Pakistan J. Zool., vol. 48(5), pp. 1465-1476, 2016.

Combined Effects of *Beauveria bassiana* (Hypocreales: Clavicipitaceae) and Insecticide Mixtures on Biological Parameters of *Musca domestica* (Diptera: Muscidae)

Muzammil Farooq and Shoaib Freed*
Department of Entomology, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan.

ABSTRACT

The current study was conducted to evaluate the effect of *Beauveria bassiana* (Balsamo) Vuillemin in mixtures with six different insecticides separately against *Musca domestica* (L). Preliminary experiments were conducted to determine the mortality rates caused by the application of fungal inoculums and different doses of insecticides separately on laboratory reared *M. domestica* populations. Later, these fungal inoculums and insecticides in mixtures were applied on adults of *M. domestica* using the bait method. Flies showed concentration/dose dependent response and insecticides *i.e.* acetamiprid, emamectin benzoate, imidacloprid and lufenuron showed higher mortality in combination with insect pathogenic fungi than expected with significantly synergistic interactions. The effects of insect pathogenic fungi and insecticides mixtures were assessed on the biological parameters *i.e.*, longevity, fecundity, egg hatching, larval duration, percent pupation, pupal weight, pupal duration, adult emergence and sex ratio of surviving *M. domestica* populations. The results showed significant effects of pathogenic fungi and insecticides mixtures on all parameters ($P<0.05$). As a result of application of fungi and insecticide mixtures, a significant decrease in longevity, fecundity, egg hatching, percent pupation, pupal weight and adult emergence was observed, while larval duration and pupal period were prolonged. The entomopathogenic fungi integrated along with insecticides *i.e.*, acetamiprid, emamectin benzoate, imidacloprid and lufenuron could be a viable option for establishing an integrated pest management program for managing populations of *M. domestica*.

Article Information
Received 2 September 2015
Revised 21 December 2015
Accepted 3 April 2016
Available online 1 August 2016

Authors' Contributions
MF performed the experiments, analyzed the data and wrote the article. SF supervised the work and helped in article writing.

Key words
Beauveria bassiana,
Musca domestica
Biological aspects
Sublethal effects

INTRODUCTION

The house fly, *Musca domestica* (L.) (Diptera: Muscidae), is a well-known pest and serious threat to human and livestock health by acting as a mechanical vector of pathogens that causes diseases in man and animals (Khan and Ahmed, 2000; Lecouna *et al*., 2005). In addition to disease transmission, *M. domestica* causes food spoilage and adults can be a source of nuisance (Ande, 2001; Forester *et al*., 2009). As human's and livestock health both are put at risk by this pest (Saleh and Elmosa, 2002), therefore, it is critical to control this pest, which is chiefly done by the use of conventional insecticides (Cao *et al*., 2006; Malik *et al*., 2007). However, due to the indiscriminate use of insecticides, serious problems like insecticide resistance and residual effects of chemicals are on the rise. Biological control could be promising and eco-friendly alternative for the control of *M. domestica* (Mishra *et al*., 2011). In comparison to insecticides, entomopathogenic fungi provide the potential for the management of *M. domestica* due to its natural prevalence of in *M. domestica* populations (Skovgård and Steenberg, 2002; Khan *et al*., 2012).

Entomopathogenic fungi, such as *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikof) Sorokin result in rapid killing and high infection rates to control *M. domestica* (Watson *et al*., 1995; Lecouna *et al*., 2005; Kaufman *et al*., 2005; Sharififard *et al*., 2011a). In these studies ultimate mortality of *M. domestica* populations was reached within a time period of 5-15 days. To improve the efficacy of biological control, fungal pathogens can be incorporated with lower doses of insecticides (Pachamuthu and Kamble, 2000). Several studies have indicated that combined usage of insecticides and entomopathogenic fungi such as *M. anisopliae* are compatible approaches (Pachamuthu and Kamble, 2000; Zurek *et al*., 2002; Ericsson *et al*., 2007). It is not clear how the combination of insecticides and entomopathogenic fungi treatments interact but the physiological effects caused by one agent may increase the effects of the other resulting in higher mortality. The above mentioned

* Corresponding author: sfareed@bzu.edu.pk
0030-9923/2016/0005-1465 $ 8.00/0

studies also indicate that such combined formulations of entomopathogenic fungi and insecticides make less use of active ingredient than what applied separately to achieve the same outcome. These combined approaches interact synergistically and result in significantly higher mortality (Ericsson *et al*., 2007). The purpose of this study was to determine the effect of different concentrations of *B. bassiana* and different doses of insecticides against the *M. domestica.* Additionally, the effects of combined mixtures of *B. bassiana* concentrations and insecticides doses on different biological parameters *i.e*., longevity, fecundity, egg hatching, larval duration, percent pupation, pupal weight, pupal duration, adult emergence and sex ratio among surviving members of *M. domestica* population was also studied.

MATERIALS AND METHODS

Musca domestica *culture*

Adult flies were collected from a poultry house and transferred to the Laboratory of Insect Microbiology and Biotechnology, Department of Entomology, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan, where flies were reared at 26±2°C, 50±5% RH (relative humidity) and photo period (12:12) h in cages ($30\times30\times30cm^3$) with mesh screen on the opposite sides and a cloth sleeve opening in the front. Adults were provided food in the form of sugar and powdered milk (3:1) in a Petri dish (9 cm) and water, while the larval medium (egg laying medium) was a water based mixture of wheat bran, rice meal, yeast, sugar and dry milk powder (40:10:3:3:1) as reported by Bell *et al*. (2010) with slight modifications. The diet was changed after 2-4 days depending on the number of larvae present. In order to establish a laboratory population, the flies were reared up to fourth generation then later 2-3 days old adults were used for bioassays.

Entomopathogenic fungi

Two different isolates of *B. bassiana* (Table I) that caused maximum mortalities in a preliminary experimentation were selected (Data not shown). The slants of monoconidial cultures of the isolates were cultured on potato dextrose agar (PDA) at 25°C in darkness and then stored at 4°C until needed. For further propagation, the spores from these slants were spread on to the surface of PDA plates (9 cm diameter) and kept at 25°C in darkness at 70-75% RH for 14 days (Freed *et al*., 2011a, b). After 14 days of growth, the fungal spores were used to treat *M. domestica* or stored at 4°C until used for this bioassay. The conidia were scraped from the plates and mixed with a sterile Tween-80 (0.05%) solution, while the conidial concentration was determined by haemocytometer for further feeding experiments. Conidial viability was determined by enumerating the percentage of germinated conidia 24 h after spreading in fresh PDA medium. A conidial suspension of 1×10^7 spores/ml (0.01 ml) was spread on 9cm perti plate containing 15 mL of PDA medium and incubated at 27 °C for 24 h for germination. Three 15mm square cover slips were placed on the surface of medium, and percentage germination was determined by counting the number of germinated conidia and total number of conidia per field view at 250X magnification (Quesada- Moraga *et al.*, 2006). The conidial germination was above 95% (Data not shown).

Entomopathogenic fungi bioassay

Different concentrations of conidial suspensions (3×10^8, 2×10^8, 1×10^8, 1×10^7 and 1×10^6 spores ml^{-1}) of *B. bassiana* isolates (Bb-01 and Bb-08) were prepared by serial dilution with 0.05% Tween 80. Conidial suspension (1 ml) was dispersed on surface of adult diet (1g) (sugar and powdered milk, 3:1) for better distribution of spores. In control, flies were provided with diet containing Tween 80 (0.05%) solution only and water was provided as *ad libitum* to all flies. Forty adults (2-3 days old) with sex ratio (1:1) were treated separately with each concentration which was replicated four times and placed in jars ($15\times6\times6$ cm^3) at 26±2°C, 50±5% RH. The mortality data was recorded every 24 h for seven consecutive days. Different levels of lethal concentrations (LC_{10}, LC_{30} and LC_{50}) necessary to kill different percentages of fly population (*i.e.*, 10%, 30% and 50%) were experimentally determined for each fungal isolate (Table I).

Insecticide bioassay

Six different formulated insecticides (Table 1) were used for comparison with insect pathogenic fungi. The insecticides were chosen randomly as these were recommended to control *M. domestica* population in the field. Preliminary experimentation was done for the measurement of LC_{10}, LC_{30} and LC_{50} against *M. domestica* population for each insecticide separately. The procedure used for the measurement of lethal concentration of insecticides was the same as for the application of fungi.

Effects of fungi and insecticides on biological parameters of the adult M. domestica

500µl of each fungus and insecticide (collectively 1 ml) was added to the 1g of diet (in ratio of 1:1) for adult's treatment, while in the control group insects were provided with diet containing 0.05% Tween 80 and

Table I. - Different isolates of *Beauveria bassiana* and insecticides with LC_{10}, LC_{30} and LC_{50} values used against laboratory reared house flies.

Fungal Species	Source	LC_{10} (spores ml^{-1})	LC_{30} (spores ml^{-1})	LC_{50} (spores ml^{-1})
B. bassiana (Isolate Bb-01)	Cotton field (Multan, Punjab)	5.71×10^5	9.32×10^5	3.78×10^6
B. bassiana (Isolate Bb-08)	Pine forest soil (Mansehra, KPK)	1.03×10^6	5.53×10^6	9.05×10^6
Insecticides	**Manufacturer**	**LC_{10} (ppm)**	**LC_{30} (ppm)**	**LC_{50} (ppm)**
Acetamprid (20SP)	Arysta Life Sciences	0.03	0.14	0.39
Bifenthrin (10EC)	FMC United	0.02	0.08	0.22
Emamectin benzoate (019) EC	Syngenta	0.00002	0.0002	0.001
Fipronil (5EC)	Bayer Crop Sciences	0.00003	0.0004	0.002
Imidacloprid (20SL)	Bayer Crop Sciences	0.022	0.09	0.27
Lufenuron (050EC)	Syngenta	0.00002	0.0002	0.001

LC_{10}, LC_{30} and LC_{50} for fungi = 7 days after treatment
LC_{10}, LC_{30} and LC_{50} for insecticides = 3 days after treatment

water. 500µl of each fungi and insecticide was adjusted to get the final desired concentration in the diet. The $LC_{10,}$ LC_{30} and LC_{50} of fungus were taken as three different levels of concentrations. On the other hand, $LC_{10,}$ LC_{30} and LC_{50} of insecticides were considered as different dose levels for combination with entomopathogenic fungi. LC_{10} of fungus and LC_{10} of one insecticide were taken as one treatment, LC_{30} of fungus and LC_{30} of insecticide as second and LC_{50} of fungus and LC_{50} of insecticide as third for each insecticide in combination with fungal isolate (Bb-01). The same experimental scheme was applied to isolate Bb-08 and all other insecticides. The experiment was carried out on forty adults (sex ratio 1:1) with 4 replications in each treatment and placed in jars ($15\times6\times6$ cm^3). The mortality data was recorded for seven consecutive days. Insects were provided with same rearing conditions as described earlier.

In addition to mortality, the sublethal effects of different fungi and insecticides mixtures on surviving *M. domestica* were observed. The male and female adults were provided with oviposition medium, longevity of each sex was determined according to the method by Fletcher *et al*. (1990). The oviposition medium (described earlier) was examined daily for egg laying and subsequent counting with aid of hand lens. The medium was changed after every two days depending upon the number of eggs. The total number of eggs was recorded and fecundity was calculated, while percent fecundity was determined according to Crystal (1964) by dividing total number of eggs laid to total number of female over the entire experiment. After counting, the eggs were again left in egg laying medium for hatching, which was checked on daily basis for hatching if any. The larvae on hatching were counted and percent hatching was calculated. The larvae were provided with food and kept until pupation to estimate larval duration. The resultant pupae were separated from larval medium and counted for percent pupation and pupal weight was also recorded. Later pupae were kept separate in jars until the emergence of adults. The adult emergence was measured in accordance to Khazanie (1979), while number of males and females were recorded in order to calculate sex ratio.

Data analysis

The control mortality ranged from 5% to 10%, the observed mortality data was corrected by using Abbott's formula (Abbott, 1925). Later, a statistical program POLO-PC (Lerora Software, 2003) was used to measure different lethal concentrations for each fungal isolate and lethal doses for insecticides separately. Similarly, mortality data for fungi and mixtures was also corrected as explained above. The synergetic effect to mortality of *M. domestica* by combine use of fungi and insecticides mixtures was analyzed by comparing mortality rates induced by fungi and insecticide mixture (observed) with sum of mortalities caused by fungi and insecticides individually (expected). Following formulae was used for measurement of expected mortality

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

where M_f and M_i were the observed percent mortalities caused by the fungus and the insecticide separately

(Farenhorst *et al.*, 2010). Paired samples T-test in Statistix 8.1 was used for pair-wise comparisons between each treatment and to eliminate potential treatment variations *i.e.*, differences between fungi applications and insecticides effectiveness. Positive M_{fi}-M_e values were considered synergistic (Koppenhöfer and Kaya, 1998).

The entire experiment was performed twice for confirmation of results and to avoid ambiguity. The means for longevity, fecundity and other parameters were analyzed with the help of analysis of variance (ANOVA) and separated with the help of LSD at significance level of 5% using Statistix 8.1 software.

RESULTS

Mortality after individual and binary application of fungi and insecticides

Mortality of *M. domestica* population after application of different fungal isolates, insecticides, individually and mixtures of entomopathogenic fungi and insecticides at different concentrations levels are presented in (Table I, II). The toxicity of tested insecticides in combination with fungi showed impacts on the survival of *M. domestica* population. The mortality rate was higher in insecticides and fungus mixtures as compared to those caused by insecticides and fungi alone while an increasing trend was found towards higher concentration in a dose dependent manner. In addition, insecticides *i.e.*, acetamiprid, emamectin, imidacloprid and lufenuron showed higher mortality than expected when combined with fungi and effects seemed to be synergetic for isolates (Bb-01, Bb-08) of insect pathogenic fungi due to positive Mfi-Me values after seven days of treatment (Table I).

In the case of a mixture of insect pathogenic fungi (Bb-01) and insecticides, the highest percent mortality (±SE) 91.43 (± 0.64) was observed in case of combined use of higher dose of isolate Bb-01 (LC_{50}, 3.78×10^6 spores ml^{-1}) and acetamiprid (LC_{50}, 0.3 ppm) followed by LC_{50} of isolate Bb-01 and LC_{50} of emamectin, (87.28 ± 2.64). These findings were significantly higher as compared to other treatments. The results regarding entomopathogenic fungi (LC_{30}) and insecticides (LC_{30}) mixture, showed highest percent mortality (63.80 ± 0.90) in isolate Bb-01(LC_{30}) and emamectin (LC_{30}). Moreover, in case of LC_{10} of insect pathogenic fungi and LC_{10} of insecticide, highest percent mortality (30.84 ± 0.52) was observed for isolate Bb-01(LC_{10}) and acetamiprid (LC_{10}).

Similar result were also observed for insect pathogenic fungi (Bb-08) and insecticides mixture where highest percent mortality (±SE) 87.02 (±1.08) was observed in case of combined use of higher dose of isolate Bb-08 (LC_{50}) and acetamiprid (LC_{50}). Moreover, (LC_{30}) of Bb-08 and insecticides (LC_{30}) mixture, showed highest percent mortality (43.26 ± 1.35) when isolate Bb-08(LC_{30}) was combined with acetamiprid (LC_{30}). While, in case of LC_{10} (Bb-08) and LC_{10} of insecticide, highest percent mortality (28.14 ± 0.67) was observed for isolate Bb-08(LC_{10}) and acetamiprid (LC_{10}).

Combined effects of fungi and insecticides

Longevity

In general, results indicated that male longevity decreased significantly as a result of treatment with fungi and insecticides mixtures especially at higher concentrations (Figs. 1a, b). Male longevity significantly varied among all treatments. A significant reduction was observed in male longevity at LC_{50} of isolate Bb-01 and LC_{50} of emamectin, reducing male longevity (±SE) of *M. domestica* to 7.83 (± 0.44) days followed by 8.18 (±0.41) days in LC_{50} of isolate Bb-01 and LC_{50} of lufenuron far less than all treatments as compared to the control ($F=13.25$, $P<0.0001$) (Fig. 1a).

Similar results were observed in case of Bb-08 where maximum reduction in male longevity was observed at LC_{50} of isolate Bb-01 and LC_{50} of emamectin reducing male longevity to 9.13 (±0.54) days ($F=6.43$, $P<0.0001$) (Fig. 1b).

In female longevity, highest reduction was observed in case of LC_{50} of isolate Bb-01 and LC_{50} of emamectin, 8.79 (± 0.35) days, followed by 8.88 (±0.25) days in combine treatment of LC_{50} of isolate Bb-01 and LC_{50} of acetamiprid as compared to all treatments ($F=31.29$, $P<0.0001$) (Fig. 1c). In addition, similar trend was observed in case of combine use of Bb-08 and insecticides, where the combine treatment of LC_{50} of Bb-08 and LC_{50} of emamectin caused least longevity (11.28 ±0.25) days of females ($F=6.37$, $P<0.0001$) (Fig. 1d).

Fecundity and percent hatching

A wide variation was observed in the fecundity among all the treatment levels of fungi and insecticides mixtures (Fig. 2a,b). Significant differences were observed in all treatments especially at highest level of fungal and insecticidal concentrations. Overall, the least number of eggs 113.50 (±5.72) and 123.00 (±8.25) were recorded in the treatment with LC_{50} of isolate Bb-01+ LC_{50} of emamectin (Fig. 2a) and LC_{50} of Bb-08+ LC_{50} of emamectin (Fig. 2b), respectively far less than that of other treatments including control.

The results showed wide variation in hatching percentage for all treatments (Fig. 2 c, d). The least egg hatching percentage was recorded in LC_{50} of Bb-01+LC_{50} of emamectin (68.80 ± 0.24) ($F=4.69$, $P=0.0001$) (Fig. 2c). While, in case of Bb-08, LC_{50} of isolate Bb-08 +LC_{50} of lufenuron (70.40 ± 0.59) ($F=4.88$, $P=0.0001$) showed

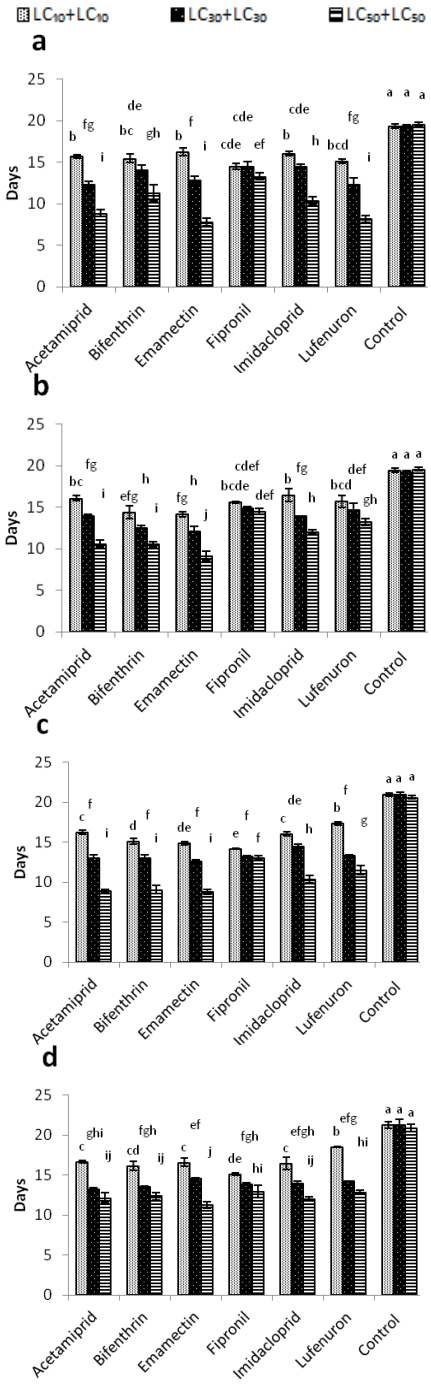

Fig. 1. Longevity (mean ± SE) of *M. domestica* adults after exposure to combined treatments of insect pathogenic fungi and insecticides. (a) Male longevity after application of Bb-01 and insecticides, (b) Male longevity after application of Bb-08 and insecticides, (c) Female longevity after application of Bb-01 and insecticides and (d) Female longevity after application of Bb-08 and insecticides. Same letters indicate that means are not significantly different.

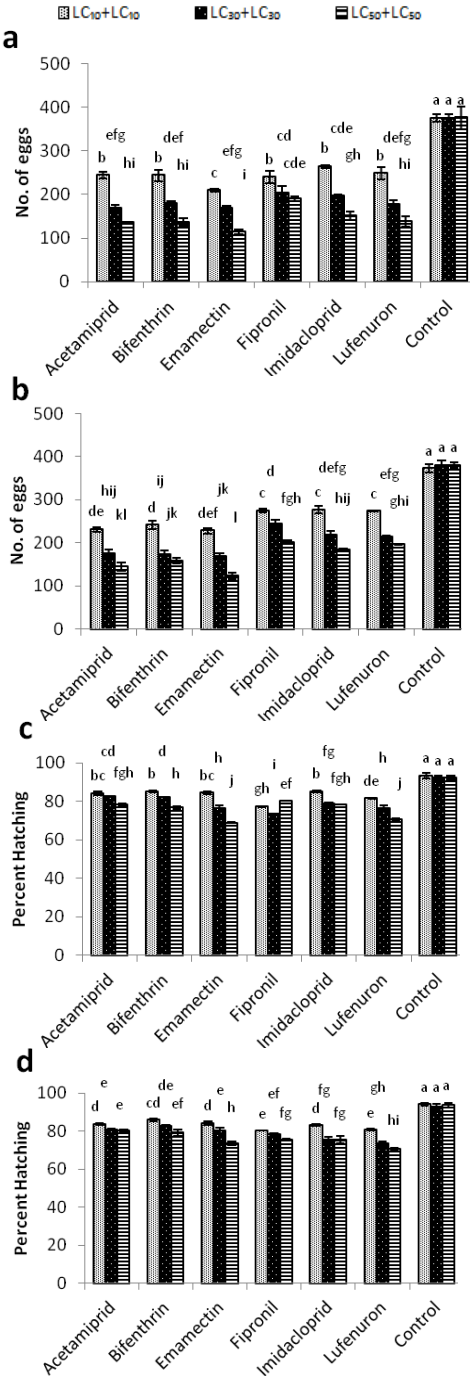

Fig. 2. Fecundity (mean ± SE) and percent hatching (mean ± SE) of *M. domestica* adults after exposure to combined treatments of insect pathogenic fungi and insecticides. **(a)** Fecundity after application of Bb-01 and insecticides, **(b)** Fecundity after application of Bb-08 and insecticides, **(c)** Percent hatching after application of Bb-01 and insecticides and **(d)** Percent hatching after application of Bb-08 and insecticides. Same letters indicate that means are not significantly different.

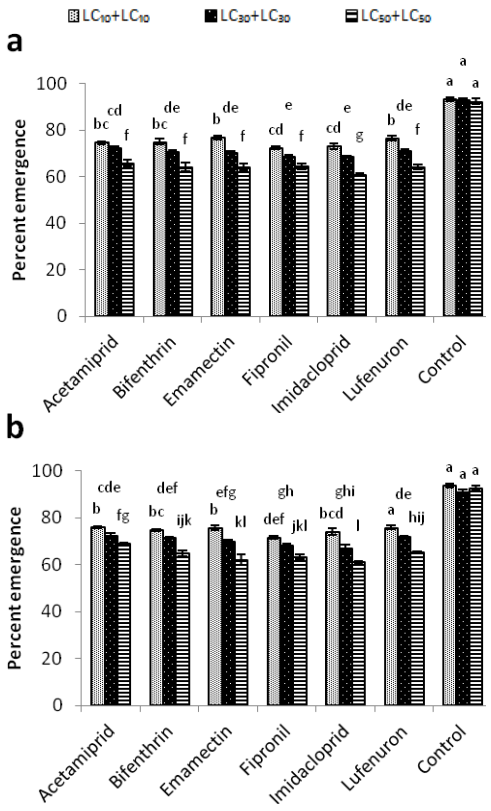

Fig. 3. Adult emergence (mean ± SE) of *M. domestica* adults after exposure to combined treatments of insect pathogenic fungi and insecticides. **(a)** Adult emergence after application of Bb-01 and insecticides. **(b)** Adult emergence after application of Bb-08 and insecticides. Same letters indicate that means are not significantly different.

maximum reduction in the hatching percentage of eggs of *M. domestica* (Fig. 2d).

Larval duration, percent pupation, pupal weight and pupal duration

The impact of fungi and insecticides mixtures on larval duration and percent pupation were observed (Table IV). The larval duration ranged from 6.60 to 8.75 days and significant prolongation in larval duration was observed in all treatments. In case of Bb-01, the maximum prolongation 8.56 (± 0.12) days was observed in treatment with LC_{50} (Bb-01)+LC_{50} of lufenuron (F=11.04, P.0001), while in case of Bb-08 maximum prolongation (8.90±0.26) days was observed in treatment with LC_{50} (Bb-08)+LC_{50} of imidacloprid as compared to other treatments including control (F=2.75, P=0.007).

Conversely, a significant decrease in percent pupation was observed for increasing level of fungi and insecticides mixtures. The minimum percent pupation in Bb-01 and insecticide mixtures was recorded in LC_{50} (Bb-01)+LC_{50} of emamectin (55.48±1.53) followed by LC_{50} (Bb-01)+LC_{50} of acetamiprid (56.53±1.53) (F=15.80, P<0.0001). Moreover in case of Bb-08, minimum percent pupation (57.55 ± 0.61) was observed in treatment with LC_{50} of isolate Bb-08+LC_{50} of acetamiprid (F=20.30, P<0.0001) as compared to other treatments and control.

The combined effects of fungi and insecticides mixtures caused significant decrease in pupal weight (Table V). In case of combined treatments of Bb-01 and insecticides, the least pupal weight (mg) 10.48 (± 0.30 mg) was observed in treatment with LC_{50} (Bb-01)+LC_{50} of imidacloprid followed by 10.86 (± 0.51 mg) in case of LC_{50} (Bb-01)+LC_{50} of bifenthrin (F=10.53, P<0.0001). In addition, for combined treatments of Bb-08 and insecticides, least pupal weight (11.15 ± 0.35) was observed in treatment containing LC_{50} (Bb-08)+LC_{50} of acetamiprid.

In general, a significant prolongation was observed in pupal duration in all treatments. For isolate Bb-01 and insecticides combinations, maximum pupal duration was observed in treatment with LC_{50} (Bb-01)+LC_{50} of lufenuron 8.29 (± 0.11) days (F=5.39, P<0.0001), while for isolate Bb-08, LC_{50} (Bb-08)+LC_{50} of emamectin showed maximum prolongation in pupal duration of 7.58 (± 0.05) days (F=5.53, P<0.0001) (Table V).

Adult emergence and sex ratio

The data showed a significant difference in the adult emergence as result of combined effects of fungi and insecticides mixtures on all treatment levels (Figs. 3a, b). For Bb-01, the lowest adult emergence (60.92±0.58) was observed in LC_{50} (Bb-01)+LC_{50} of imidacloprid, while in case of Bb-08, similar results were observed in treatment with LC_{50} (Bb-08)+LC_{50} of imidacloprid (61.25±0.42).

The combined treatment of fungi and insecticides showed significant difference in sex ratio. For Bb-01, the lowest female ratio was observed in LC_{50} (Bb-01)+LC_{50} of bifenthrin (F=1.81., P=0.03) (Table VI). While for Bb-08, lowest percentage of females was found in combined treatment of LC_{50} of Bb-08+LC_{50} of emamectin (F=2.21,P=0.02) (Table VI).

DISCUSSION

Insect pathogenic fungi have shown to be an effective biological control agent against *M. domestica* (Kaufman *et al*., 2005; Sharififard *et al.*, 2011a). In order to enhance the effectiveness, insect pathogenic fungi can be incorporated with the insecticides doses (Pachamuthu and Kamble, 2000). In our study, concentrations of pathogenic fungi and insecticides that caused 10%, 30%

Table II.- Mortality rates of housefly after individual application of fungus and insecticides (LC_{10}, LC_{30} andLC_{50}).

Name	LC_{10} (%±SE)	LC_{30} (%±SE)	LC_{50} (%±SE)
Bb-01	8.60 ± 0.30d	21.64 ± 0.38c	39.35 ± 0.32e
Bb-08	6.30 ± 0.41e	19.70 ± 0.45d	36.70 ± 0.48f
Acetamiprid	11.30 ± 0.14c	31.40 ± 0.46ab	53.20 ± 0.32b
Bifenthrin	13.30 ± 0.20bc	31.40 ± 0.12ab	51.60 ± 0.25c
Emamectin	15.80 ± 0.25ab	33.61 ± 0.23a	55.40 ± 0.68a
Fipronil	17.6± 0.48a	34.30 ± 0.25a	56.30± 0.61a
Imidacloprid	11.62 ± 0.68c	28.53 ± 0.61	49.50 ± 0.47d
Lufenuron	16.17 ± 0.21a	31.60 ± 0.78ab	51.30 ± 0.12c
F-value	34.5	121.3	65.7
P-value	0.02	0.00	0.01
LSD-value	1.35	3.21	2.24

Where LC_{10},LC_{30} and LC_{50} represent different levels of fungal and insecticides concentrations
Mortality data for fungi =7 days after treatment
Mortality data for insecticides =3 days after treatment

Table III.- Effect of combinations of entomopathogenic fungi and insecticides on percent mortality (±SE) of *M. domestica*

	Bb-01					Bb-08				
	Expected mortality	**Observed Mortality**	**T-test**	**p-value**	**M_{fi}-M_e**	**Expected mortality**	**Observed Mortality**	**T-test**	**P-value**	**M_{fi}-M_e**
LC_{10}+LC_{10}										
Acetamiprid	18.62±0.41	30.84±0.52	2.46	**0.032**	12.2169	16.32±0.82	28.14±0.67	3.45	**0.034**	11.8169
Bifenthrin	20.13±0.45	21.55±1.09	1.1	0.17	1.4189	17.83±0.21	11.78±0.63	0.36	0.49	-6.0511
Emamectin	21.90±1.24	28.54±1.66	3.1	**0.01**	6.6364	19.60±0.76	23.98±1.24	4.52	**0.021**	4.3764
Fipronil	23.10±0.32	17.60±0.95	1.21	0.21	-5.5024	20.80±0.51	15.18±0.30	0.75	0.31	-5.6224
Imidacloprid	18.87±0.21	21.33±0.35	1.02	**0.01**	2.460244	16.57±0.48	20.48±0.31	4.22	**0.021**	3.910244
Lufenuron	22.15±0.46	34.57±2.06	2.31	**0.012**	12.41469	19.86±0.43	21.53±0.90	4.31	**0.012**	1.674689
LC_{30}+LC_{30}										
Acetamiprid	43.18±0.88	56.71±0.24	4.78	**0.042**	13.5296	41.24±0.33	43.26±1.35	5.43	**0.031**	2.0196
Bifenthrin	43.18±1.34	32.33±1.50	0.42	0.83	-10.8504	41.24±0.45	21.75±0.94	0.71	0.94	-19.4904
Emamectin	43.95±1.78	63.80±0.90	1.78	**0.012**	19.84632	42.01±0.81	42.75±1.85	2.32	**0.041**	0.736321
Fipronil	44.18±2.45	26.24±1.34	0.61	0.71	-17.9351	42.24±0.18	22.9±1.20	0.21	0.74	-19.3351
Imidacloprid	42.03±1.57	43.01±1.31	0.82	**0.01**	0.979609	40.09±0.51	40.80±0.96	1.02	**0.02**	0.709609
Lufenuron	43.25±0.95	52.68±1.93	2.51	**0.014**	9.4256	41.31±0.32	48.08±1.52	2.11	**0.024**	6.7656
LC_{50}+LC_{50}										
Acetamiprid	64.25±2.11	91.43±0.64	8.31	**0.021**	27.1824	61.59±0.54	87.02±1.08	0.89	**0.013**	25.4224
Bifenthrin	64.32±1.56	49.63±3.06	0.69	0.53	-14.6944	61.67±0.31	37.76±3.15	0.71	0.73	-23.9144
Emamectin	64.05±1.89	87.28±2.64	4.81	**0.032**	23.2216	61.41±0.98	78.33±1.84	1.53	**0.023**	16.9216
Fipronil	63.95±0.93	43.08±1.54	9.72	0.24	-20.8731	61.30±1.23	33.10±0.66	0.13	0.92	-28.2031
Imidacloprid	64.34±1.03	68.33±1.01	0.43	**0.019**	3.9825	61.69±0.67	62.85±2.82	1.01	**0.021**	1.1525
Lufenuron	64.33±2.45	75.10±1.40	5.31	**0.035**	10.7669	61.68±2.34	67.73±2.58	1.56	**0.035**	6.0469

M_{fi} = observed mortality of mixture
*****Expected Mortality Me = Mf + Mi (1 - Mf/100) with Mf and Mi observed mortalities caused by fungus and insecticides alone respectively. Results shows outcome of paired sample T-test comparison of both observed and expected mortality rates (means ±SE), with significant effect in bold. Mortality data was accounted for 7 consecutive days after treatment

Table IV.- Sublethal effects of entomopathogenic fungi and insecticides mixtures on larval duration and percentage pupation of *M. domestica*.

	Larval duration (days ± SE)						Percent pupation (%± SE)					
	Bb-01			Bb-08			Bb-01			Bb-08		
	$LC_{10}+LC_{10}$	$LC_{30}+LC_{30}$	$LC_{50}+LC_{50}$	$LC_{10}+LC_{10}$	$LC_{30}+LC_{30}$	$LC_{50}+LC_{50}$	$LC_{10}+LC_{10}$	$LC_{30}+LC_{30}$	$LC_{50}+LC_{50}$	$LC_{10}+LC_{10}$	$LC_{30}+LC_{30}$	$LC_{50}+LC_{50}$
Acetamiprid	7.63 ± 0.04ef	7.72 ± 0.03de	7.81 ± 0.05de	7.47 ± 0.21hi	7.78 ± 0.02fgh	8.00 ± 0.06efg	75.25 ± 0.69f	65.18 ± 1.10hi	56.53 ± 1.53k	75.48 ± 1.1cde	63.98 ± 0.26hi	57.55 ± 0.61k
Bifenthrin	7.20 ± 0.04h	7.47 ± 0.06fg	8.29 ± 0.05ab	7.33 ± 0.05i	7.73 ± 0.04gh	8.42 ± 0.12bcd	77.39 ± 0.76ef	69.36 ± 1.52gh	61.81 ± 1.70ij	81.03 ± 0.24b	73.30 ± 1.07e	62.08 ± 0.50ij
Emamectin	7.33 ± 0.04gh	7.91 ± 0.03cd	8.55 ± 0.05a	7.68 ± 0.09ghi	8.35 ± 0.05cde	8.82 ± 0.04ab	83.16 ± 0.69cd	75.06 ± 1.10f	55.48 ± 1.53kl	82.30 ± 0.43b	73.03 ± 0.65ef	61.03 ± 0.48j
Fipronil	7.32 ± 0.13gh	7.76 ± 0.04de	8.42 ± 0.08a	8.16 ± 0.21def	8.50 ± 0.11abcd	8.57 ± 0.08abc	80.88 ± 0.52de	77.73 ± 0.25ef	65.81 ± 0.97hi	80.68 ± 0.30b	78.03 ± 0.76c	73.58 ± 0.88de
Imidacloprid	7.34 ± 0.13gh	7.74 ± 0.08def	8.34 ± 0.07ab	8.57 ± 0.23abc	8.75 ± 0.09ab	8.90 ± 0.26a	83.23 ± 0.88cd	75.56 ± 2.01ef	71.96 ± 0.30fg	81.50 ± 1.12b	70.48 ± 0.46f	64.60 ± 1.72hi
Lufenuron	7.68 ± 0.13def	8.10 ± 0.04bc	8.56 ± 0.12a	7.53 ± 0.12hi	8.16 ± 0.02def	8.55 ± 0.21abc	75.11 ± 0.97f	71.00 ± 0.56g	62.79 ± 1.72ij	76.25 ± 1.16cd	69.18 ± 0.64g	65.23 ± 0.93h
Control	6.61 ± 0.19i	6.60 ± 0.39i	6.56 ± 0.30i	6.66 ± 0.28j	6.52 ± 0.21j	6.76 ± 0.37j	93.68 ± 3.58a	92.58 ± 5.72a	93.35 ± 4.61a	92.40 ± 4.11a	93.08 ± 3.27a	91.60 ± 5.10a
F		11.04			2.75			15.80			20.30	
P		0.000			0.007			0.000			0.000	
LSD		0.27			0.41			3.63			2.74	

Means followed by same letters in rows and columns are not statistically different; LSD, P<0.05
LC_{10}, LC_{30} and LC_{50} represent different doses of insecticides
LC_{10},LC_{30} and LC_{50} represent different fungal concentrations levels
ns; represents non significance at 5% level of significance

Table V.- Sublethal effects of entomopathogenic fungi and insecticides mixtures on pupal weight and pupal duration of *M. domestica*

	Pupal weight (days ± SE)						Pupal duration (days ± SE)					
	Bb-01			Bb-08			Bb-01			Bb-08		
	$LC_{10}+LC_{10}$	$LC_{30}+LC_{30}$	$LC_{50}+LC_{50}$	$LC_{10}+LC_{10}$	$LC_{30}+LC_{30}$	$LC_{50}+LC_{50}$	$LC_{10}+LC_{10}$	$LC_{30}+LC_{30}$	$LC_{50}+LC_{50}$	$LC_{10}+LC_{10}$	$LC_{30}+LC_{30}$	$LC_{50}+LC_{50}$
Acetamiprid	16.10 ± 0.37cd	14.86 ± 0.37f	13.18 ± 0.39g	15.85 ± 0.21de	13.50 ± 0.47gh	11.15 ± 0.35k	6.14 ± 0.09g	7.28 ± 0.20cd	7.75 ± 0.10bc	5.95 ± 0.10g	6.83 ± 0.22bc	7.23 ± 0.13ab
Bifenthrin	16.86 ± 0.25bc	15.13 ± 0.22def	10.86 ± 0.51h	17.33 ± 0.09bc	14.25 ± 0.03fg	11.73 ± 0.65jk	6.06 ± 0.16g	7.40 ± 0.09cd	6.94 ± 0.34de	6.03 ± 0.19f	7.48 ± 0.15a	7.53 ± 0.09a
Emamectin	17.40 ± 0.17b	16.17 ± 0.21cd	11.42 ± 0.44h	16.20 ± 0.51cde	14.30 ± 0.71fg	13.25 ± 0.41hi	5.95 ± 0.26g	6.80 ± 0.28ef	7.55 ± 0.20bc	6.25 ± 0.06e	7.28 ± 0.05a	7.58 ± 0.05a
Fipronil	15.50 ± 0.16def	15.35 ± 0.35def	13.28 ± 1.04g	16.80 ± 0.35bc	16.15 ± 0.23cde	15.63 ± 0.13e	6.19 ± 0.04g	7.45 ± 0.10bcd	7.42 ± 0.49bcd	6.26 ± 0.04e	7.26 ± 0.05a	7.24 ± 0.02ab
Imidacloprid	15.85 ± 0.22cde	14.93 ± 0.29ef	10.48 ± 0.3h	16.65 ± 0.16bcd	15.58 ± 0.17e	12.45 ± 0.18ij	6.10 ± 0.10g	7.28 ± 0.08cde	7.93 ± 0.13ab	5.98 ± 0.16g	6.60 ± 0.12cde	6.70 ± 0.14c
Lufenuron	16.67 ± 0.24bc	16.15 ± 0.10cd	11.46 ± 0.45h	16.33 ± 0.37cde	16.25 ± 0.06cde	14.63 ± 0.20f	6.34 ± 0.12fg	7.55 ± 0.16bc	8.29 ± 0.11a	6.20 ± 0.23e	6.44 ± 0.33cde	6.69 ± 0.17cd
Control	19.44 ± 0.15a	19.34 ± 0.24a	19.11 ± 0.19a	19.41 ± 0.17a	19.29 ± 0.23a	19.19 ± 0.18a	5.35 ± 0.16h	5.33 ± 0.09h	5.40 ± 0.09h	5.43 ± 0.11h	5.43 ± 0.11h	5.22 ± 0.11h
F		10.53			14.05			5.39			5.53	
P		0.000			0.000			0.000			0.000	
LSD		1.04			0.85			0.51			0.43	

Means followed by same letters in rows and columns are not statistically different; LSD, P<0.05
LC_{10}, LC_{30} and LC_{50} represent different doses of insecticides
LC_{10}, LC_{30} and LC_{50} represent different fungal concentrations levels
ns; represents non significance at 5% level of significance

Table VI.- Sublethal effects of entomopathogenic fungi and insecticides mixtures on sex ratio (♀/(♀+♂))(%± SE) of *M. domestica*

	♀ (%± SE)					
	Bb-01			Bb-08		
	$LC_{10}+LC_{10}$	$LC_{30}+LC_{30}$	$LC_{50}+LC_{50}$	$LC_{10}+LC_{10}$	$LC_{30}+LC_{30}$	$LC_{50}+LC_{50}$
Acetamiprid	49.50 ± 1.94bc	49.25 ± 2.02bc	48.75 ± 1.80bc	50.75 ± 1.84abc	49.75 ± 1.03bcd	48.25 ± 1.60cde
Bifenthrin	52.25 ± 1.80ab	51.00 ± 2.6ab8	46.75 ± 1.03bcd	47.00 ± 0.82cde	51.00 ± 1.78abc	46.50 ± 0.96de
Emamectin	50.75 ± 2.17ab	49.75 ± 2.50 bc	48.00 ± 2.12bc	48.25 ± 1.97 cde	50.00 ± 2.42bcd	45.00 ± 0.58e
Fipronil	51.25 ± 1.65ab	48.05 ± 0.68bc	49.60 ± 1.61bc	48.22 ± 0.48 cde	48.47 ± 1.38 cde	52.82 ± 2.05ab
Imidacloprid	50.20 ± 1.61b	48.00 ± 0.67bc	49.65 ± 1.60bc	47.50 ± 0.69 cde	53.05 ± 1.88a	50.35 ± 2.60bcd
Lufenuron	48.18 ± 0.28cd	51.20 ± 1.47ab	54.75 ± 1.03a	54.25 ± 0.95a	50.35 ± 1.46bcd	48.05 ± 0.68 cde
Control	48.50 ± 0.65cd	48.88 ± 0.66cd	49.25 ± 0.48bc	48.50 ± 0.65 cde	49.35 ± 0.41bcd	48.50 ± 0.65 cde
F		1.81			2.21	
P		0.03			0.02	
LSD		4.51			4.25	

Means followed by same letters in rows and columns are not statistically different; LSD, P<0.05
LC_{10},LC_{30} and LC_{50} represent different fungal concentrations levels
LC_{10}, LC_{30} and LC_{50} represent different doses of insecticides
ns; represents non significance at 5% level of significance

and 50% mortality were measured and these arbitrary concentrations of insecticides and entomopathogenic fungi were considered for further experimentation. Later, these fungal and insecticides concentrations were mixed and tested against *M. domestica* for the effects on different biological parameters of house fly. Mortality of binary treatments of entomopathogenic fungi and insecticides showed a considerably higher trend than the mortality caused by entomopathogenic fungi and insecticides alone (Tables II, III).

The differences among the efficacy of fungi and insecticides is due to different mode of actions when applied alone. However, the exact mechanisms for effects of fungi and insecticide mixtures are unclear, insecticides may influence the insect cuticle and facilitate penetration for fungal spores, or possibly restrain immune response and facilitate fungal infection process (Hiromori and Nishigaki, 2001). Moreover, insecticides inhibit vegetative growth and spore germination of fungi depending upon nature of active ingredient. In addition, higher toxicity in vitro does not indicate that similar phenomena should occur in field (Archana and Ramaswamy, 2012). Similarly, according to the LC_{10} ranking the most toxic insecticides are much less efficient when combined with *B. bassiana* highlights the influence of insecticides on the pathogenic fungi and make it is less compatible as compared to other insecticides, which show more toxicity when used in combination with *B. bassiana.*

The effectiveness of *B. bassiana* has been demonstrated for controlling *M. domestica.* Besides effectiveness of pathogenic fungi against *M. domestica*, it is important to find approaches that reduce the lethal time by entomopathogenic fungi (Kaufman *et al*., 2005; Mishra *et al*., 2011; Sharififard *et al*., 2011a). The potential use fungi and insecticides in combination have been focused in previous studies (Pachamuthu and Kamble, 2000; Zurek *et al*., 2002; Jaramillo *et al*., 2005; Thompson and Brandebburg, 2006; Ericsson *et al*., 2007; Sharififard *et al*., 2011b). Most of the studies regarding combined mixtures of insect pathogenic fungi and insecticides were mainly concerned to the mortality of host insect. However, sublethal effects on biological parameters have been overlooked especially for the *M. domestica*, which may increase the susceptibility of affected insects to other natural enemies.

In addition to direct effect on adult mortality, combined treatments of fungus and insecticide significantly reduced the adult longevity as compared to the average survival time of adult. Due to the reduced life expectancy of the female, the number of eggs were also affected by the treatment. Similar results have been reported for other dipterans where pathogenic fungi resulted in the decreased survival and fecundity with the increasing dose (Flores *et al*., 2004; Pelizza *et al*., 2013), while egg hatching percentage was significantly different among all the treatments. In addition to this, similar results were reported by Khodadad *et al.* (2007) where significant effects of *M. anisopliae, B. bassiana* and *Lecanicillium psalliotae* on the percent egg hatchability of *Rhipicephalus* (*Boophilus*) *annulatu* were observed.

Larval duration of *M. domestica* in the current study was significantly prolonged in all treatments. However, the larval duration was not significantly different at higher doses of fungi and insecticides mixtures. These results are not in accordance with Poprawski *et al.* (1998)

where no significant effect was observed on larval duration of *Serangium parcesetosum* Sicard. (Coleoptera: Coccinellidae) when tested against entomopathogenic fungi. The results showed significantly different pupation percentage among all treatments as compared to the control. In addition to this, pupal weight was also influenced by the sublethal effects of combined mixtures of fungi and insecticides. Similar kind of results were observed when *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) was investigated for the effect of sublethal concentrations of spinosad, where percentage pupation and pupal weight significantly differed from the control group (Abouelghar *et al.*, 2013; Rehan and Freed, 2015). The findings of the current research showed a significant prolongation in pupal duration in all treatments as compared to the control which are in accordance to results of Hafez *et al*. (1997) where pupae of *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae) showed prolonged duration when treated with *B. bassiana*. These findings are contrary to Poprawski *et al*. (1998) where no significant differences were observed in pupal duration as compared to the control.

In the current research adult emergence was not significantly affected on all levels of combined mixtures. This is contrary to other research, where adult emergence was observed to be affected in treatments with plant materials for *M. domestica* and other dipertans (Muse *et al*., 2003; Khalaf *et al.*, 2009; Elkattan *et al.*, 2011). For sex ratio, significant difference was found only at lower levels of fungus/insecticide mixtures. Disturbance in sex ratio was detected due to mixture of fungi and insecticides as recorded by other studies (Robert and Olson, 1989; Shaalan *et al*., 2005).

The results of the present study regarding entomopathogenic fungi and insecticides mixtures on biological parameters of *M. domestica* suggest that the combination of fungi and insecticides prevented the normal developmental stages and duration of *M. domestica*. The dosage of synthetic insecticides can act as physiological stressors and/or behavioral modifiers, thereby predisposing insects to diseases (Inglis *et al*., 2001). The combined mixtures of isolate Bb-01 with acetamiprid, emamectin, imidacloprid, or lufenuron significantly altered the normal development of *M. domestica*. Integrating entomopathogenic fungi with insecticides may have advantages. This approach will not only increase mortality in pest and possibility can reduce the time to kill.

CONCLUSION

In conclusion, the combined use of insect pathogenic fungi and a chemical insecticide may be an important component of integrated pest management of *M. domestica*, which needs to be tested under field conditions to determine its efficacy.

Statement of conflict of interest

Authors have declared no conflict of interest.

REFERENCES

Abbott, W.S., 1925. A method for computing the effectiveness of an insecticide. *J. econ. Ent*., **18**: 265–267.

Abouelghar, G.E., Sakr, H., Ammar, H.A., Yousef, A. and Nassar, M., 2013. Sublethal effects of spinosad (Tracer®) on the cotton leafworm (Lepidoptera: Noctuidae). *J. Pl. Prot. Res*., **53**: 275–284.

Ande, A.T., 2001. Biological activities of some plant materials against the house fly *Musca domestica*. *Nigerian Soc. Exp. Biol. (NISEB) J*., **1**: 293–296.

Archana, M.R. and Ramaswamy, K., 2012. Interactive effect of entomopathogenic fungi *Paecilomyces fumosoroseus* with few organophosphate and pyrethroid pesticides: an in vitro study. *Indian J. Fund. appl. Life Sci*., **2**: 10–17.

Bell, H.A., Robinson, K.A. and Weaver, R.J., 2010. First report of cyromazine resistance in a population of UK house fly (*Musca domestica*) associated with intensive livestock production. *Pest Manage. Sci*., **66**: 693–695.

Cao, M.X., Song, F.L., Zhao, T.Y., Dong, Y.D., Sun, Ch, X. and Lu, B.L., 2006. Survey of deltamethrin resistance in houseflies (*Musca domestica*) from urban garbage dumps in Northern China. *Environ. Ent*., **35**: 1–9.

Crystal, M.M., 1964. Insect fertility: inhibition by folic acid derivatives. *Science, N. S.*, **144**: 308–309.

Elkattan, N.A.I., Ahmed, K.S., Elbermawy, S.M. and Abdel-Gawad, R.M., 2011. Effect of some botanical materials on certain biological aspects of the house fly, *Musca domestica* L. *Egyptian J. Hosp. Med*., **42**: 33–48.

Ericsson, J.D., Kabaluk, J.T., Goettel, M.S. and Myers, J.H., 2007. Spinosad interacts synergistically with the insect pathogen *Metarhizium anisopliae* against the exitic wireworm *Agriotes lineatus* and *Agriotes obscurus* (Coleoptera: Elatridae). *J. econ. Ent*., **100**: 31–38.

Farenhorst, M., Knols, B.G.J., Thomas, M.B., Howard, A.F.V., Takken, W., Rowland, M. and Guessan, R., 2010. Synergy in efficacy of fungal entomopathogens and permethrin against west African insecticide-resistant *Anopheles gambiae* mosquitoes. *PLoS ONE*, **5**: e12081.

Fletcher, M.G., Axtell, R.C. and Stinner, R.E., 1990. Longevity and fecundity of *Musca domestica* (Diptera: Muscidae) as a function of temperature. *J. med. Ent*., **27**: 922–926.

Flores, A.E., Garcia, G.P., Badii, M.H., Tovar, L.R. and Salas, I.F., 2004. Effects of sublethal concentrations of vectovac® on biological parameters of *Aedes aegypti*. *J. Am. Mosq. Contr. Assoc*., **20**: 412–417.

Forester, M., Klimpel, S. and Sievert, K., 2009. The house fly (*Musca domestica*) as a potential vector of metazoan parasites caught in a pig-pen in Germany. *Vet. Parasitol*., **160**: 163–167.

Freed, S., Jin, F.L. and Ren, S.X., 2011a. Determination of genetic variability among the isolates of *Metarhizium anisopliae* var. *anisopliae* from different geographical origins. *World J. Microbiol. Biotechnol*., **27**: 359–370.

Freed, S., Jin, F.L. and Ren, S.X., 2011b. Phylogenetics of entomopathogenic fungi isolated from the soils of different ecosystems. *Pakistan J. Zool*., **43**: 417–425.

Hafez, M., Zaki, F.N., Moursy, A. and Sabbour, M., 1997. Biological effects of the entomopathogenic fungus, *Beauveria bassiana* on the potato tuber moth, *Phthorimaea operculella* (Seller). *J. Pestic. Sci*., **70**: 158–159.

Hiromori, H. and Nishigaki, J., 2001. Factor analysis of synergistic effect between the entomopathogenic fungus *Metarhizium anisopliae* and synthetic insecticides. *Appl. Ent. Zool*., **36**: 231–236.

Inglis, G.D., Goettel, M.S., Butt, T.M. and Strasser, H., 2001. Use of hyphomycetous fungi for managing insect pests. In: *Fungi as biocontrol agents. Progress, problems and potential* (eds. T.M. Butt, C. Jackson and N. Magan), CABI Publishing NY. pp. 23–69.

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

Kaufman, P.E., Reasor, C., Rutz, D.A., Ketzis, J.K. and Arends, J.J., 2005. Evaluation of *Beauveria bassiana* applications against adult house fly, *Musca domestica*, in commercial caged-layer poultry facilities in New York State. *Biol. Contr*, **33**: 360–367.

Khalaf, A.F.A., Hussein, K.T. and Shoukry, K.K., 2009. Biocidal activity of two botanical volatile oils against the larvae of *Synthesiomyia nudiseta* (Wulp) (Diptera: Muscidae). *Egypt. Acad. J. biol. Sci*., **2**: 89–101.

Khan, M.F. and Ahmed, S.M., 2000. Toxicity of crude neem leaf extract against houseflies *Musca domestica* adult as compared with DDVP, dichlorvos. *Turk. J. Zool*., **24**: 219–223.

Khan, S., Guo, L.H., Maimaiti, Y., Mijit, M. and Qiu, D.W., 2012. Entomopathogenic fungi as microbial biocontrol agent. *Mol. Pl. Breed*., **3**: 63–79.

Khazanie, R., 1979. *Elementary statistics*. Good Year Publishing Co., California, U.S.A.

Khodadad, P.K., Hamidreza, H., Mehdi, R.A., Saeed, B., Rasoul, Z., Mehran, G. and Masoomeh, S.G., 2007. Biological control of *Rhipicephalus* (Boophilus) *annulatus* by different strains of *Metarhizium anisopliae*, *Beauveria bassiana* and *Lecanicillium psalliotae* fungi. *Parasitol. Res*., **100**: 1297–1302.

Koppenhöfer, A.M. and Kaya, H.K., 1998. Synergism of imidacloprid and entomopathogenic nematodes: A novel approach to white grub control in turfgrass. *J. Econ. Ent*., **91**: 618-623.

Lecouna, R.E., Turica, M., Tarocco, F. and Crespo, D.C., 2005. Microbial control of *Musca domestica* (Diptera: Muscidae) with selected strains of *Beauveria bassiana*. *J. Med. Ent*., **42**: 332–336.

Leora Software, Polo PC. 2003. *A user's guide to probit and logit analysis*. LeOra Software, Berkeley, CA, USA.

Malik, A., Singh, N. and Satya, S., 2007. House fly (*Musca domestica*): A review of control strategies for a challenging pest. *J. Environ. Sci. Hlth. B*., **42**: 453–469.

Mishra, S., Kumar, P., Malik, A. and Satya, S., 2011. Adulticidal and larvicidal activity of *Beauveria bassiana* and *Metarhizium anisopliae* against house fly, *Musca domestica* (Diptera: Muscidae), in laboratory and simulated field bioassays. *Parasitol. Res*., **108**: 1483–1492.

Muse, W.A., Lajide, L. and Adediri, C.O., 2003. Effect of some Nigerian plants on survival, oviposition, and emergence of adult blowfly, *Chrysomya chloropyga* (Wied.) (Diptera: Calliphoridae). *J. Asia-Pacific Ent*., **6**: 69–75.

Pachamuthu, P. and Kamble, S.T., 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. Ent*., **93**: 60–70.

Pelizza, S.A., Scorsetti, A.C. and Tranchida, M.C., 2013. The sublethal effects of the entomopathic fungus *Leptolegnia chapmanii* on some biological parameters of the dengue vector *Aedes aegypti*. *J. Insect Sci*., **13**: 1–8.

Poprawski, T.J., Legaspi, J.C. and Parker, P.E., 1998. Influence of fungi on *Serangium parcesetosum* (Coleoptera: Coccinellidae), an important predator of whiteflies (Homoptera: Alyerodidae). *Environ. Ent*., **27**: 785–795.

Quesada-Moraga, E., Maranhao, E.A.A,, Valverde-GarcÍa, P. and Santiago-Álvarez, C., 2006. Selection of *Beauveria bassiana* isolates for control of the whiteflies *Bemisia tabaci* and *Trialeurodes vaporarium* on the basis of their virulence, thermal requirement and toxicogenic activity. *Biol. Contr*., **36**: 274–287.

Rehan, A. and Freed, S., 2015. Lethal and sub-lethal effects of spinosad on the life-history traits of army worm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae), and its fitness cost of resistance. *Entomol. Res*., **45**: 247-253

Robert, L.L. and Olson, J.K., 1989. Effects of sublethal dosages of insecticides on *Culex quinquefasciatus*. *J. Am. Mosq. Contr. Assoc*., **5**: 239–246.

Saleh, A. and, Elmosa, H., 2002. Toxicities of several insecticides to the house fly *Musca domestica* from different regions in Jordon. *Sarhad J. Agric.*, **18**: 69–75.

Shaalan, E.A.S., Canyon, D.V., Younes, M.W.F., Abdel-Wahab, H. and Mansour, A.H., 2005. Effects of sub-lethal concentrations of synthetic insecticides and *Callitris glaucophylla* extracts on the development of *Aedes aegypti*. *J. Vector Ecol*., **30**: 295–298.

Sharififard, M., Mossadegh, M.S., Vazirianzadeh, B. and Mahmoudabadi, A.Z., 2011a. Laboratory evaluation of pathogenicity of entomopathogenic fungi, *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metch.) Sorok. to larvae and adults of the house fly, *Musca domestica* L. (Diptera: Muscidae). *Asian J. biol. Sci*., **4**: 128–137.

Sharififard, M., Mossadegh, M.S., Vazirianzadeh, B. and Mahmoudabadi, A.Z., 2011b. Interactions between entomopathogenic fungus, *Metarhizium anisopliae* and sublethal doses of spinosad for control of house fly, *Musca domestica*. *Iran. J. Arthrop. Born. Dis.*, **5**: 28–36.

Skovgård, H. and Steenberg, T., 2002. Activity of pupal parasitoids of the stable fly *Stomoxys calcitrans* and prevalence of entomopathogenic fungi in the stable fly and the house fly *Musca domestica* in Denmark. *BioControl*, **47**: 45–60.

Statistix Version 8.1: *Analytical software, User's manual Analytical Software*, Tallahassee, Florida; 2005.

Thompson, S.R. and Brandebburg, R.L., 2006. Effect of combining imidacloprid and Diatomaceous earth with *Beauveria bassiana* on mole cricket (Orthoptera: Gryllotapiade) mortality. *J. Econ. Ent*., **99**: 1948–1954.

Watson, D.W., Geden, C.G., Long, S.J. and Rutz, D.A., 1995. Efficacy of *Beauveria bassiana* for controlling the house fly and stable fly (Diptera: Musciadae). *Biol. Contr*., **5**: 405–411.

Zurek, L., Watson, D.W. and Schal, C., 2002. Synergism between *Metarhizium anisopliae* (Deuteromycotia: Hyphomycetes) and boric acid against the German cockroach (Dictyoptera: Blattellidae). *Biol. Contr*., **23**: 296–302.