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Original article (Orijinal araştırma)

Lethal and sublethal effects of mixtures of entomopathogenic fungi and synthetic insecticides on biological aspects of *Musca domestica* L.

Entomopatojen fungus ve sentetik insektisit karışımlarının *Musca domestica* L.'nın biyolojik dönemleri üzerinde lethal ve sublethal etkileri

Muzammil FAROOQ[1] **Shoaib FREED[1]***

Summary

The current study was performed to assess the effectiveness of the entomopathogenic fungi *Metarhizium anisopliae* var. *anisopliae* (Metschnikof) Sorokin and *Isaria fumosorosea* (Wize) Brown&Smith applied in combination with some synthetic insecticides against the house fly, *Musca domestica* L. (Diptera: Muscidae) at Department of Entomology, Bahauddin Zakariya University, Multan, Pakistan during 2015. An extensive, typically dose-dependent range of responses was shown by flies towards fungus-synthetic insecticide combinations using a bait method. The insecticides acetamiprid, emamectin benzoate, imidacloprid and lufenuron in combination with insect pathogenic fungi showed higher mortality than expected with significant synergistic interactions. In addition, the mixtures were also assessed for sublethal effects on biological parameters of *M. domestica*, namely adult longevity, fecundity, egg hatching, larval duration, percent pupation, pupal weight, pupal duration, adult emergence and sex ratio. The results showed a considerable impact of fungi and synthetic insecticide mixtures on all biological parameters of *M. domestica* ($P<0.05$) except adult emergence. A noteworthy reduction in adult longevity, fecundity, egg hatching, percent pupation and pupal weight was observed, while larval and pupal durations were prolonged. The results highlight the potential of combined use of entomopathogenic fungi and synthetic insecticides for the control of *M. domestica*. Moreover, the combination of entomopathogenic fungi and synthetic insecticides can reduce the concentrations of the active ingredient required to achieve the same result as when these are applied separately. However, field trials are required to validate the effects of entomopathogenic fungi and synthetic insecticides mixtures before they can be recommended as elements of an integrated pest management program for *M. domestica*.

Keywords: Biological parameters*, Isaria fumosorosea, Metarhizium anisopliae* var. *anisopliae*, *Musca domestica*

Özet

Bu çalışma, *Metarhizium anisopliae* var. *anisopliae* (Metschnikof) Sorokin ve *Isaria fumosorosea* (Wize) Brown&Smith entomopatojen funguslarının bazı sentetik insektisitler ile birlikte kullanımıyla *Musca domestica* L. (Diptera: Muscidae)'ya karşı etkinliğini değerlendirmek amacı ile 2015 yılında Bahauddin Zakariya Üniversitesi, department of Entomoloji Bölümü (Multan, Pakistan)'nde yapılmıştır. Kapsamlı, tipik bir doza bağlı tepki aralığı, sineklerde fungus ve sentetik insektisit kombinasyonlarına karşı bir tuzak yem metodu kullanılarak gösterilmiştir. Acetamiprid, emamectin benzoate, imidacloprid ve lufenuron gibi insektisitlerin böcek patojeni funguslar ile birlikte kombinasyonu, önemli düzeyde sinerjistik etkileşimlerle beklenenden daha yüksek ölüm göstermiştir. Ayrıca, karışımların *Musca domestica*'nın, ömür, doğurganlık, yumurta açılımı, larva dönemleri süresi, pupa oluşturma yüzdesi, pupa ağırlığı, pupa süresi, ergin çıkışı ve eşey oranı gibi biyolojik parametreleri üzerinde subletal etkileri de değerlendirilmiştir. Sonuçlar, ergin çıkışı dışında *M. domestica* tüm biyolojik parametreleri üzerinde fungus ve sentetik insektisit karışımlarının önemli bir etkisi ($P <0.05$) olduğunu göstermiştir. Ömür, doğurganlık, yumurtadan açılımı, pupa oluşturma yüzdesi ve pupa ağırlığında dikkate değer bir azalma gözlenirken, larva ve pupa dönemlerinin süreleri uzamıştır. Sonuçlar *M. domestica* kontrolü için entomopatojen fungus ve sentetik insektisitlerin kombine kullanımının potansiyelini vurgulamaktadır. Bundan başka, entomopatojen fungus ve sentetik insektisit kombinasyonu, aynı sonuca ulaşmak için gerekli olan aktif bileşen konsantrasyonları ayrı olarak uygulandığına göre daha azaltılabilir. Ancak, *M. domestica* için entegre zararlı yönetimi programının bir bileşeni olarak tavsiye edilmeden önce, entomopatojen fungus ve sentetik insektisit karışımlarının etkilerini doğrulamak için arazi denemeleri de yapılmalıdır.

Anahtar sözcükler: Biyolojik parametreler, *Isaria fumosorosea, Metarhizium anisopliae* var. *anisopliae*, *Musca domestica*

[1] Department of Entomology, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan
* Corresponding author (Sorumlu yazar) e-mail: sfareed@bzu.edu.pk
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Introduction

The house fly, *Musca domestica* L. (Diptera: Muscidae), is one of the most important insect pests of dairy and poultry farms and due to its pestiferous nature, and it is responsible for transmitting pathogens causing serious diseases such as cholera, diarrhea, dysentery, gastroenteritis and peptic ulcer in both man and animals (Li & Sutzenberger, 2000; Khan et al., 2012). Being a potential danger to human and livestock health, the control of *M. domestica* is essential (Mansour et al., 2011). The chief method used worldwide for the control of *M. domestica* is the utilization of insecticides belonging to various groups i.e., carbamates, organophosphates, pyrethroids and new chemicals (Cao et al., 2006; Shi et al., 2011). Unfortunately, the extensive use of insecticides has caused serious problems such as resistance in target insects and residual effects in the atmosphere (Phillip et al., 2001; Akiner & Caglar, 2006). In Pakistan, *M. domestica* resistance to organophosphates, pyrethroids and new chemistry insecticides has been documented in earlier studies (Khan et al., 2013; Abbas et al., 2015), making it necessary to seek alternative measures for controlling *M. domestica*. Biological control may be a promising and environment friendly alternative for *M. domestica* management (Mishra et al., 2011). *Metarhizium anisopliae* (Metschnikof) Sorokin and *Isaria fumosorosea* (Wize) Brown & Smith have been reported to have high infection rates in insect populations (Kaufman et al., 2005; Sharififard et al., 2011). Compared to many synthetic insecticides, the speed of kill associated with many entomopathogenic fungi is slow, thus often limiting their use. However, the combined use of entomopathogenic fungi with synthetic insecticides may have additive effects (Serebrov et al., 2005; Purwar & Sachan, 2006; Ericsson et al., 2007; Kassab et al., 2014) and as such, promote the application of insecticide at lower doses resulting in reduced environmental pollution and decreased risk of resistance development.

A study was undertaken to determine the effect of different concentrations of two isolates of insect pathogenic fungi, *M. anisopliae* var*. anisopliae* and one isolate of *I. fumosorosea* alone and in combination with corresponding doses of six synthetic insecticides against *M. domestica*. In addition, the sublethal effects of each fungus-insecticide combination were investigated on different biological parameters of *M. domestica*.

Materials and Methods

Musca domestica **rearing**

Adults of *M. domestica* were reared in the Laboratory of Insect Microbiology and Biotechnology, Department of Entomology, Bahauddin Zakariya University, Multan, Pakistan during 2015. The flies were kept in a rearing cage of 30×30×30 cm with mesh screen and cloth sleeve at the front. The rearing conditions were maintained at 25±2°C, relative humidity of 65±2% and 12L:12D h photoperiod. Sugar and powdered milk in a ratio of 3:1 was provided as food for the adult flies. Medium containing wheat bran, rice meal, yeast, sugar and dry milk powder in a ratio of 40:10:3:3:1 by weight as water based paste (Bell et al., 2010) was also provided in cages as an egg laying substrate and food for hatched larvae. The diet was changed after 2-4 d depending upon the number of larvae.

Entomopathogenic fungi culture and synthetic insecticides

Two isolates of *M. anisopliae* var. *anisopliae* and one isolate *I. fumosorosea* already available in Laboratory of Insect Microbiology and Biotechnology, Department of Entomology, Bahauddin Zakariya University, Multan, Pakistan (Table 1) were selected based on maximum mortalities caused in the preliminary experimentation. Fungal spores used as the infectious propagules were cultured on PDA (potato dextrose agar media) (Freed et al., 2011a, b). Briefly, the fungi were inoculated on PDA and cultured at 25°C for 14 d. The conidia were then scraped from the plates and mixed with a sterile solution of Tween-80 (0.05%). Conidial concentrations were estimated by using a hemocytometer. LC_{10}, LC_{30} and LC_{50} for each fungal isolate were measured separately (data unpublished).

Six synthetic insecticides (Table 1) were included for toxicity bioassay. Preliminary experiments determined lethal concentrations (LC_{10}, LC_{30} and LC_{50}) for each chemical insecticide separately (Table 1).

Table 1. Concentrations of entomopathogenic fungi and synthetic insecticides assessed against *Musca domestica* in this study

Fungal Species	Source	LC_{10} (spores ml^{-1})	LC_{30} (spores ml^{-1})	LC_{50} (spores ml^{-1})
Metarhizium anisopliae var.	cotton field (Multan)	8.78×10^{6}	1.64×10^{7}	3.38×10^{7}
M. anisopliae var. *anisopliae*	maize field (Mansehra)	9.21×10^{6}	4.56×10^{6}	1.30×10^{6}
Isaria fumosorosea (If-03)	cotton field (Multan)	9.70×10^{7}	1.20×10^{8}	1.41×10^{8}
Insecticides	Manufacturer	$LC_{10\ (ppm)}$	$LC_{30\ (ppm)}$	$LC_{50\ (ppm)}$
Acetamiprid	Arysta LifeScience	0.03	0.14	0.39
Bifenthrin	FMC United	0.02	0.08	0.22
Emamectin benzoate	Syngenta	0.00002	0.0002	0.001
Fipronil	Bayer CropScience	0.00003	0.0004	0.002
Imidacloprid	Bayer CropScience	0.022	0.09	0.27
Lufenuron	Syngenta	0.00002	0.0002	0.001

Lethal effects of entomopathogenic fungi and synthetic insecticides combinations

Suspensions of fungi and synthetic insecticides were mixed in corresponding concentrations (LC_{10}, LC_{30} and LC_{50}) and incorporated into the adult diet, and an insecticide- and fungus-free diet was provided in control treatments.

For each fungus-insecticide combination, there were three treatment doses, LC_{10} of fungus+ LC_{10} of insecticide (low dose), LC_{30} of fungus+ LC_{30} of insecticide (intermediate dose) and LC_{50} of fungus+ LC_{50} of insecticide (high dose). Each treatment combination was replicated four times with ten adults (2-3 day old, male to female ratio 50:50) per replication placed in plastic jars (15×6×6 cm).

Musca domestica mortality was recorded every 24 h for 7 d. For effective comparison, following the same procedure as described above, the LC_{10}, LC_{30} and LC_{50} of fungi and LC_{10}, LC_{30} and LC_{50} of synthetic insecticides were applied individually.

Sublethal effects

In addition to mortality caused by the fungus-insecticide combinations, the sublethal effects of these mixtures were also assessed on different biological parameters of the surviving *M. domestica* population, namely adult longevity, fecundity, egg hatching, larval duration, percent pupation, pupal weight, pupal duration, adult emergence and sex ratio. The adult flies were provided with egg laying medium as described above, while, male and female longevity was recorded separately as described by Fletcher et al. (1990). The egg laying substrate was examined daily for eggs count and changed every 2 d. If eggs were present, they were counted and left in same medium for hatching and larval development. Fecundity was calculated by dividing the total number of eggs laid to the total number of surviving females over the entire experiment. Following egg hatching, the larvae were counted and percent hatching was calculated. The larvae were provided with food and kept until pupation to estimate the duration of the larval period. Once pupation had occurred, the pupae were separated from the medium, cleaned, counted and weighed to calculate percent pupation and determine pupal weight. The pupae were placed in petri dishes and kept separately in plastic jars and observed until adult emergence from which percent emergence was measured according to Khazanie (1979) and the sex ratio was determined by counting males and females separately. Each experiment, including the controls, was repeated twice. The data was pooled for analysis.

Data analysis

Mortality data was corrected with the help of Abbott's formula to account for natural mortality recorded in the control treatment (Abbott, 1925). The statistical program POLO-PC (LeOra Software, Berkeley, CA, USA) was used to determine the different concentrations (LC_{10}, LC_{30} and LC_{50}) for each fungal isolate and different doses (LC_{10}, LC_{30} and LC_{50}) for synthetic insecticides separately. The synergistic effect of mortality was analyzed by comparing mortality rates induced by mixtures of fungi and synthetic insecticides (observed) with the sum of mortalities by fungi and synthetic insecticides separately (expected). For measurement of expected mortality (M_e), the following formula was used:

$M_e = M_f + M_i(1 - M_f/100)$

Where, M_f and M_i were the observed percent mortalities caused by the fungus and the chemical insecticide separately (Farenhorst et al., 2010). Paired samples t-test was used for pair-wise comparisons between each treatment and to eliminate potential treatment variations i.e., differences between fungi and synthetic insecticides effectiveness by using Statistix 8.1 (Analytical Software, Tallahassee, USA). Positive M_{fi}-M_e values were considered synergistic (Koppenhöfer & Kaya, 1998). The means for longevity, fecundity and other parameters were added and the average was taken from both repetitions. Any differences amongst fungus-insecticide combinations on the sublethal parameters were assessed, and analyzed with the analysis of variance coupled with the LSD separation of means at the 5% level of significance.

Results

Mortality bioassays

Mortality of *M. domestica* after application of insect pathogenic fungi and different synthetic insecticides individually are presented in Table 2. The expected and observed mortality rates were also recorded for mixtures of entomopathogenic fungi and synthetic insecticides at different doses (Table 3). The insecticides in combination with fungi affected the survival rate of *M. domestica*. The mortality rate increased at higher concentrations of fungi and synthetic insecticides. The maximum mortality percentage (97.2±1.7) was observed at the high dose of Ma-4.1 (1.30×10^6 spores ml^{-1}) with acetamiprid (0.3 ppm) followed by Ma-4.1 with emamectin benzoate (high dose) (86.9±1.8). Moreover, significant synergetic interactions were observed between pathogenic fungi and synthetic insecticides especially, when both were applied at the highest dose. All synthetic insecticides, except bifenthrin and fipronil, showed higher mortality than expected and effects seemed to be synergistic on all three doses of fungal isolates.

Table 2. Percentage mortality (±SE) caused by *Metarhizium anisopliae* var. *anisopliae* (Ma-2.3 and Ma-4.1), *Isaria fumosorosea* (If-03) and synthetic insecticides applied individually against the *Musca domestica* at each of the three tested doses

	Concentrations		
Treatment	LC_{10}	LC_{30}	LC_{50}
Ma-2.3	9.20±0.43	20.65±1.21	43.80±1.56
Ma-4.1	11.32±0.54	26.70±0.67	46.70±2.10
If-03	10.10±0.32	20.30±1.23	41.20±2.12
Acetamiprid	11.30±0.14	31.40±0.46	53.20±0.32
Bifenthrin	13.30±0.20	31.40± 0.12	51.60±0.25
Emamectin	15.80± 0.25	33.61±0.23	55.40±0.68
Fipronil	17.60± 0.48	34.30±0.25	56.30±0.61
Imidacloprid	11.62±0.68	28.53±0.61	49.50±0.47
Lufenuron	16.17±0.21	31.60±0.78	51.30±0.12
Control	3.45±0.54	2.43±0.23	2.49±0.31

Table 3. Effect of combinations of *Metarhizium anisopliae* var. *anisopliae* (Ma-2.3 and Ma-4.1), *Isaria fumosorosea* (If-03) and synthetic insecticides on percent mortality (±SE) of *Musca domestica*

	Ma-2.3					Ma-4.1					IF-03				
	EXP.	OBS.	T-test	P-value	M_{fi}-M_e	EXP.	OBS.	T-test	P-value	M_{fi}-M_e	EXP.	OBS.	T-test	P-value	M_{fi}-M_e
Low dose (LC_{10})															
Acetamiprid	19.2±0.5	27.8±0.8	3.31	0.04*	8.6	21.6±0.4	32.3±0.8	4.21	0.02*	10.9	20.4±0.3	26.5±0.5	1.56	0.01*	6.35
Bifenthrin	20.7±0.5	18.4±1.9	1.21	0.21	-2.4	22.9±0.9	21.7±0.7	1.23	0.71	-1.20	21.6±1.2	19.5±1.0	0.82	0.53	-2.18
Emamectin benzoate	22.5±1.2	24.2±1.4	2.12	0.13	1.7	24.6±1.2	28.2±1.4	3.45	0.03*	3.61	23.4±0.3	23.7±1.4	2.14	0.11	0.29
Fipronil	23.9±0.5	16.3±0.5	0.67	0.31	-7.4	25.0±0.7	17.9±1.3	0.91	0.35	-7.97	24.8±0.7	16.3±0.6	0.76	0.75	-8.27
Imidacloprid	19.5±0.3	20.8±0.3	1.02	0.12	1.4	21.6±0.5	21.7±0.7	2.02	0.13	0.09	20.4±1.2	21.4±0.7	1.87	0.21	1.01
Lufenuron	22.8±0.7	22.9±0.7	1.13	0.23	0.18	24.9±0.5	31.3±0.6	4.32	0.02*	6.45	23.7±0.9	23.9±0.9	1.1	0.24	0.24
Intermediate dose (LC_{30})															
	Ma-2.3					Ma-4.1					IF-03				
	EXP.	OBS.	T-test	P-value	M_{fi}-M_e	EXP.	OBS.	T-test	P-value	M_{fi}-M_e	EXP.	OBS.	T-test	P-value	M_{fi}-M_e
Acetamiprid	42.1±0.9	50.9±1.4	5.43	0.01*	8.76	48.1±1.3	61.2±1.8	6.21	0.03*	12.95	41.7±1.2	49.7±1.6	4.78	0.04*	7.83
Bifenthrin	42.5±2.1	29.3±3.2	0.71	0.43	-12.89	48.5±2.2	35.3±2.6	0.31	0.28	-12.91	41.1±1.7	29.2±2.6	0.42	0.12	-12.66
Emamectin benzoate	42.6±1.3	43.6±1.6	2.32	0.13	0.67	49.0±0.4	60.8±0.4	3.27	0.04	11.78	42.3±0.8	42.75±0.9	1.78	0.21	0.13
Fipronil	43.4±1.5	30.1±2.1	0.21	0.71	-13.08	49.5±1.9	30.8±1.4	0.62	0.71	-18.41	42.1±0.7	31.9±0.8	0.61	0.51	-10.98
Imidacloprid	41.0±0.3	41.5±0.6	1.02	0.12	0.41	47.1±1.2	47.5±0.9	1.34	0.32	0.35	40.7±0.9	40.9±0.2	1.82	0.42	0.21
Lufenuron	42.3±2.1	45.9±3.9	2.11	0.04*	3.72	48.3±1.3	54.1±0.9	3.41	0.02*	5.76	41.9±3.2	44.1±4.5	2.51	0.01*	2.17
High dose (LC_{50})															
	Ma-2.3					Ma-4.1					IF-03				
	EXP.	OBS.	T-test	P-value	M_{fi}-M_e	EXP.	OBS.	T-test	P-value	M_{fi}-M_e	EXP.	OBS.	T-test	P-value	M_{fi}-M_e
Acetamiprid	67.1±0.9	86.3±0.8	9.21	0.02*	17.63	69.9±1.3	97.2±1.7	9.72	0.04*	25.60	64.5±1.2	83.8±1.1	8.31	0.02*	17.65
Bifenthrin	66.7±1.9	40.3±2.9	0.51	0.32	-28.51	69.6±0.8	53.3±1.0	0.43	0.52	-18.42	64.1±1.8	38.7±2.6	0.69	0.43	-27.49
Emamectin benzoate	68.4±1.1	80.3±1.4	5.14	0.01*	11.76	71.3±0.6	86.9±1.8	5.31	0.03*	15.44	65.8±0.6	79.4±0.9	4.81	0.021*	13.52
Fipronil	68.2±2.5	48.2±5.0	0.89	0.46	-20.20	71.1±1.4	49.8±2.6	0.13	0.61	-21.55	65.6±3.2	46.9±5.2	0.74	0.81	-18.87
Imidacloprid	68.4±2.1	69.3±1.8	0.71	0.04	0.45	71.3±0.3	73.2±1.2	1.01	0.04*	1.50	65.8±3.2	71.3±1.1	3.45	0.03*	5.13
Lufenuron	68.8±2.1	72.1±2.4	1.53	0.03	3.29	71.7±1.5	76.3±0.5	1.56	0.03*	4.56	66.2±1.8	73.4±1.1	4.35	0.02*	7.24

Exp= expected mortality; Obs= observed mortality; M_{fi} = observed mortality of mixture; ^Expected mortality Me= Mf+ Mi(1 - Mf/100) with Mf and Mi observed mortalities caused by fungus and synthetic insecticides alone respectively; *Results show significant paired samplest-test comparisons for both observed and expected mortality rates (means ±SE); LC_{10}, LC_{30} and LC_{50}= fungus and insecticide dose.

Sublethal effects on *Musca domestica* progeny

Adult longevity

A decreasing trend was found for male longevity of *M. domestica* in response to fungi and synthetic insecticides combinations (Table 4). Ma-4.1 with acetamiprid followed by Ma-4.1 with emamectin benzoate at the high dose, caused significant reduction in the male longevity of *M. domestica* in comparison to all the other treatments and the control ($F = 6.64$; df = 6,12; $P < 0.0001$). A similar trend was observed for female longevity, in which the application of Ma-4.1 with acetamiprid (high dose) caused significant reduction in the female longevity of *M. domestica* from 20.8 to 8.5 d followed by 8.6±0.3 due to the application of Ma-4.1 with emamectin benzoate (high dose) as compared to all other treatments ($F = 3.32$; df = 6,12; $P = 0.001$) (Table 4).

Fecundity and hatching percentage

A broad range of variation in fecundity of *M. domestica* was observed in all treatments (Table 5). A significant difference was observed for mixtures of different doses of fungi and synthetic insecticides especially at the high dose. The least number of eggs (118±4.1, 121±4.6 and 123±4.4) were recorded for treatments Ma-4.1 with lufenuron (high dose), Ma-4.1 with acetamiprid (high dose), Ma-4.1 with emamectin benzoate (high dose), respectively. Hatching percentage also varied significantly among the treatments (Table 5). The lowest egg hatching percentages, 67.9±1.0, 70.0±0.7 and 70.1±0.5, was recorded for Ma-4.1 with emamectin benzoate (high dose), If-03 with lufenuron (high dose) and Ma-4.1 with lufenuron (high dose) ($F = 3.81$; df = 6,12; $P = 0.0003$), respectively.

Larval period, pupation percentage, pupal weight and pupal duration

The larval period ranged from 6.3-9.1 d which showed significant prolongation among all treatments. If-03 with imidacloprid (high dose) (9.1±0.1), Ma-2.3 with imidacloprid (high dose) (9.1±0.1) and If-03 with emamectin benzoate (high dose) (8.9±0.1) ($F = 2.35$; df = 6,12; $P < 0.01$) caused maximum prolongation of the larval period (Table 6).

A significant reduction in the pupation percentage was recorded with increasing concentrations of fungus-insecticide mixtures (intermediate and high dose). The lowest pupation percentage was recorded for If-03 with acetamiprid (high dose) (60.0±2.4), Ma-4.1 with emamectin benzoate (high dose) (60.2±2.2) and Ma-2.3 with acetamiprid (high dose) (60.8±1.5) ($F = 2.18$; df = 6,12; $P = 0.03$) (Table 6).

A similar inverse trend was observed for pupal weight (mg), decreasing as the fungus-insecticide concentration combinations increased. The minimum pupal weight was recorded for Ma-4.1 with imidacloprid (high dose) (10.3±0.3), Ma-2.3 with acetamiprid (high dose) (10.8 ±0.1) and If-03 with acetamiprid (high dose) (11.0 ±0.2) ($F=3.46$; df=6,12; $P=0.0008$) (Table 7). Moreover, significant prolongation was observed in the pupal period in all treatments compared to the control. The treatment of Ma-4.1 with lufenuron (high dose) (8.4±0.1), Ma-4.1 with imidacloprid (high dose) (8.0±0.1) and Ma-4.1 with acetamiprid (high dose) (7.8±0.1) ($F = 3.81$; df = 6,12; $P = 0.0003$) (Table 7) caused maximum prolongation in pupal period.

Table 4. Sublethal effects of *Metarhizium anisopliae* var. *anisopliae* (Ma-2.3 and Ma-4.1), *Isaria fumosorosea* (If-03) and synthetic insecticide mixture on longevity of adults of *Musca domestica*

MALE LONGEVITY (days) (±SE)									
	Low dose (LC_{10})			Intermediate dose (LC_{30})			High dose (LC_{50})		
Insecticide/fungus	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03
Acetamiprid	16.1±0.3	14.8±0.3	16.1±0.3	13.9±0.3cd	11.6±0.4g	14.1±0.1bcd	10.6±0.4fgh	7.9±0.1j	10.9±0.5efgh
Bifenthrin	14.4±0.5	16.1±0.2	14.8±0.3	12.6±0.2efg	14.3±0.5bc	12.9±0.2def	10.6±0.3fgh	12.1±0.5de	11.3±0.2defg
Emamectin benzoate	14.1±0.3	15.0±0.3	14.3±0.1	12.2±0.5fg	12.9±0.3def	12.2±0.5fg	9.1±0.5ij	8.1±0.3j	9.9±0.0hi
Fipronil	15.6±0.1	16.1±0.3	15.8±0.1	14.9±0.1bc	13.9±0.7cd	15.3±0.2b	14.5±0.3b	13.4±0.4bc	14.6±0.2b
Imidacloprid	16.5±0.8	15.9±0.2	16.8±0.6	13.9±0.3cd	13.8±0.2cde	14.2±0.1bcd	12.1±0.2de	10.2±0.4ghi	12.3±0.1cd
Lufenuron	15.7±0.8	14.9±0.3	15.8±0.7	14.7±0.8bc	12.4±0.5fg	14.3±1.1bc	12.4±0.9cd	8.5±0.4j	11.9±0.8def
Control	19.2±0.2	19.4±0.5	19.5±0.3	19.5±0.3a	20.3±0.7a	20.0±0.4a	19.6±0.2a	19.9±0.5a	19.8±0.1a
P		0.06			0.0005			<0.0001	
F		1.88			3.62			6.64	
LSD value		ns			1.31			1.33	

FEMALE LONGEVITY (days) (±SE)									
	Low dose (LC_{10})			Intermediate dose (LC_{30})			High dose (LC_{50})		
Insecticide/fungus	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03
Acetamiprid	16.5±0.2	15.8±0.3	16.6±0.3	13.0±0.4hij	12.8±0.3ij	13.3±0.1efghi	11.9±0.5cdef	8.5±0.3h	12.0±0.5 bcdef
Bifenthrin	15.9±0.4	15.6±0.3	15.9±0.3	13.2±0.4ghi	13.0±0.2hij	13.4±0.1efghi	12.0±0.6 bcdef	10.3±0.7g	12.3±0.4bcde
Emamectin benzoate	16.2±0.5	15.2±0.1	16.4±0.4	14.2±0.4bcd	12.4±0.1j	14.4±0.1b	10.9±0.5fg	8.6±0.3h	11.1±0.5efg
Fipronil	14.8±0.3	15.3±0.5	14.8±0.3	13.5±0.2defgh	13.2±0.0fghi	13.5±0.1defgh	12.7±0.6bc	13.1±0.2b	12.7±0.6bc
Imidacloprid	16.1±1.0	15.9±0.2	15.5±0.9	13.7±0.3cdefgh	14.2±0.1bc	13.9±0.2bcdef	11.7±0.3cdef	11.3±0.3efg	11.4±0.3defg
Lufenuron	15.5±0.7	16.9±0.3	15.3±0.5	13.9±0.3bcde	13.2±0.0fghi	13.8±0.3bcdefg	12.5±0.5bcd	11.3±0.5 defg	12.2±0.4bcde
Control	20.7±0.2	20.4±0.4	20.9±0.1	20.3±0.1a	20.9±0.1a	20.6±0.1a	20.6±0.1a	20.5±0.5a	20.8±0.4a
P		0.21			0.0004			0.001	
F		1.36			3.73			3.32	
LSD value		ns			0.68			1.20	

Means in rows and columns followed by same letters are not statistically different; LSD, P<0.05; ns = not significant, LC_{10}, LC_{30} and LC_{50}=fungus and insecticide dose.

Table 5. Effects of binary mixture of *Metarhizium anisopliae* var. *anisopliae* (Ma-2.3 and Ma-4.1), *Isaria fumosorosea* (If-03) and synthetic insecticide on fecundity and percent hatching of *Musca domestica* progeny

	FECUNDITY (±SE)								
	Low dose (LC_{10})			Intermediate dose (LC_{30})			High dose (LC_{50})		
Insecticide/fungus	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03
Acetamiprid	234±4.7fg	239±7.3efg	237±2.6efg	182±3.1efg	165±10.2gh	180±2.1efg	142±9.6cd	121±4.6ef	136±5.1de
Bifenthrin	247±6.0def	233±13.9fg	250±3.3de	184±2.1efg	174±10.6fgh	178±8.1efgh	157±7.0c	137±3.5de	157±7.0c
Emamectin benzoate	232±4.6fg	206±3.0h	232±4.3fg	174±4.1fgh	158±6.2h	169±4.3fgh	129±9.5def	123±4.4ef	142±2.5cd
Fipronil	273±3.3bc	231±7.1g	273±3.0bc	247±6.3b	196±9.1de	249±5.3b	194±9.5b	190±5.7b	196±10.5b
Imidacloprid	280±6.5b	262±2.2cd	277±4.3bc	224±6.7c	189±8.2ef	224±6.7c	185±2.5b	145±9.0cd	188±0.6b
Lufenuron	267±6.9bc	238±6.5efg	272±2.1bc	217±5.6cd	175±11.9fgh	219±5.8c	198±1.8b	118±4.0f	201±0.8b
Control	382±3.9a	380±5.5a	382±2.6a	375±8.2a	377±7.8a	381±5.5a	383±3.6a	380±5.6a	384±3.2a
P		0.003			0.02			<0.0001	
F		2.96			2.33			6.42	
LSD value		15.88			20.96			17.48	
	PERCENT HATCHING (±SE)								
	Low dose (LC_{10})			Intermediate dose (LC_{30})			High dose (LC_{50})		
Insecticide/fungus	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03
Acetamiprid	82.6±0.5d	85.4±1.0 bc	84.0±0.6 cd	81.1±0.4bc	83.3±0.4b	81.1±0.4bc	79.4±0.7bc	77.5±0.7cd	80.3±0.5b
Bifenthrin	85.8±0.5bc	84.6±0.6 c	86.9±0.5b	83.2±0.7b	81.9±0.3bc	82.9±0.3b	78.6±0.7bc	75.9±1.3de	79.5±1.6bc
Emamectin benzoate	84.9±0.8 bc	83.4±0.8 c	84.7±0.9 bcd	80.8±1.2bc	77.9±2.0e	80.8±1.3c	74.1±0.5e	67.9±1.0g	74.4±0.4e
Fipronil	80.6±0.2def	78.9±0.7f	79.6±0.6f	77.5±0.5e	76.0±0.9f	77.8±0.7e	75.8±0.4de	75.5±0.6de	75.7±0.6de
Imidacloprid	82.3±0.7 cd	86.2±0.6b	83.0±0.3 cd	74.9±1.4fg	79.8±0.8c	76.4±1.4f	74.7±1.9e	77.8±0.6bcd	73.9±2.0e
Lufenuron	81.4±0.9de	82.2±0.6d	81.6±0.9de	72.8±0.4g	78.0±1.6cd	73.9±1.1fg	70.7±0.3f	70.1±0.5fg	70.0±0.7fg
Control	95.7±0.9a	96.2±0.8a	95.9±0.9a	93.5±0.2a	95.3±1.2a	93.9±0.68a	95.2±0.8a	94.7±0.8a	94.5±0.9a
P		0.002			0.008			0.0003	
F		3.10			2.61			3.81	
LSD value		2.04			2.82			2.59	

Means in rows and columns followed by same letters are not statistically different; LSD, $P<0.05$; ns =not significance at 5% level; LC_{10}, LC_{30} and LC_{50}=fungus and insecticide dose.

Table 6. Effects of *Metarhizium anisopliae* var. *anisopliae* (Ma-2.3 and Ma-4.1), *Isaria fumosorosea* (If-03) and synthetic insecticide mixture on larval duration and percent pupation of *Musca domestica* progeny

LARVAL DURATION (days) (±SE)									
	Low dose (LC_{10})			Intermediate dose (LC_{30})			High dose (LC_{50})		
Insecticide/fungus	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03
Acetamiprid	7.4±0.3cd	7.5±0.1cd	7.5±0.2cd	7.6±0.2 gh	7.7±0.1 gh	7.7±0.2 gh	8.1±0.1h	8.1±0.7h	8.2±0.1gh
Bifenthrin	7.5±0.1cd	7.3±0.1de	7.5±0.1cd	7.6±0.10gh	7.6±0.2h	7.7±0.4 gh	8.5±0.1efg	8.3±0.1fgh	8.5±0.1 cdef
Emamectin benzoate	7.7±0.1bcd	7.3±0.1de	7.8±0.1bcd	8.4±0.03bcd	7.8±0.7gh	8.4±0.1bc	8.8±0.1abc	8.5±0.3 cdef	8.9±0.1ab
Fipronil	8.1±0.2ab	7.3±0.1de	7.9±0.4abc	8.4±0.04bcd	7.9±0.1fg	8.3±0.1cde	8.6±0.1 bcdef	8.5±0.1defg	8.8±0.1abcd
Imidacloprid	8.4±0.2a	7.4±0.1cd	7.8±0.4abc	8.8±0.1a	7.7±0.1 gh	8.6±0.2ab	9.1±0.1a	8.4±0.2fg	9.1±0.1a
Lufenuron	7.6±0.1bcd	7.6±0.1bcd	7.6±0.1cd	8.1±0.1ef	8.1±0.2def	8.1±0.1def	8.6±0.2cdef	8.8±0.2bcde	8.5±0.2 cdef
Control	6.3±0.2f	6.8±0.1ef	6.4±0.2f	6.5±0.3i	6.6±0.3i	6.5±0.3i	6.7±0.4i	6.7±0.4i	6.4±0.40i
P		0.007			<0.0001			0.01	
F		2.66			5.59			2.35	
LSD value		0.53			0.26			0.31	
PERCENT PUPATION(±SE)									
	Low dose (LC_{10})			Intermediate dose (LC_{30})			High dose (LC_{50})		
Insecticide/fungus	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03
Acetamiprid	76.2±0.5	74.6±0.9	74.2±1.5	65.2±1.3ij	68.6±1.0hi	64.4±0.5j	60.8±1.5g	61.2±2.2fg	60.0±2.4g
Bifenthrin	79.1±1.0	78.3±1.2	81.0±0.2	71.8±2.4efgh	70.3±1.1fgh	74.7±1.8de	62.5±0.9efg	64.5±1.4 defg	63.3±1.5 defg
Emamectin benzoate	82.5±0.5	82.6±0.9	82.9±0.4	72.7±0.9efg	75.2±1.0cde	73.9±1.4def	62.6±1.2efg	60.2±2.2g	64.5±2.0 defg
Fipronil	81.1±0.7	80.1±0.4	81.4±0.5	79.7±0.6b	76.6±1.2bcd	78.2±1.5bc	73.2±0.8b	66.9±1.1 cde	74.8±2.6b
Imidacloprid	79.9±1.3	81.5±1.2	83.2±1.2	68.6±1.5h	75.0±1.9cde	71.4±0.6efgh	67.8±0.4cd	71.1±0.5bc	65.8±2.0def
Lufenuron	76.4±1.0	74.2±0.9	77.6±0.8	69.9±0.6gh	70.4±0.9fgh	70.7±0.7fgh	66.2±1.3 cde	63.8±1.2 defg	67.4±1.4cde
Control	92.7±3.2	94.9±3.9	94.0±3.1	94.0±4.6a	93.5±4.4a	93.4±4.3a	93.0±5.2a	94.2±5.8a	93.2±5.0a
P		0.06			0.003			0.03	
F		1.86			2.95			2.18	
LSD value		ns			3.87			4.97	

Means in rows and columns followed by same letters are not statistically different; LSD, $P<0.05$;ns =not significant;LC_{10}, LC_{30} and LC_{50}=fungus and insecticide dose.

Table 7. Effects of *Metarhizium anisopliae* var. *anisopliae* (Ma-2.3 and Ma-4.1), *Isaria fumosorosea* (If-03) and synthetic insecticide mixture on pupal weight and pupal duration of *Musca domestica* progeny

PUPAL WEIGHT (mg)(±SE)									
	Low dose (LC_{10})			Intermediate dose (LC_{30})			High dose (LC_{50})		
Insecticide/fungus	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03
Acetamiprid	15.7±0.1c	15.9±0.2c	15.6±0.4cd	13.8±0.3d	14.7±0.3cd	13.7±0.3de	10.8±0.1g	12.6±0.3ef	11.0±0.2fg
Bifenthrin	16.9±0.3bc	16.7±0.4bc	17.1±0.3b	13.9±0.3d	14.8±0.3cd	14.2±0.1cd	11.4±0.6f	12.9±0.5f	11.4±0.6f
Emamectin benzoate	15.8±0.2c	17.2±0.3ab	16.1±0.5bc	13.9±0.6d	16.1±0.2b	14.0±0.6c	12.6±0.3d	11.2±0.4f	13.1±0.5c
Fipronil	16.1±0.4bc	15.4±0.2cd	16.4±0.6bc	15.7±0.2c	15.1±0.4cd	15.9±0.3bc	14.6±0.7b	12.7±1.0e	14.9±0.6b
Imidacloprid	16.5±0.2bc	15.7±0.2c	16.5±0.3bc	14.8±0.4c	13.9±0.5de	15.5±0.2bc	12.1±0.4ef	10.3±0.3g	12.1±0.4ef
Lufenuron	15.9±0.4c	16.2±0.4bc	16.1±0.4bc	16.2±0.1b	15.9±0.3bc	16.1±0.2b	13.4±0.5c	11.3±0.4fg	14.3±0.1b
Control	19.1±0.3a	19.2±0.2a	19.4±0.2a	18.9±0.2a	19.3±0.2a	19.2±0.1a	19.1±0.2a	18.9±0.4a	19.1±0.2a
P		0.02			0.0001			0.0008	
F		2.35			4.15			3.46	
LSD value		1.14			1.03			1.36	
PUPAL DURATION (days)(±SE)									
	Low dose (LC_{10})			Intermediate dose (LC_{30})			High dose (LC_{50})		
Insecticide/fungus	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03
Acetamiprid	6.1±0.1	6.1±0.1	6.0±0.1	7.1±0.1cd	7.4±0.1abc	6.8±0.2de	7.5±0.1bcde	7.8±0.1bc	7.3±0.1cdefg
Bifenthrin	6.1±0.2	6.2±0.4	6.1±0.2	7.3±0.1abc	7.5±0.6ab	7.4±0.2abc	7.6±0.1bcd	7.2±0.5defg	7.4±0.1bcde
Emamectin benzoate	6.2±0.1	5.9±0.3	6.2±0.1	7.3±0.1abc	6.6±0.1e	7.2±0.1abc	7.7±0.1bcd	7.6±0.2bcd	7.5±0.3bcd
Fipronil	6.3±0.1	6.3±0.1	6.2±0.1	7.4±0.1abc	7.5±0.1ab	7.2±0.1bc	7.5±0.3bcde	7.8±0.5bcd	7.3±0.1cdef
Imidacloprid	6.2±0.1	6.0±0.1	6.0±0.2	6.5±0.1e	7.3±0.8abc	6.7±0.2e	6.8±0.6g	8.0±0.1ab	6.9±0.1efg
Lufenuron	6.3±0.2	6.4±0.1	6.3±0.2	6.7±0.2e	7.6±0.6a	6.8±0.2de	6.8±0.3fg	8.4±0.1a	6.9±0.1efg
Control	5.4±0.1	5.4±0.2	5.3±0.1	5.6±0.2f	5.7±0.2f	5.6±0.1f	5.2±0.5h	5.5±0.2h	5.3±0.1h
P		0.97			<0.0001			0.0003	
F		0.36			5.01			3.81	
LSD value		ns			0.38			0.60	

Means in rows and columns followed by same letters are not statistically different; LSD, $P<0.05$;ns = not significant; LC_{10}, LC_{30} and LC_{50}=fungus and insecticide dose.

Percent adult emergence and sex ratio

The data regarding fungi and synthetic insecticides mixtures showed no significant differences at any of three tested concentrations (Table 8). Moreover, there was no difference in sex ratio except when fungi and synthetic insecticides were applied simultaneously at the low dose. The lowest male percentage (47.0±0.8) was observed in treatment If-03 with lufenuron (low dose), while minimum female emergence (46.7±0.6) was observed in Ma-2.3 with bifenthrin (low dose) ($F = 2.31$; $df = 6,12$; $P = 0.02$) (Table 9).

Table 8. Effects of binary mixture of *Metarhizium anisopliae* var. *anisopliae* (Ma-2.3 and Ma-4.1), *Isaria fumosorosea* (If-03) and synthetic insecticides on adult emergence (±SE) of *Musca domestica* progeny

Insecticide/fungus	Low dose (LC_{10})			Intermediate dose (LC_{30})			High dose (LC_{50})		
	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03
Acetamiprid	76.4±0.4	75.7±0.5	76.4±0.4	71.9±0.4	72.4±0.5	71.9±0.4	68.6±0.5	66.2±1.0	68.7±0.7
Bifenthrin	74.1±0.6	73.6±1.1	75.1±0.4	71.1±0.5	70.7±0.8	71.6±0.3	65.6±0.7	69.3±1.7	65.9±0.5
Emamectin benzoate	75.9±0.9	76.4±0.8	75.8±0.9	70.4±0.9	70.3±0.3	70.4±0.7	64.2±1.4	66.9±0.6	63.7±1.4
Fipronil	71.8±0.7	74.5±1.2	73.1±1.1	68.7±0.5	69.7±1.2	69.5±1.0	62.4±0.5	64.6±1.6	64.3±1.3
Imidacloprid	72.6±0.6	76.3±1.7	75.6±1.8	65.3±1.6	69.2±0.7	68.4±0.5	60.9±0.7	62.2±1.0	62.2±1.0
Lufenuron	75.9±1.0	74.6±1.5	77.5±2.0	72.1±0.4	70.3±0.5	71.7±0.3	66.2±1.3	67.4±0.9	65.9±0.8
Control	92.5±1.1	94.1±1.2	93.0±1.5	94.1±1.1	94.5±0.9	93.2±0.8	94.1±0.8	94.4±1.1	93.3±0.5
P		0.52			0.11			0.22	
F		0.94			1.61			1.34	
LSD value		ns			ns			ns	

Means in rows and columns followed by same letters are not statistically different; LSD, $P<0.05$; ns =not significant; LC_{10}, LC_{30} and LC_{50}=fungus and insecticide dose.

Discussion

Keeping in mind the short life cycle and high reproduction potential of *M. domestica*, it would be beneficial to find an approach for increasing pest mortality and reducing the lethal time using entomopathogenic fungi. In the current study, synthetic insecticides were applied in mixtures with entomopathogenic fungi. A synergistic effect was observed for all three fungal isolates and the synthetic insecticides, acetamiprid, emamectin benzoate, imidacloprid and lufenuron, at the three doses by comparison of observed and expected mortality. Synthetic insecticides have been mixed fungi in previous studies (Pachamuthu & Kamble, 2000; Ericsson et al. 2007; Asi et al., 2010; Sharififard et al., 2011; Archana & Ramaswamy, 2012; Kassab et al., 2014). These studies mainly focused on the mortality of the pest whereas the current study also examined sublethal doses, LC_{10} and LC_{30}. The comparison of the percent mortality of binary mixture and single products was evaluated and significant differences were observed in the mortality of binary mixture as compared to single products. The insecticides, acetamiprid, emamectin benzoate, imidacloprid and lufenuron, increased pest mortality and showed potential against *M. domestica* when combined with entomopathogenic fungi.

In cases where fungi and synthetic insecticides are applied alone, the difference in effectiveness is due to different mode of action. Although, the mechanisms for effects of fungi and synthetic insecticides mixtures are unclear, synthetic insecticides may influence the insect cuticle and facilitate penetration for fungal spores, or possibly restrain immune response and facilitate fungal infection process (Hiromori & Nishigaki, 2001). As indicated by the $LC_{10,}$ the most toxic synthetic insecticide (fipronil) was much less efficient when combined with entomopathogenic fungi, which highlights the influence of insecticide on the pathogenic fungi and make it is less compatible as compared to other synthetic insecticides.

Table 9. Effects of *Metarhizium anisopliae* var. *anisopliae* (Ma-2.3 and Ma-4.1), *Isaria fumosorosea* (If-03) and synthetic insecticides mixture on sex ratio of *Musca domestica* progeny

MALE RATIO (±SE)									
	Low dose (LC_{10})			Intermediate dose (LC_{30})			High dose (LC_{50})		
Insecticide/fungus	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03
Acetamiprid	51.0±1.2ab	53.0±0.7a	51.0±1.5ab	51.0±0.7	53.3±0.9	52.5±0.5	53.0±0.4	58.0±2.5	54.0±0.7
Bifenthrin	53.3±0.6a	48.3±2.1bcd	50.8±0.5ab	48.8±1.8	51.0±1.8	48.8±1.8	53.3±1.1	52.5±0.5	52.8±1.1
Emamectin benzoate	52.0±1.7a	50.8±0.9ab	53.0±0.8a	51.0±1.5	49.8±2.3	50.8±1.7	54.8±0.8	51.5±2.6	54.3±0.6
Fipronil	51.9±0.4a	50.3±1.1abc	51.7±0.5a	52.8±0.6	51.9±0.7	51.0±1.3	49.5±3.2	51.0±1.0	52.5±2.2
Imidacloprid	53.2±1.3a	50.3±1.1abc	51.9±0.7a	49.8±1.4	51.5±0.2	51.5±1.4	50.1±2.7	50.6±1.7	53.3±1.1
Lufenuron	47.3±2.1cd	51.5±0.5a	47.0±0.8da	48.0±2.3	49.7±1.0	49.8±0.8	53.1±1.2	46.5±1.6	50.4±0.6
Control	50.8±0.5ab	50.8±0.5ab	50.3±0.3abc	50.5±0.3	50.9±0.4	50.9±0.5	51.8±0.5	50.5±0.3	51.1±0.4
P		0.02			0.87			0.05	
F		2.31			0.55			1.93	
LSD value		3.12			ns			ns	

FEMALE RATIO (±SE)									
	Low dose (LC_{10})			Intermediate dose (LC_{30})			High dose (LC_{50})		
Insecticide/fungus	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03
Acetamiprid	49.0±1.2cd	47.0±0.7d	49.0±1.5cd	49.0±0.7	46.7±0.9	47.5±0.5	47.0±0.4	42.0±2.5	46.0±0.7
Bifenthrin	46.7±0.6d	51.7±2.1abc	49.2±0.5cd	51.2±1.8	49.0±1.8	51.2±1.8	46.7±1.1	47.3±0.5	47.2±1.1
Emamectin benzoate	48.0±1.7d	49.2±0.9cd	47.0±0.8d	49.0±1.5	50.2±2.3	49.2±1.7	45.2±0.8	49.5 ±2.6	46.7±0.6
Fipronil	48.1±0.44d	49.7±1.1bcd	48.3±0.5d	47.2±0.6	48.1±0.7	49.0±1.3	50.5±3.2	49.0±1.0	47.5±2.2
Imidacloprid	46.8±1.3d	49.7±1.1bcd	48.1±0.7d	50.2±1.4	48.5±0.2	48.5±1.4	49.9±2.7	49.4±1.7	46.7±1.0
Lufenuron	52.7±2.1ab	48.5±0.5d	53.0±0.8a	52.0±2.3	50.3±1.0	50.1±0.8	46.9±1.2	53.5±1.6	49.6±0.6
Control	49.2±0.5cd	49.2±0.5cd	50.7±0.3bcd	49.5±0.3	49.1±0.4	49.1±0.5	48.2±0.5	50.5±0.3	48.9±0.4
P		0.02			0.87			0.05	
F		2.31			0.55			1.93	
LSD value		3.12			ns			ns	

Means in rows and columns followed by same letters are not statistically different; LSD, $P<0.05$;ns= not significant; LC_{10}, LC_{30} and LC_{50}=fungus and insecticide dose.

In addition to lethal effects, binary mixtures of pathogenic fungi and synthetic insecticides significantly reduced the adult longevity in comparison to the normal adult life. The shortened life of females affected the number of eggs laid. A similar trend was observed by Flores et al. (2004) and Pelizza et al. (2013) who reported decreased survival and reduced fecundity in *Aedes aegypti* L. (Diptera: Culicidae) as a sublethal effect of entomopathogenic fungi. In addition, females of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), *Ceratitis fasciventris* (Bezzi) (Diptera: Tephritidae) and *Ceratitis cosyra* (Walker) (Diptera: Tephritidae) suffered 82, 73 and 37% reduction in fecundity, respectively, following infection by *M. anisopliae* (Dimbi et al., 2013). The percent hatch was reduced at higher doses of insect pathogenic fungi and synthetic insecticide mixture in comparison with control, which is consistent with an earlier study in which percent hatch of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) was reduced compared to the control when treated with LC_{25} concentration of methoxyfenozide (Enríquez et al., 2010). The insect pathogenic fungi and synthetic insecticide mixtures significantly influenced and prolonged the time to pupation in *M. domestica* at high doses. Similarly, *Muntingia calabura* L. prolonged the larval duration of diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae) (Bandeira et al., 2013).

In the current study, percent pupation of *M. domestica* at the high dose was significantly different from the control. Similar results were observed when *Anopheles stephensi* L. (Diptera: Culicidae) was tested against *Beauveria bassiana* (Prasad & Veerwal, 2012). Also, pupal weight was negatively affected by application of fungi and insecticide mixtures, which is similar to findings of Ruiu et al. (2006) where pupal weight reduced after larvae of *M. domestica* were fed on diet containing sublethal doses of *Brevibacillus laterosporus* (Laubach). The current study found prolonged pupal duration which is consistent with the study of Malarvannan et al. (2010), where *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) had prolonged pupal duration when treated with fungi in comparison to the control.

No significant differences in adult emergence were found in any of the treatments. This result is consistent with the study of El-Razik et al. (2013), where adult emergence in *Callosobruchus maculatus* (F) (Coleoptera: Chrysomelidae) was reduced by a synthetic insecticide and oil mixture. Moreover, significant differences in the sex ratio was observed only in the low dose treatment, which was similar to the study of Shaalan et al. (2005), where disturbance in sex ratio was noted after the binary application of *Callitris glaucophylla* (Thompson and Johnson) (Callitris: Cupressaceae) extracts and synthetic insecticides.

The present study of *M. anisopliae* var. *anisopliae*, *I. fumosorosea* and chemical insecticide mixtures and their sublethal effects on life parameters of *M. domestica* revealed that normal developmental stages and time periods were affected. The mixture of insect pathogenic fungi and synthetic insecticides can significantly alter the average development of *M. domestica*. However, field studies are important to validate the efficacy of mixtures of entomopathogenic fungi and synthetic insecticides observed to have high mortality and reduced lethal time against *M. domestica* in laboratory studies. In conclusion, entomopathogenic fungi and synthetic insecticides mixture, if validated under field conditions, might become a key element of integrated pest management for *M. domestica*.

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