Original Article

Interactions between Entomopathogenic Fungus, *Metarhizium anisopliae* and Sublethal Doses of Spinosad for Control of House Fly, *Musca domestica*

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Abstract

Background: *Metarhizium anisopliae* strain IRAN 437C is one of the most virulent fungal isolates against house fly, *Musca domestica*. The objective of this study was to determine the interaction of this isolate with sublethal doses of spinosad against housefly.

Methods: In adult bioassay, conidia of entomopathogenic fungus were applied as inoculated bait at 10^5 and 10^7 spore per gram and spinosad at 0.5, 1 and 1.5 µg (A.I.) per gram bait. In larval bioassay, conidia were applied as combination of spore with larval bedding at 10^6 and 10^8 spore per gram and spinosad at sublethals of 0.002, 0.004 and 0.006 µg (AI) per gram medium.

Results: Adult mortality was 48% and 72% for fungus alone but ranged from 66–87% and 89–95% in combination treatments of 10^5 and 10^7 spore/g with sublethal doses of spinosad respectively. The interaction between 10^5 spore/g with sublethals exhibited synergistic effect, but in combination of 10^7 spore in spite of higher mortality, the interaction was additive. There was significant difference in LT_{50} among various treatments. LT_{50} values in all combination treatments were smaller than LT_{50} values in alone ones. Larval mortality was 36% and 69% for fungus alone but ranged from 58%–78% and 81%–100% in combination treatments of 10^6 and 10^8 spore/g medium with sublethals of spinosad respectively. The interaction was synergistic in all combination treatments of larvae.

Conclusion: The interaction between *M. anispliae* and spinosad indicated a synergetic effect that increased the house fly mortality as well as reduced the lethal time.

Keywords: *Metarhizium anisopliae*, *Musca domestica*, spinosad, Iran

Introduction

Housefly, *Musca domestica* L that is well known as poultry and livestock pest is also word-wide mechanical vector of human pathogens (Lecouna et al. 2005). High level of insecticide resistance in the housefly and public demands for reducing pesticide use around animal food have promoted interest in the development of other control strategies of this pest (Geden et al. 1995). An important strategy is integrated pest management (IPM) programs, which includes biological, cultural, and/ or chemical methods to control the population of this pest (Crespo et al. 1998, Lecouna et al. 2005). Although biological control of

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housefly is currently focused mostly on pupal parasitoids, entomopathogenic fungi are ubiquitous in nature and could be considered for manipulation in IPM programs (Stainkraus et al. 1990, Barson et al. 1994, 1995, Bywater et al. 1994, Watson et al. 1995, 1996, Renn et al. 1999, Lecouna et al. 2005, Kaufman et al. 2005). The entomopathogenic fungus, *Metarhizium anisopliae* (Metch) Sorok. has been isolated from 200 insect species including the orders of Lepidoptera, Coleoptera, Orthoptera, and Hemiptera. There are few reports on the use of this fungus for urban pest management (Pachamuthu and Kamble 2000). The major limitations in the use of entomopathogenic fungi such as *M*. *anisopliae* have been an extended time to cause sufficient insect mortality and its inconsistent performance under field conditions. One of the options for improving the efficacy of the entomopathogenic fungi is to incorporate the fungus pathogens with sublethal doses of insecticides (Pachamuthu and Kamble 2000). Data from in vivo compatibility studies have indicated that *M. anisopliae* and insecticides are compatible, and their combination can have synergistic, antagonistic, or additive effect (Pachamuthu and Kamble 2000, Zurek et al. 2002, Ericsson et al. 2007).

Spinosad is a novel macrolide-class insecticide produced by the soil bactrium *Saccharopolyspora spinosa* and is known to be active against many noxious pests. The mechanism of action of spinosad appears to be unique, with a primary site of attack being the nicotinic acetycholine receptor and a secondary site of attack possibly being GABA receptors (Scott 1998, Kristensen and Jepersen 2004). In contrast to other commonly used insecticides where the technical active ingredients are classified as moderately or high hazardous, spinosad is classified as a reduced-risk pesticide and has been determined to pose little to no mammalian toxicity (White et al. 2007). There have been no reports of resistance or cross-resistance in field population housefly (Scott 1998, Liu and Yue 2000, Kristensen and Jepersen 2004, White et al. 2007).

The research objectives were to enhance the lethal effect of *M. anisopliae* strain IRAN 437C by using it in combination with different sublethal doses of spinosad against house fly, *M. domestica*. The aim was to determine which kind of interaction (synergistic, antagonistic, and additive) occurs between *M. anisopliae* and spinosad and to ascertain the LT_{50} in control of larvae and adult.

Materials and Methods

Musca domestica culture

Adult house flies were collected from a poultry house by sweeping net and transferred to the laboratory where they were reared at 26° C, 50±5% Rh and photoperiod of 14:10 (L: D). Adults were maintained in cages (40×40× 40 cm^3) covered by gauze. Water and food in the form of sugar and powdered milk were provided and replenished every 24–48h. Larval medium comprised 55 g wheat bran, 3g date extract and 2g dried alfalfa suspended in 140 ml water. One cup (250ml volume) of this medium was left in each cage for adult oviposition and subsequent development of larvae. The food was replaced every 24–48h.

Fungus

Ten Iranian isolates of *Beauveria bassiana* (Bals) Vuill. and *Metarhizium anisopliae* (Metch) Sorok. were obtained as cultures from the Ministry of Jihad Keshavarzy of Iran. Previous study indicated that *M. anisopliae* strain IRAN 437° C was the most virulent against house fly, *M. domestica* that caused higher mortality in the shorter time than the others (Sharififard et al. 2011), so this isolate was selected for current study. It was cultured on sabouraud dextrose agar with yeast extract (SDAY) for 2 weeks at 27°C, 75±5% Rh and photoperiod of 12:12 (L:D). Sporulating cultures were harvested by scraping the dry conidia from the surface of the culture plate with a scalpel and transferring them to sterile distilled water containing 0.01% Tween–80. The concentration of the suspension was determined using a hemocytometer.

Adult Bioassay

Spinosad concentrations that caused zero mortality after 48 h in the adult house fly were selected as sublethal based on conducting several pretests. There were 0, 0.5, 1 and 1.5 µg (AI) per gram bait. Selected *M. anisopliae* concentrations were 0, 10^5 and 10^7 conidia per gram bait. Adult bait containing sugar, powdered milk and distilled water were prepared and treated with different combinations of spinosad and conidial concentrations. Cohorts of twenty-five 2-3 day old house flies were housed in small cage (20×20×20 cm^3). Each cage contained a 9 cm diameter Petri dish lined with Watman filter paper and 10 g treated bait. Adults in the control groups were feed with untreated bait. Each treatment was replicated 5 times. Cages were maintained in room conditions and checked daily over a period of 9 days for mortality recording.

Larval Bioassay

Concentrations of spinosad that produced less than 30% mortality of the larval housefly larvae were determined using several pretests and classified as sublethal doses. There were 0, 0.002, 0.004, and 0.006 µg (AI) per gram larval bedding. In another treatment, we have also determined that 10^6 and 10^8 conidia/g larval bedding as sub lethal concentrations of *M. anisopliae* strain IRAN 437C in the control of house fly larvae. Plastic 150-ml containers were filled with 50 g larval bedding, containing of wheat bran, dry alfalfa, Date extract and water. The stock suspension of fungi was adjusted to a concentration of 0, 5×10^7 and 5×10^9 conidia/ml with an improved hemocytometer. One milliliter of each stock fungi suspension was added to each larval container to raise the larval bedding concentration to 0, 10^6 and 10^8 conidia/g bedding. Both spinosad and *M. anisopliae* treatments were mixed into the larval bedding with a glass rod. In total, the treatments evaluated in this bioassay included 12 different combinations of the insecticide and fungi concentrations. Twenty larvae were used per treatment and each treatment was replicated 4 times. Larva in the control groups were treated with distilled water. However, mortality was observed daily for all treatments and the dead larva were removed.

Statistical Analysis

Data from this study were analyzed by factorial analysis of variance (ANOVA) by using two factor complete randomized design of MSTATC software. Percentage mean of mortality were compared using Duncan's multiple range test at α= 0.05. Significant differences among the combination treatments by factorial analysis indicated that there was an interaction between *M. anisopliae* and insecticide and the effect observed might be synergistic or antagonistic. In contrast, if there was no significant difference in *M. anisopliae* plus insecticide treatment, it implied that the effects were additive (Pachamuthu et al. 2000). Chi-squared tests were performed to determine the type of interaction (additive, synergistic or antagonistic). Expected mortality (E) was generated from the following formula: $E= O_{spin} + O_{Met}\,(1– O_{spin})$, where E is the expected mortality, and O_{Spin} and O_{Met} represent the proportion mortality due to treatments of pure spinosad and pure *M. anisopliae*, respectively. The predicted effects of spinosad and M. anisopliae treatments (E) were compared with the observed mortality of the binary treatments (O) with following formula, $\chi^2 = \{(O-E)^2\}/E$ (Ericsson et al. 2007). If the calculated chi-squared value exceeds the tabular value, then it indicates either synergistic or antagonistic interaction. In contrast, if the tabular value exceeds calculated chi- square value, then it indicates an additive effect. LT_{50} values and 95% confidence limits of each value for different treatments were calculated by using probit method of SAS software. When there was no overlap in the 95% CL of lethal time values, the treatments difference were considered significant.

Results

Adult Bioassay

These sub lethal concentrations of spinosad were classified as 0, 0.5, 1 and 1.5 µg (AI) per gram bait. The results of analyze variance showed that adult mortality was significantly affected by insecticide concentration (F= 90.7, df= 3, P< 0.0001), conidial concentration (F= 623.86, df= 2, P< 0.001) and interaction of insecticide and fungi (F=3.19, df= 6, P< 0.011). Higher mortality was observed in *M. anisopliae* plus spinosad combination treatments than sole treatment of fungi or insecticide (Table 1). Mixing of 10^5 conidia/g with 0.5, 1 and 1.5 μg (AI)/g of spinosad caused higher mortality of adult housefly than alone treatments. Estimation of Chi-squared showed synergistic interaction in combination of 10^5 conidia/g combined with 1 and 1.5 µg (AI)/g. In the combination treatments of 10^7 conidia/g with sublethals of spinosad, there was no significant interaction between insecticide and *M. anisopliae*. The increased mortality was the result of an additive effect (Table 2). Based on individual treatment levels, the greatest synergistic effect occurred when 10^5 conidia/g bait were used with 1.5 µg (AI)/g.

Calculated LT_{50} values and 95% confidence limits of each value for different treatments in adult bioassay showed that the LT_{50} values were lower in all combination treatments of *M. anisopliae*+spinosad in comparison with *M. anisopliae* alone (Table 3). Combination treatments caused faster mortality than the alone ones. When there was no overlap in the 95% CL of lethal time values, the treatments difference were considered significant. While the interaction was additive in the combination of 10^7 conidia/g with sublethals of spinosad, but there was significant difference in LT_{50} values between *M. anisopliae* (10^7) and *M. anisopliae* (10^7) plus spinosad (0.5, 1, 1.5 µg). The shortest lethal time for causing 50% mortality in adult population was observed in 10^7 conidia of *M.anisopliae* +1.5 µg of spinosad. There was no significant difference in LT_{50} values of *M. anisopliae* (10^7) + spinosad (0.5 and 1 µg).

There was a significant difference in LT_{50} between *M. anisopliae* (10^5) + spinosad (0.5, 1 and 1.5µl) and *M. anisopliae* (10^5) alone, but there was no difference in the LT_{50} values among 10^5 conidia of *M. anisopliae* +1 and 1.5 μg of spinosad, also between *M. anisopliae* (10^5)+ spinosad (1.5) and *M. anisopliae* (10^7)+ spinosad(1.5). Therefore, due to the greatest synergistic effect occurred when 10^5 conidia were used with 1.5 µg (AI) of spinosad and no significantly difference in LT_{50} value of this treatments with *M. anisopliae* (10^7) + spinosad (1.5), mentioned combination of *M. anisopliae* and spinosad was the best combination for control of adult housefly.

Larval Bioassay

The results of analyze variance showed that larval mortality was significantly affected by insecticide concentration (F= 149.84, df= 3, P< 0.0001), conidial concentration (F= 895.83, df= 2, P< 0.001) and interaction of insecticide and fungi (F= 12.78, df= 6, P= 0.025). The percent of mortality of medium size larvae was significantly difference among all 11 treatments (Table 4). The greatest mortality was recorded in the combination treatments of 10^8 spores of *M. anisopliae* plus sublethals of spinosad. A synergistic interaction between *M. anisopliae* and spinosad was always found when the fungus was applied at a dosage of 10^6 and 10^8 conidia/g larval bedding in combination with 0.002, 0.004 and 0.006 µg (AI)/g of spinosad.

But in the combination of 10^6 spores of *M. anisopliae*+sublethals of spinosad chi-squared values were greater than 10^8 spores with same sublethal of spinosad (Table 5). The greatest synergetic effect observed when 10^6 conidia of *M. anisopliae* were combined with 0.006 µg (AI), so this was the best combination of *M. anisopliae* with spinosad for larval control.

Table 1. Toxicity of spinosad (µg (AI)/g) and *M. anisopliae* (Conidia/g) alone and in combination treatments on adult house fly after 9 days

Treatment[a]	n	%Mortality(±SE)[b]
***M. anisopliae*(10^5)**	150	44±4.20G
***M. anisopliae* (10^7)**	150	72.4±1.79E
Spinosad (0.5)	150	21±1.24J
Spinosad(1)	150	32±1.7I
Spinosad (1.5)	150	39±1.7H
10^5+0.5	150	66.4±2.68F
10^5+1	150	80.6±3.13D
10^5+1.5	150	87±1.22C
10^7+0.5	150	89±4.02BC
10^7+1	150	90.4±1.79B
10^7+1.5	150	95±3.3A

[a] Each treatment (containing 30 adults) were replicated 5 times.
[b] Means followed by the same letters were not significantly different (Duncan's test;α= 0.05).

Table 2. Synergy bioassay: adult house fly mortality from Combination Traetments of Spinosad and *M. anisopliae* after 9 days

Treatment		%Mortality				
Fungi (Conidia/g)	**Spinosad µg (AI)/g**	**Fungi**	**Spinosad**	**Expected**	**Observed**	χ^2 *
10^5	0.5	44	21	56	66	1.79
10^5	1	44	32	62	81	5.82*
10^5	1.5	44	39	66	87	6.68*
10^7	0.5	72	21	78	89	1.55
10^7	1	72	32	81	90	1.00
10^7	1.5	72	39	83	95	1.73

*A chi-square comparison that exceeds 3.84 with df= 1 and α= 0.05 is considered synergistic and is denoted by an asterisk (*).

Table 3. Calculated LT_{50} values for *M. anisopliae* (conidia/ g) and its combination with sublethal doses of spinosad (µg (AI)/g) bait

Treatment[a]	n	Slope±SE	LT_{50}[b]	95%CL[c]	χ^2 (df)
***M. anisopliae* (10^7)**	150	6.9 ± 0.58	6.4	6.12 – 6.67	4.85(2)
***M. anisopliae* 10^7+Spinosad 1.5**	150	4.08 ± 0.33	2.6	2.36 – 2.83	4.91(2)
***M. anisopliae* 10^7+Spinosad 1**	150	3.39 ± 0.31	3.7	3.34 – 4.02	0.96(2)
***M. anisopliae* 10^7+Spinosad 0.5**	150	3.81 ± .032	4.1	3.69 – 4.35	1.05(2)
***M. anisopliae* (10^5)**	150	6.58 ± 1.77	8.08	7.69 – 8.73	8.42(2)
***M. anisopliae* 10^5+Spinosad 1.5**	150	4.37 ± 0.78	3.1	2.71 – 3.55	10.05(2)
***M. anisopliae* 10^5+Spinosad 1**	150	2.71 ± 0.31	3.9	1.84 – 5.96	0.33(2)
***M. anisopliae* 10^5+Spinosad 0.5**	150	2.74 ± 0.27	4.9	4.47 – 5.44	0.94(2)
Spinosad 1.5	150	1.74 ± 0.28	12.4	9.59 – 19.6	1.01(2)
Spinosad 1	150	2.01 ± 0.32	14.1	10.79 – 22.8	1.03(2)
Spinosad 0.5	150	1.74 ± 0.35	21.2	13.98 – 53.17	1.53(2)

[a]Each treatment (containing 30 adults) were replicated 5 times.
[b] Number of days until 50% mortality occured after different treatments.
[c] Treatments will have significant effect on LT_{50} if there was no overlap of 95% CL.

Table 4. Toxicity of spinosad (µg (AI)/g) and *M. anisopliae* (Conidia/g) alone and in Combination Traetments against house fly larvae

Treatment[a]	n	%Mortality(±SE)[b]
M. anisopliae **(10^6)**	100	36±1.93 I
M. anisopliae **10^6 +Spinosad 0.002**	100	58±2.58 G
M. anisopliae **10^6 +Spinosad 0.004**	100	65± 3.42 F
M. anisopliae **10^6 +Spinosad 0.006**	100	78±2.5 D
M. anisopliae **(10^8)**	100	69±1.91 E
M. anisopliae **10^8+Spinosad 0.002**	100	81±2.52 C
M. anisopliae **10^8 +Spinosad 0.006**	100	95± 1.91 B
M. anisopliae **10^8+Spinosad 0.006**	100	100±0.00 A
Spinosad 0.002	100	14± 2.58 K
Spinosad 0.004	100	23±1.91 J
Spinosad 0.006	100	41±1.91 H

[a] Each treatment (containing 25 larvae) were replicated 4 times.
[b] Means followed by the same letters were not significantly different (Duncan's test; α= 0.05).

Table 5. Synergy bioassay: larval mortality from combined treatments of Spinosad and *M. anisopliae* after 9 day

Treatment		**%Mortality**				
Fungi (Conidia/g)	**Spinosad µg (AI)/g**	**Fungi**	**Spinosad**	**Expected**	**Observed**	χ^2
10^6	0.002	35	14	44	58	4.38*
10^6	0.004	35	23	50	65	9.90*
10^6	0.006	35	41	62	78	15.75*
10^8	0.002	69	14	65	81	3.94*
10^8	0.004	69	23	76	96	5.19*
10^8	0.006	69	41	82	100	4.09*

*A chi-square comparison that exceeds 3.84 with df= 1 and α= 0.05 is considered synergistic and is denoted by an asterisk (*).

Discussion

Because conidia require at least 12–24 h for development of germ tube, appressoria and penetration to insect cuticle, so the doses of spinosad that caused <40% mortality 48h after exposure in the adults were selected as sublethals. These doses would allow sufficient time for conidia to form the germ tube and appressoria. High mortality by insecticide during this period affects the effectiveness of fungus. In our study, the *M. anisopliae* strain IRAN 437C was effective and caused 44% and 72% mortality in adult population at the concentrations of 10^5 and 10^7 spores per gram bait in 9 days after exposure. Synergistic interaction was observed in combination treatments of 10^5 spore with sublethal doses of insecticide but in combination of 10^7 spore the interaction was additive. Lethal time in all combination treatments were reduced in comparison with alone treatments of fungi. Thus, increased mortality and lowered LT_{50} values were a general pattern observed in most of Insecticide + *M. anisopliae* combinations against house fly in our study.

In larval test, this fungal strain caused 35 and 69% mortality at 10^6 and 10^8 spores per gram bedding in larval population in the end of larval cycle. When spinosad and *M. anisopliae* were applied together as a mixture, larval mortality was significantly higher than the expected value of their additive effect, which indicated a synergistic interaction in all treatments. Lower dosages of spinosad not only enhanced the efficacy of *M. anisopliae*, but also lead to a reduced quantity of inoculum needed to cause high levels of mortality in house fly adult and larvae. The time to mortality of larvae could not be accurately assessed as a proportion of infected larvae subsequently died in the pupal stage. Moreover, it was not considered in larval bioassay because the eventually aim of larval control is decreasing of adult population and lethal period of larvae is not too important.

Earlier studies by Barson et al. (1994), Renn et al. (1999) also demonstrated the effectiveness of *M.anisopliae* in controlling house fly. In spite of effectiveness of entomopathogenic fungi against house fly, different strains require different times to achieve high mortality. With due attention to high reproduction rate and short life cycle of *M. domestica*, it is necessary to find approach for increasing pest mortality as well as reducing the lethal time by biopesticide agents. So, in this study, we evaluated the effect of combined applications of *M. anisopliae* and spinosad against *M. domestica* under laboratory conditions. Several studies have focused on the potential use of entomopathogenic fungi in combination with sublethal doses of organic insecticides against various insect pests such as compatibility of *M. anisopliae* with sublethals of chlorpyrifos, propetamphos and cyfluthrin against the German cockroach (Pachamuthu et al. 2000), *M. anisopliae* with Boric Acid against German cockroach (Zurek et al. 2002), combination of Imidiaclopride and Diatomaceous Earth with *Beauveria bassiana* on mole cricket (Thompson et al. 2006), sublethals of spinosad with *M. anisopliae* against exotic wireworms (Ericsson et al. 2007) and *M. anisopliae* in combination with sublethal doses of imidiacloprid on the subterranean burrower bug *Cyrtomenus bergi* (Jaramillo et al. 2005). Sublethal dosage of synthetic insecticides can act as physiological stressors and/ or behavioral modifiers, thereby predisposing insects to diseases (Inglis et al. 2001).

Integrating insecticides and entomopathogens has a few advantages: 1) such approach will increase pest mortality as well as reduce the lethal time, 2) prolong the use of a particular insecticide by reducing the total amount of insecticide using, 3) minimizing environmental contamination and increasing human safety, 4) it accelerates the mode of action of fungus without compromising the fungus growth from cadavers that is crucial for inducing epizootic in house fly population particularly in larval bedding that humidity and temperature of bed supported the growth of muscardine on larval cadavers.

In conclusion, our results indicated that the use of combination of *M. anisopliae* with lower dosage of spinosad might become an important component of *M. domestica* IPM but at first, this approach must be testing under field conditions.

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References

Barson G, Renn N, Bywater AF (1994) Laboratory evaluation of six species of entomopathogenic fungi for control of House fly *Musca domestica* L, a pest of intensive animal units. J Inverter Pathol. 64: 107–113.

Bywater AF, Barson G, Renn N (1994) The potential of oil-based suspension of *Metarhizium anisopliae* conidia for the control of the housefly (*Musca domes-*

tica), a pest of intensive animal units. In: Proc. Brighton Crop Protection Conf. The British Crop Protection Council, Farnham, UK, 3: 1097–1102.

Crespo DC, Lecuona RE, Hogsette JA (1998) An important component in integrated management of *Musca domestica* (Diptera: Muscidae) in caged-layer poultry houses in Buenos Aires, Argentina. J Bio Control. 13: 16–24.

Deacutis JM, Leichter CA, Gerry, AC, Rutz DA, Watson WD, Geden C, Scott JG (2006) Susceptibility of field collected houseflies to Spinosad before and after a season of use. J Agricul Urb Entomol. 23(2): 105–110.

Ericsson JD, kabaluk JT, Goettel MS, Myers JH (2007) Spinosad interacts synergistically with the insect pathogen *Metarhizium anisopliae* against the exitic wireworm *Agriotes lineatus* and *Agriotes obscurus* (Coleoptera: Elatridae). J Econ Entomol. 100(1): 31–38.

Geden CG, Steinkraus DC, Rutz DA (1993) Evaluation of two methods for release of *Entomophthora muscae* (Entomophthorales: Entomophthoraceae) to infect house flies (Diptera: Muscidae) on dairy farms. J Environ Entomol. 22(5): 1201–1208.

Geden CG, Rutz DA, Steinkraus DC (1995) Virulence of different isolates and formulation of *Beauveria bassiana* for house flies and the parasitoid *Muscidofurax raptor*. J Bio Control. 5: 615–621.

Jaramillo J, Borgemeister C, Ebssa L, Gaigl A, Tobon R, Zimmermann G (2005) Effect of combined application of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) strain CIAT 224 and different dosages of imidiacloprid on the subterranean burrower bug *Cyrtomenus bergi* Froeschner (Hemiptera: Cydnidae). J Bio Conrol. 34: 12–20.

Kaufman PE, Reasor C, Rutz DA, Ketzis JK, Arends J (2005) Evaluation of *Beauveria bassiana* applications against adult house fly, *Musca domestica*, in commercial caged-layer poultry facilities in New York State. J Bio Control. 33: 360–367.

Kristensen M, Jespersen JB (2004) Susceptibility of Spinosad in *Musca domestica* L. field population. J Econ Entomol. 97(3): 42–1048.

Lecouna RE, Turica M, Tarocco F, Crespo DC (2005) Microbial control of *Musca domestica* (Diptera: Muscidae) with selected strains of *Beauveria bassiana*. J Med Entomol. 42(3): 332–336.

Pachamuthu P, Kamble ST (2000) In vivo study on combined toxicity of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) strain ESC-1 sublethal doses of chlorpyrifos, propetamphos, and cyfluthrin against German cockroach (Dictyoptera: Blattellidae). J Econ Entomol. 93(1): 60–70.

Renn N, Bywater AF, Barson F (1999) A bait formulation with *Metarhizium anisopliae* for the control of *Musca domestica* (Diptera: Muscidae) assessed in large scale laboratory enclosures. J Appl Entomol. 123: 309–314.

Scott JG (1998) Toxicity of Spinosad to susceptible and resistant strains of house fly, *Musca domestica*. Pestic Sci. 54**:** 131–133.

Sharififard M, Mossadegh MS, Vazirianzadhe B, Zarei Mahmoudabadi A (2011) Laboratory pathogenicity of Entomopathogenic Fungi, *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisoplae* (Metch.) Sorok. to larvae and adult of house fly, *Musca domestica* L. (Diptera: Muscidae). Asian J Bio Sci. 4(2): 128–137.

Shono T, Scott JG (2003) Spinosad resistance in the housefly, *Musca domestica*, is due to a recessive factor on outosome 1. Pestic Biochem Physiol. 75:1–7.

Steinkraus DC, Geden CJ, Rutz DA, Kramer JP (1990) First report of the natural occurrence of *Beauveria bassiana* (Moniliales: Moniliaceae) in *Musca domestica* (Diptera: Muscidae). J Med Entomol. 27: 309–312.

Thompson SR, Brandebburg RL (2006). Effect of combining Imidacloprid and Diatomaceous Earth with *Beauveria bassiana* on mole cricket (Orthoptera: Gryllotapiade) mortality. J Econ Entomol. 99(6): 1948–1954.

Watson, DW, Geden CG, Long SJ, Rutz DA (1995) Efficacy of *Beauveria bassiana* for controlling the house fly and stable fly (Diptera: Muscidae). J Bio Control. 5: 405–411.

Watson DW, Rutz DA, Long SJ (1996) *Beauveria bassiana* and sawdust bedding for the management of the house fly, *Musca domestica* (Diptera: Muscidae) in calf hutches. J Bio Control. 7: 221–227.

White WH, McCoy CM, Meyer J, Winkle, JR (2007) Knockdown and mortality comparisons among Spinosad-Imidacloprid and Methomyl-containing baits against susceptible *Musca domestica* L. under laboratory conditions. J Econ Entomol. 100(1): 155–163.

Zurek L, Watson DW, Schal C (2002) Synergism between *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) and boric acid against the German cockroach (Dictyoptera: Blattellidae). J Bio Control. 23: 296–302.