even at two weeks after treatment. Treated surface with conidia as aqueous suspension or oil-in-water formulation was more effective than the bait formulation against the cockroach caused 39.4–97.2% mortality compared with 2.5% mortality in control group after two days. Spraying the conidia formulated with sunflower oil was an effective formulation causing 76.1% reduction in the cockroach density on the third day post treatment in the houses.

Conclusion: The oil-in-water formulation of *M. anisopliae* IRAN 437C could be recommended as a promising alternative for cockroach control.

Keywords: *Supella longipalpa*, *Metarhizium anisopliae*, Entomopathogenic fungus, Biocontrol

Introduction

Cockroaches are recognized as cosmopolitan and trouble-maker insect pests in the different premises such as: homes, restaurants, hospitals, warehouses, offices, and other buildings regarding stores of food materials (Stankus et al. 1990, Nasirian et al. 2006, Vazirianzadeh et al. 2014).

They move among different premises and transmit the pathogenic and non-patho-

genic microorganisms from waste material to the food and kitchen stuffs. In this way, they carry bacterial disease agents such as cholera, leprosy, plague, typhoid fever, viral diseases such as poliomyelitis and parasitic worms and protozoa disease agents (Stankus et al. 1990, Vazirianzadeh et al. 2014).

In addition, they are considered as insect pests, which cause allergic reactions includ-

ing dermatitis, itching, swelling of the eyelids, and more serious respiratory conditions like asthma by their feces and disintegrated dead bodies (Stankus et al. 1990, Savoldelli and Luciano 2005).

The brown-banded cockroach, *Supella supellectilium (longipalpa)* (F.) (Blattaria: Blattellidae) as a small species of cockroach, measuring about 10 to 14 mm long, carries a variety of microorganisms (Le Guyader et al. 1989, Ehdæ 2014). It is a vector of pathogenic bacteria in urban environments and hospitals (Rivault et al. 1994, Ehdæ 2014, Vazirianzadeh et al. 2014). *Supella longipalpa* is also reported as an allergen source (Eggleston and Arruda 2001, Savoldelli and Luciano 2005).

High amounts and numbers of chemical insecticides are used annually to reduce cockroach infestations in urban areas. This fact leads to development of resistance in these filth pests especially the German cockroaches.

They are a major threat to chemical and pest control industries worldwide (Lee et al. 1996, Nasirian et al. 2006, Ladonni et al. 2013). In all field-collected strains of Iran, cockroaches have showed different level of resistance to different insecticides of organochlorine, organophosphorus and carbamate insecticides. Therefore, application of these insecticides should be stopped as a recommendation (Nasirian et al. 2006, Ladonni et al. 2013).

Insecticide resistance, environmental concerns, and increased optimal costs of new insecticides have promoted searching for safer compounds with novel modes of action and environmentally safe biological products such as biocontrol agents (Pachamuthu et al. 2000). Biological control uses one organism to control another. Where insecticides are undesirable or it is not necessary to eliminate cockroaches, biological control program is considered.

Cockroaches as animal pests like almost all animals have natural enemies with no

exception. Biological control plays an important role in managing cockroach populations. Natural cockroach enemies include long list agents which entomopathogenic fungi are one of them. *Metarhizium* species are known to attack a wide range of arthropods: greater than 200 species in over 50 families. These include many species of agricultural, medical and veterinary importance. *Metarhizium anisopliae* is a fungus that grows naturally in soils throughout the world and causes disease in various insects. *M. anisopliae* does not appear to infect humans or other animals and is considered safe as an insecticide. The disease caused by the fungus is sometimes called green muscardine disease because of the green color of its spores (Freimoser 2003, Bischoff et al. 2009).

Microbial control agents, such as entomopathogenic fungi, can be effective and serve as alternatives to broad-spectrum chemical insecticides (Zimmermann 1993, Lacey et al. 2001). Earlier studies have shown the efficacy of different *M. anisopliae* isolates in cockroach control, especially the German cockroach, *Blattella germanica*, (Gunner et al. 1991, Kaakeh et al. 1996, Pachamuthu et al. 1999, Zurek et al. 2002, Quesada-Moraga et al. 2004, Lopez and Alves 2011).

There are rare studies about control of the brown-banded cockroach by entomopathogens. Evaluation of conidia-dust formulation of *M. anisopliae* strain Iran 437 C exhibited high performance against this cockroach (Sharififard et al. 2014). This filth pest has recently become wide distribution in the city of Ahwaz, Khuzestan Province, South West of Iran, especially in apartments and hospitals (Vazirianzadeh et al. 2014, Sharififard et al. 2014).

The current research was undertaken to evaluate different formulation of the most virulence isolate of *M. anisopliae* including aqueous suspension, inoculated bait and oil-in-water formulations against the brown-banded cockroach, *S. longipalpa*, under labor-

atory and field conditions.

Materials and Methods

Insects

This study was carried out from April 2011 to March 2012 in medical lab of Ahvaz Joundishapur University of Medical Sciences (AJUMS) as small scale and in apartment houses as field scales. The Brown-banded cockroaches originally were collected from kitchen area of human dwellings. They were reared in Plexiglas containers (25 cm high×17 cm diameter) and maintained at 27±2 °C, 50±5% RH for a photoperiod of 12:12 (L: D) h. They were fed on bread or dry crumbled Saghe Talaei biscuits and water. Pieces of facial tissue were provided as harborage. Cockroaches were anesthetized with extreme cold to facilitate handling.

Fungus

Four strains of the entomopathogenic fungus, *M. anisopliae* (*M. anisopliae* strain Iran 437C, *M. anisopliae* strain Iran 715C, *M. anisopliae* strain 1018, and *M. anisopliae* strain *Rhynchophorus*), were provided from the fungi collection of the Plant Protection Institute of Iran. They were cultured on SDAY plates, kept at 27 °C and in a photoperiod condition of 12:12 (L: D) h. Sporulating cultures were harvested by scraping dry conidia from the surface of the culture plate with a scalpel (Talwar 2005).

Experiments

Virulence Screening of *Metarhizium anisopliae* isolates

Aqueous suspensions of four isolates of *M. anisopliae* were prepared and the concentrations were adjusted to 10⁸ conidia ml⁻¹. In order to determine the virulence of fungal isolates against the brown-banded cockroach, a group of 20 adults (10 males and 10 females) in 4 replicates were dipped in the conidial suspension for 10 sec and were then

placed on damp filter paper in Plexiglas containers. The control group was dipped in sterile distilled water. All containers were incubated at 27±1 °C, 75±5% RH and a photoperiod of 12:12 (L: D) h. The cockroaches were fed during the test and mortality was recorded daily for a week. Cockroach cadavers were collected, surface sterilized, and transferred to sterile Petri dishes containing damp filter paper. True mortality was taken to occur for those cadavers on which fungal sporulation were visible (Butt and Goettle 2007).

Surface treated with aqueous suspension

Stock aqueous suspension of *M. anisopliae* isolates Iran 437C with Tween 80 (0.01 percent) was prepared and diluted serially to give the concentrations of 5×10⁸, 10⁸, 5×10⁷, 10⁷ and 5×10⁶ conidia ml⁻¹. Whatman filter paper (No.1, 8cm diameter) was dipped into the suspension and then placed in the bottom of a glass jar (with 600 ml capacity which 2 ml of suspension was nearly absorbed by a filter paper). The last instar cockroach nymphs (3 and 4 instars) were released simultaneously into each glass jar and confined to the floor. The control group was exposed to a surface treated with distilled water. The experiment was repeated four times. Cockroaches were fed on bread and water during the trial period. Mortality was recorded daily for a week (Butt and Goettle 2007).

Bait inoculated with the most virulent isolate

Attractive bait consisting of bread wetted in beer was used in this test. This bait was recommended for cockroach live trapping by Cochran, (1999). Ten grams of the bait in a Petri dish was inoculated with 1ml of the conidia suspension of *M. anisopliae* isolate Iran 437C with 5 doses of 5×10⁶, 10⁷, 5×10⁷, 10⁸ and 5×10⁸ conidia ml⁻¹. Twenty large brown-banded cockroach nymphs (3 and 4 instars) were released into Plexiglas containers (25 cm high×17 cm diameter) and al-

lowed to adapt for one day before the introduction of treated baits. After that, inoculated bait was introduced to each container and remained as the only food source for 72 h. Treated bait was then replaced with untreated food. The control group was fed on untreated bait. There were four replicates for each treatment. Mortality was recorded daily for two weeks.

Evaluation of conidia oil- in-water formulation against *Supella longipalpa*

Oil-in-water emulsions were prepared with sesame oil, sunflower oil, coconut oil, and aqueous suspension of 2.5%, 5%, and 10% (v/v). The surfactant Tween 80 was added to each formulation at a concentration of 50 percent of the amount of oil. All compounds were previously mixed and agitated at 250 rpm (Albernaz et al. 2008) for 20 min at room condition. Then a conidia suspension was added to give the defined concentration (10^8 conidia ml^{-1}). Large instar cockroach nymphs (3 and 4 instars) were released into glass jars (600 ml beakers) and exposed directly to 2.5 ml of different oil-in-water or aqueous formulations using a hand-held pressure sprayer. The nozzle of the sprayer was held 45 cm away from and at a right angle to the application surface. The control group was exposed to distilled water. There were four replicates for each treatment. The treated cockroaches were maintained at 27 ± 1 °C, with a photoperiod of 12:12 L: D, and RH: 45 ± 5 percent.

Field evaluation of the fungus

The sunflower oil-in-water formulation of conidia (proportion of 10%) of *M. anisopliae* strain IRAN 437C (equal to 10^9 spore $\text{ml}^{-1} \approx 10$ times the laboratory concentration) was selected for further evaluation at house-scale, because it showed the highest efficiency against the brown-banded cockroach. The method of Thavara et al. (2007) with a little modification was used to evalu-

ate cockroach infestations in houses. Eight houses with brown-banded infestation were selected and surveyed for cockroach density using sticky traps before and after treatment (four houses for treatment and four houses for control). After the preliminary survey, to assess the degree of cockroach infestation, the sticky traps were placed in each house and left there for a night. One sticky trap per 10 cm^2 was located at the selected house. Then, all the traps were collected and the cockroaches caught in each trap were counted. After the initial infestation evaluation, the conidia oil-in-water formulation at the standard dosage of 40 ml/m^2 for residual treatment (Rozendal 1997) was applied by a hand-held pressure sprayer. The treatment was carried out only once in each house. Cockroach density was assessed on the seventh day after treatment by sticky trap. The average number of collected cockroaches per house (mean no.) and standard error of the mean (SE) were calculated. The percentage of reduction in the number of cockroaches following treatment at each treated site was calculated by Mulla's formula (Mulla et al. 1971): Reduction (%) = $100 - [(C_1/T_1) \times (T_2/C_2)] \times 100$

Where: C_1 = average number of cockroaches per house at the control site (pre-treatment), T_1 = average number of cockroaches per house at the treated site (pre-treatment), C_2 = average number of cockroaches per house at the control site (post-treatment), T_2 = average number of cockroaches per house at the treated site (post-treatment).

Statistical analysis

Firstly, all mortality data was transferred to percentages. Mean and standard error mortality for each dose of conidia per each method were calculated. Kruskal-Wallis and Mann-Whitney tests and Probit analysis were used to compare the treatments and to create the toxicity values of conidia doses (LD_{50} , LD_{90} , LT_{50} and confidence intervals),

respectively. The significant level tests were $P < 0.05$. The SPSS version 16 and SAS software were used for data analysis. On the field treatment, the percentage of reduction in cockroach infestation was calculated using Mulla's formula (Mulla et al. 1971).

Results

Virulence Screening of *Metarhizium anisopliae* isolates

As shown in Table 1, *M. anisopliae* strain Iran 437C was the most virulent isolate against *S. longipalpa*. It caused 82% and 100% mortality in adults in the third and seventh day after treatment, respectively. The mortality percentage of this isolate was significantly different from the other isolates ($P = 0.0001$). Green moscardin were observed on the all sterilized cockroach cadavers (Fig. 1). Therefore, this more virulent isolate was selected for further evaluation.

Surface treated with aqueous suspension

Results showed that cockroach mortality rates increased by an increase in the concentration of conidia suspension. Mortality rates of the treated cockroach nymphs ranged from 34.2 to 97.8% at seventh day post-exposure at the lowest and the highest doses, respectively. It was also significant at the second day post-exposure and ranged from 9.2%–65.3% (Table 2). The mortality rates were not significantly different at the doses of 2×10^7 and 4×10^6 conidia cm^{-2} on the seventh day, and they did not differ on the fifth and seventh day at the dose of 2×10^7 conidia cm^{-2} . The average mortality rate of the control group was significantly different from the treated groups and was always lower than 5%. The LD_{50} and LD_{90} values were 7.7×10^6 and 10^8 conidia cm^{-2} at third day post-exposure but reduced to 5.6×10^5 and 2.7×10^6 conidia cm^{-2} at seventh days post-exposure (Table 3).

Calculation and comparison of lethal time

values (LT_{50}) also showed a significant differences between applications of different doses of conidia, because there was nearly no overlap in their confidence intervals. The shortest time for killing cockroach nymphs was recorded when they were exposed to 2×10^7 conidia per cm^2 , although the mortality rates using this dose was not significantly different with 4×10^6 conidia per cm^2 on the seventh day. The other obtained LT_{50} s with their confidence intervals according to the treated surface using 2×10^7 , 4×10^6 , 2×10^6 and 4×10^5 conidia/ cm^2 were 1.4(1.1–1.7), 2.6(2.3–2.8), 2.9(2.6–3.2), 11.4(8.2–14.9) days after a week, respectively.

Bait inoculated with the most virulent isolate

Bait inoculated with fungus did not show high performance against the cockroach nymphs, even at the highest conidia dose when compared with untreated bait. Although mortality was recorded up to two weeks, the average mortality rate did not exceed to 20 percent, even at the highest dose. The LD_{50} and LD_{90} were not calculated, since the highest mortality rate was lower than 50 percent (Table 2).

The various concentration of the conidia oil-in-water formulation

The oil-in-water formulation of conidia with different proportions increased the mortality rates of the brown-banded cockroach nymphs compared with the aqueous formulation and the control group. Significant differences in mortality rates were obtained due to the treatments with formulated and non-formulated conidia or the control group. Mortality percentages of different oil formulations were significantly different from those of the aqueous suspension or the control group ($P_{\text{value}} = 0.003$, $P_{\text{value}} = 0.003$ and $P_{\text{value}} = 0.005$ and $P_{\text{value}} = 0.002$ for sesame, coconut, paraffin, and sunflower oils, respectively). There were no significant differences, however, between the mortality rates of three concentrations of each oil

($P_{\text{value}}= 0.151$, $P_{\text{value}}= 0.165$, $P_{\text{value}}= 0.123$ and $P_{\text{value}}= 0.012$ for sesame, coconut, paraffin, and sunflower oils respectively). Comparison of the same proportion of different oils didn't show any difference between the mortality rates of the 10 percent proportion of all oils, but the differences were significant in 5 ($P_{\text{value}}= 0.017$) and 2.5 ($P_{\text{value}}= 0.016$) percent concentrations. The highest level of mortality rate was obtained when cockroach nymphs were exposed to 10 percent sunflower oil concentration (97.2%), although there were no significant differences between proportions of 5% and 10% of sesame, sunflower, and paraffin oil concentrations. Coconut oil formulation performed the lowest mortality compare to the other oil formulations and the lowest mortality rate was observed in the aqueous suspension treatment (39.4%) (Table 4). The observed mortality in all conidia oil formulations and aqueous suspension treatments

were significantly different from the control group.

Field evaluation of the fungus

Twelve houses were surveyed for cockroach infestation during the preliminary inspection before treatment, and totally eight houses were selected because of their relatively severe cockroach infestation. The average number of cockroaches collected at each house ranged from 15.2–27 cockroaches per house prior to treatment. On the seventh day after treatment with the oil-in-water formulation of *M. anisopliae*, the cockroach density decreased to 4.6–7.5 cockroaches per house with average density reduction of 76.1 percent using the formula of Mulla et al. (1971). In the control houses, the average number of cockroaches was 24.5 cockroaches per house pre-treatment, and then it increased to 30.5 cockroaches per house after seven days (Table 5).

Table1. Virulence screening of *Metarhizium anisopliae* isolates on adult *Supella longipalpa* by immersing in conidia aqueous suspension (10^8 conidia ml^{-1})

<i>M. anisopliae</i> isolates	% Mortality	
	Mean± SE	
	After 3 days	After 5 days
IRAN 437C	82± 2.3	100± 0.0
IRAN 1018C	56±3.6	76±1.9
IRAN 715	39±1.5	67±4.2
<i>Rhynchophrus</i>	28±2.7	45±3.1

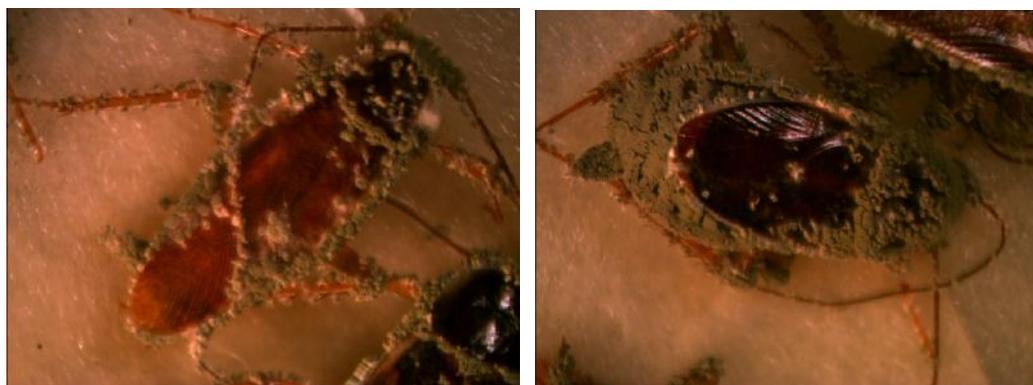


Fig. 1. The brown-banded cockroach with green muscardin (left: male, right: female)

Table 2. Cumulative mortality of *Supella longipalpa* nymphs exposed to different concentrations of *Metharhizium anisopliae* isolate Iran 437C presented as treated surface and treated bait (2011–2012)

<i>Treated surface</i> <i>Conidia/cm²</i>	% Mortality Mean ±SE			
	After 2 days	After 3 days	After 5 days	After 7 days
2×10 ⁷	65.3±4.6	88.9±3.9	97.8±0.27	97.8±0.27
4×10 ⁶	38.9±3.8	64.4±7.1	81.1±8.6	93.6±10.8
2×10 ⁶	28.2±3.1	59±3.7	72.5±2.6	83.1±3.2
4×10 ⁵	9.2±0.83	20.8±1.6	30.8±0.83	34.2±2.2
Control	0.0	0.0	0.0	3.3±2.9

<i>Inoculated bait</i> <i>Conidia g⁻¹</i>	% Mortality Mean ±SE	
	After 5 days	After 7 days
2.5×10 ⁸	3.7±2.4	18.7±2.4
5×10 ⁷	2.5±1.4	13.7±2.4
2.5×10 ⁷	0.0	5±2.04

Table 3. Probit analysis of *Metharhizium anisopliae* (IRAN 437C) against nymphs of *Supella longipalpa* presented as treated surface (conidia per cm²) (2011–2012)

Post exposure day	LD ₅₀ (95% CI)	LD ₉₀ (95% CI)	P _{value} (Probit Model)	P _{value} (X ²)
2	7.7×10 ⁶ (5.2×10 ⁶ –1.1×10 ⁷)	10 ⁸ (6.1×10 ⁷ –2.2×10 ⁸)	0.0001	0.179
3	2×10 ⁶ (8.5×10 ⁵ –3.2×10 ⁶)	1.7×10 ⁷ (10 ⁷ –3.3×10 ⁷)	0.0001	0.015
5	9.5×10 ⁵ (2.9×10 ⁵ –1.8×10 ⁶)	6.6×10 ⁶ (4.2×10 ⁶ –1.1×10 ⁷)	0.0001	0.072
7	5.6×10 ⁵ (5.3×10 ⁵ – 1.7×10 ⁶)	2.7×10 ⁶ (8.8×10 ⁵ –1.7×10 ⁷)	0.0001	0.001

CI: Confidence Intervals

Table 4. *Supella longipalpa* nymphs mortality rates exposed to different concentration of *Metharhizium anisopliae* strain Iran 437C formulations presented as treated surface at 2 days post exposure (10⁸ conidia/ ml⁻¹) (2011–2012)

Conidia-formulations	Concentration of oil (%)	Mortality Means (%)±SE (2 days post exposure)
Sesame oil	10	96.1±2.5
	5	94±2.2
	2.5	89.1±2.8
Coconut oil	10	85.2±3.3
	5	84±3
	2.5	75.5±3
Paraffin oil	10	94.4±2.7
	5	95.4±2.1
	2.5	91.8±3.4
Sunflower oil	10	97.2±1.7
	5	93.6±0.88
	2.5	86.1±4.3
Aqueous suspension	0.01	39.4±8.8
Control	-	2.5±1.6

Table 5. Field evaluation of *Metharhizium anisopliae* strain Iran 437C as oil-in-water formulation containing 10% sunflower oil applied against *Supella longipalpa* on seventh day after exposure (2011–2012)

	House	Pre-treatment	Post-treatment	Reduction ^c (%)
		Mean± SE ^a	Mean± SE ^b	
Treatment	1	20.7±3.2	7.5±0.87	71.2
	2	15.2±1.5	6±1.4	68.7
	3	25±1.4	4.7±2.1	85.1
	4	27±2.9	7±1.2	79.4
Control	1	26±2.2	34±3.1	-
	2	22±4.2	29±2.6	-
	3	23±2.5	27±5.2	-
	4	27±3	32±1.4	-

a and b: Mean number of cockroach per house pre-treatment and post-treatment

c: Average percent reduction of cockroach in each house.

Discussion

In the current study, *M. anisopliae* strain IRAN 437C was the most effective isolate causing 100% mortality rate in adult brown-banded cockroach at seven days after exposure using 10^8 conidia ml^{-1} in screening of the fungus isolate. The ability to produce the high performance of conidia and its high virulence were the reasons which this isolate was selected for the brown-banded cockroach control. This fungus isolate exhibited high virulence against *S. longipalpa* as conidia – dust formulation in dose ranges of 6.6×10^5 – 3.3×10^7 conidia per cm^2 which led to 45–97.5% mortality using treated surface bioassay method with survival time range of 3.4 to 6.7 days (Sharififard et al. 2014). Other studies have shown different efficiencies of *M. anisopliae* in the control of cockroaches. According to Gunner et al. (1991), the *M. anisopliae* strain PA-2 required 6 week to produce 90% mortality in the German cockroaches. Kaakeh et al. (1996) reported 26–30 d to achieve 90% or higher mortality in German cockroaches with the *M. anisopliae* strain ESC-1 using the contact method. Pachumathu et al. (1999) reported 4.18×10^8 spore ml^{-1} as LD_{50} of *M. anisopliae*, applying 1μ of the spore solution as a topical assay.

The average mortality of German cockroaches with *M. anisopliae* using a $4\mu\text{l}$ suspension of concentrations ranging from 4.2×10^6 to 4.2×10^9 spores ml^{-1} was determined to be 42.3 to 93.3% in the treated insects, whereas 13.6% mortality was observed in the untreated cockroaches. The LD_{50} value was recorded as 1.4×10^7 spore ml^{-1} (Quesada-Moraga et al. 2004).

We observed 97.8% and 93.6% mortality rates in the brown-banded cockroach nymphs exposed to a surface treated with 2×10^7 and 4×10^6 conidia cm^{-2} of *M. anisopliae* strain Iran 437C, respectively, at seven days post-exposure with LD_{50} and LD_{90} values of 5.6×10^5 and 2.7×10^6 conidia cm^{-2} respectively. Lopez and Alves (2011) reported 73.9% and 76.1% adult cockroach mortality rates in the German cockroach nymphs at nine and fifteen days after exposure to a surface inoculated with 6.5×10^6 conidia cm^{-2} of *M. anisopliae*. Adult cockroach mortality rates were 96.1% and 100% at the mentioned times and doses. They also reported 9.4% and 28% mortality in adults at nine and fifteen days after exposure to bait inoculated with *M. anisopliae* at 5×10^9 conidia g^{-1} .

The LT_{50} values of the brown-banded

cockroach nymphs were obtained 1.4 and 2.6 days using 2×10^7 and 4×10^6 conidia cm^{-2} in this study. Mean survival times of nymph and adult of *B. germanica* exposed to 6.5×10^6 conidia cm^{-2} on a treated surface were 6.5 and 5.6 days (Lopes and Alves, 2011). Quesada-Moraga et al. (2004) reported $\text{LT}_{50} = 5.3$ days for 4.2×10^9 spores ml^{-1} for the German cockroach by topical application of spore solution. The differences in results can be attributed to the cockroach species, virulent of the fungus isolate, the bioassay methods, conidia dose and even the exposure time. We stopped data recording on the seventh day, whereas Lopes and Alves (2011) continued their study to the fifteenth day.

It seems that the brown-banded cockroach is more susceptible to control agents than the German cockroach, so it takes a shorter time and lower doses of entomopathogenic fungi to kill them.

Our results showed that bait inoculated with conidia could not be a very effective method for the brown-banded cockroach control.

The successful development of a mycoinsecticide ultimately depends on the availability of a virulent strain, an optimized and economic production system, and a suitable formulation to optimize its application, efficacy, and storage characteristics as well as persistence after application (Kassa 2003). Oil-based formulations of fungal propagules are known to enable the infection of various insect pests at low humidity (Albernaz et al. 2009). Performance evaluation of oil-in-water formulations of *M. anisopliae* showed very promising results, because it increased cockroach mortality in shorter lethal time. Using oil formulation in arid areas (relative humidity less than 35 percent) enhances the efficacy of entomopathogenic fungi compared with aqueous formulation (Kassa 2003). Sesame oil was more effective in increasing the efficacy of *M. anisopliae* against the brown-

banded cockroach, though all tested oils were compared with the aqueous formulation.

Conclusion

In this study, we used a sunflower-oil formulation (proportion of 10%) for cockroach control in houses. Mulla's formula was used to assess the degree of cockroach infestation before and after treatment. The reduction in the number of cockroaches was significant (76.1% reduction on the seventh day post-treatment). Although there is a time lag between treatment and cockroach death compared with the rapid effect of chemical insecticides, this time lag can be reduced by combining entomopathogenic fungi with a sublethal dose of chemical insecticides (Sharififard et al. 2011a). It may also be acceptable to produce where insects have become too resistant to such chemicals, and this can lead to a reduction in the use of them. Other research on the *M. anisopliae* isolate IRAN 437C has shown high virulence against *M. domestica*, as a widespread vector of many human pathogens (Sharififard et al. 2011a). Additionally, this isolate was highly pathogenic in low humidity environments (Sharififard et al. 2012). Therefore, it can be developed as a mycoinsecticide for the safer control of houseflies and brown-banded cockroaches because of its high virulence, the effectiveness in conidial production, and the high pathogenicity in low ambient humidity.

Acknowledgements

The authors wish to acknowledge Ahvaz Jundishpur University of Medical Sciences, Ahvaz, Iran for financial support and the Iranian Plant Protection Institutes for providing the fungus isolates. The authors declare that there is no conflict of interests.

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