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Infectivity of housefly, *Musca domestica* (Diptera: Muscidae) to different entomopathogenic fungi

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ABSTRACT

The housefly *Musca domestica* is a worldwide insect pest that acts as a vector for many pathogenic diseases in both people and animals. The present study was conducted to evaluate the virulence of different local isolates of *Beauveria bassiana*, *Metarhizium anisopliae* and *Isaria fumosorosea* on *M. domestica* using two bioassay techniques: (1) adult immersion and (2) a bait method applied to both larvae and adults. The results showed evidence of a broad range of responses by both stages (larvae and adults) to the tested isolates of *B. bassiana*, *M. anisopliae* and *I. fumosorosea*. These responses were concentration-dependent, with mortality percentages ranging from 53.00% to 96.00%. Because it resulted in lower LC_{50} values and a shorter lethal time, *B. bassiana* (Bb-01) proved to be the most virulent isolate against both housefly larvae and adults. Sublethal doses of the tested isolates were also assessed to evaluate their effect on *M. domestica* fecundity and longevity. The fungal infections reduced housefly survival regardless of their sex and also decreased egg production in females.

Introduction

The housefly *Musca domestica* L. (Diptera: Muscidae) is a cosmopolitan insect responsible for causing annoyance, irritation, and food spoilage and is also an important pathogenic disease vector in both people and animals. Associations between houseflies and pathogens can result in disease outbreaks such as typhoid, cholera, tuberculosis, bacillary dysentery, infantile diarrhoea and anthrax.[1,2] Housefly habits—such as walking and feeding on trash and excrement—make them superlative agents for transferring disease-causing pathogens to human and animal populations.[3] Therefore, it is crucial to control *M. domestica* to improve the health of people, livestock and poultry.

Conventional insecticides are primarily used for control of *M. domestica* over the short term[5,6] but the haphazard use of insecticides has given rise to serious problems that include both insecticide resistance and the residual effects of the chemicals used in insecticides.[7] Insecticide resistance in houseflies has now become a global problem—and is increasing.[8] Currently, houseflies are resistant to almost all groups of conventional insecticides including organophosphates, organochlorines, carbamates and pyrethroids.[4,9–14] The problems regarding resistance, residual effects and high chemical costs have opened the door to other alternatives

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such as entomopathogenic fungi, which have the potential to control this insect pest.[15]

In comparison to synthetic insecticides, entomopathogenic fungi have low mammalian toxicity. In addition, their natural prevalence in housefly populations provides great potential for managing housefly populations.[16,17] A large number of cases have been reported to control houseflies, through rapid killing and high infection rates from fungi that include *Beauveria bassiana* (Bals.) Vuill., *Metarhizium anisopliae* (Metsch.) Sorok.[1,18–23] These studies have shown high mortality among housefly populations within 5–15 days; however, research efforts are still needed to explore which local isolates of the insect pathogenic fungi work effectively in which local environments and can thus compete with conventional insecticides. In accordance with the importance of housefly as a medical and veterinary pest, the current study was designed to investigate the effectiveness of local isolates of *B. bassiana*, *M. anisopliae* and *Isaria fumosorosea* (Wize) from Pakistan on housefly populations consisting of both larvae and adults and, additionally, to evaluate the effect of sublethal doses of fungi on the housefly fecundity and longevity.

Materials and methods

Insects

Adult *M. domestica* were collected from poultry farms in Multan, Punjab, Pakistan and reared in transparent cages (30 cm × 30 cm × 30 cm) with mesh screens on opposite sides and a cloth sleeve opening at the front. The adult flies were provided with sugar and powdered milk (3:1) in Petri dishes as diet and allowed water *ad libitum*. After 2–3 days of feeding, plastic cups containing larval diet (water based paste of wheat bran, rice meal, yeast, sugar and dry milk powder (40:10:3:3:1)) were placed in the cages as an egg laying substrate following the methods reported by Bell et al.[24] with slight modifications. When eggs became visible on the sides of cups or attached to the food, the cups were removed and kept separated for larval development. The larval food was changed every 2–4 days depending on the number of larvae per cup.

Entomopathogenic fungi

Fungal isolates

Nine different isolates of *B. bassiana*, *M. anisopliae* and *I. fumosorosea* were used for the experiments (Table 1). This study used slants of monoconidial cultures grown on potato dextrose agar (PDA) at 25 °C in darkness and then stored at 4 °C. For further propagation the spores from these slants were spread onto PDA plates (9 cm diameter) and kept at 25 °C in darkness at 70–75% RH (relative humidity) for 14 days.[25,26] After 14 days of growth the spores were used to treat the insects or stored at 4 °C until used for insect bioassays.

Conidial viability

For each isolate, conidia viability was determined by enumerating the percentage of germinated conidia 24 h after spreading on fresh PDA medium. A conidial suspension of 1×10^7 (0.01 mL) was spread on 9 cm petri plates containing 15 mL of PDA medium, incubated at 27 °C for 24 h for germination. Three 15 mm square cover slips were placed on the surface of medium. The germination percentage was determined by counting the number of germinated conidia and the total number of conidia per field of view under a microscope at 250× magnification.[27]

Fungal infections

The fungal spores were scraped from the PDA plates and mixed with sterile Tween80 (0.05%) solution. The resulting conidial concentration was determined using a haemocytometer. Insects were infected by a brief immersion in the conidial suspension of all fungal isolates. For mycosis development, the insects were maintained at high humidity (>75%) produced by artificial humidification. Insect mortality was recorded daily for seven consecutive days.

Method of infection of adult M. domestica

To assess the potential efficacy of entomopathogenic fungi against adults of *M. domestica*, the two following methods were employed as explained by Sharififard et al.[23] with slight modifications.

Immersion method

To check the infectivity of fungal isolates on 3–4-day-old *M. domestica* adults (male to female ratio 50:50), the insects were first anesthetised with CO_2. Then, batches of 28 individuals each were immersed for few seconds into each fungal suspension containing spores at different concentrations (1×10^6, 1×10^7, 1×10^8, 2×10^8, 3×10^8 spores/mL). After immersion, each batch of insects was placed on filter paper to remove excess moisture and then placed in small plastic containers

Table 1 – Isolates of entomopathogenic fungi from Pakistan and their origins/host tested for efficacy on the housefly *Musca domestica* in laboratory conditions.

S. No.	Fungal Species	Source (Habitat)	Location (Pakistan)
1.	*B. bassiana* (Bb-01)	Cotton field	Makhdoom Rasheed, Multan
2.	*B. bassiana* (Bb-08)	Pine forest soil	Naran, Mansehra
3.	*B. bassiana* (Bb-10)	River side soil	Naran, Mansehra
4.	*M. anisopliae* (Ma-2.3)	Cotton field	Makhdoom Rasheed, Multan
5.	*M. anisopliae* (Ma-4.1)	Maize field	Balakot, Mansehra
6.	*M. anisopliae* (Ma-11.1)	Canal side soil	Band Bosan, Multan
7.	*I. fumosorosea* (If-02)	Rove beetle	Multan
8.	*I. fumosorosea* (If-2.3)	Vegetable field	Makhdoom Rasheed, Multan
9.	*I. fumosorosea* (If-03)	Cotton field	Aadhi Bagh, Multan

(10 cm × 10 cm × 10 cm) at 27 ± 1 °C. Sugar, dry powdered milk and water were provided as a food source. Control flies were treated with 0.05% Tween80 solution only. All treatments were replicated four times. Mortality was recorded for 7 consecutive days at 24 h intervals. Insect cadavers were collected on a daily basis and placed in sterile petri dishes containing damp filter paper for sporulation.

Bait method

Five different doses of fungal isolates were tested on cohorts of 3–4-day-old flies (male to female ratio 50:50) in small plastic containers (10 cm × 10 cm × 10 cm). Each container held a petri dish lined with bait (powdered milk, sugar and distilled water (1:3:1)). For better fungal dispersion, 1 mL suspensions of the fungal strains at the test concentrations explained earlier were dispersed on the bait surfaces. The baits treated with different fungal concentrations were placed in the plastic containers for 48 h before being removed and replaced with dry bait (sugar + powdered milk (3:1)). Water was provided *ad libitum*. All treatments were replicated four times. Mortality data and all other procedures were performed as described earlier.

Method of infection of larvae of M. domestica

Immersion method

The virulence of different entomopathogenic fungal isolates was evaluated by directly dipping groups of 25 third instar larvae for 10 s into the conidial suspensions at different concentrations as explained earlier, while the control group was dipped into a 0.05% Tween80 solution only. Excess moisture was removed with aid of filter paper. Later, the larvae were transferred to a larval medium. There were four replications per treatment. Mortality data were recorded until pupation. All other procedures were performed as explained above.

Bait method

Different concentrations of each isolate of entomopathogenic fungi were prepared to determine the effectiveness of fungal isolates in larval medium as bait against housefly larvae. Plastic cups (5 cm × 5 cm × 5 cm) containing 10 g larval medium were treated with 1 mL of each concentration and replicated four times. Each cup was inoculated with 25 third instar larvae and maintained under conditions similar to those described earlier. Dead larvae and pupae were kept to monitor fungal sporulation.

Effect of entomopathogenic fungi on longevity and fecundity of M. domestica

Newly emerged adults over a 24 h period were taken from the already established laboratory population to assess the effect of sublethal dose of different entomopathogenic fungi on the fecundity and longevity of houseflies. A total of 40 flies with a sex ratio of 1:1 were used for each treatment with four replications. All the flies were treated by the immersion method with a sublethal dose (1×10^6 spores/mL) while control adults were dipped in Tween80, 0.05% solution. The treated and control groups were kept in small plastic containers (10 cm × 10 cm × 10 cm) and maintained at 27 ± 1 °C. All flies were provided with sugar as an adult diet and larval medium for egg laying.

Mortality and fecundity data were recorded every 24 h interval until all flies in each container were dead. Mortality was monitored by removing the dead flies, counting dead males and females separately, while fecundity data were collected by removing the larval media and counting the eggs under a dissecting microscope. The mean longevity for males and females was calculated by multiplying the number of flies that died each day by number of days that they survived, summing these values and then dividing by the initial number of flies. The mean numbers of eggs laid per female were calculated by dividing the total number of eggs laid over the entire experiment time by the initial number of flies.[28]

Data analysis

Mortality data were corrected using Abbott's formula.[29] The LC_{50} and LT_{50} values were calculated using probit analysis. The means for percent mortality, longevity and fecundity were analysed using analysis of variance (ANOVA) and means were separated by LSD at significance level of 5% using Statistix 8.1 software.

Results

Adult bioassays

Immersion test for adults

B. bassiana (Bb-01) caused maximum mortality (96.43 ± 3.57) of *M. domestica* adults with LC_{50} of 3.79×10^6 spores/mL within 4.20 days, while *I. fumosorosea* (If-02) showed the lowest LC_{50} value at 1.57×10^8 spores/mL. The results suggest that fungal isolates Bb-01, Bb-08, Bb-10, Ma-2.3, Ma-4.1 and If-2.3 caused 75.00–96.00% mortality in 4–6 days (Table 3). The data showed the least significant differences among the LC_{50} values of *M. anisopliae* (Ma-2.3, Ma-11.1) and *I. fumosorosea* (If-03, If-2.3). The mean percent mortality of housefly adults treated with different isolates *B. bassiana*, *M. anisopliae* and *I. fumosorosea* was dose dependent and increased with the increase in conidial concentration (Table 3).

Bait test for adults

Conidia of different isolates of *B. bassiana*, *M. anisopliae* and *I. fumosorosea* were dispersed on bait surfaces. The results shown in Table 2 reveal the effects of different isolates of entomopathogenic fungi on *M. domestica*. *B. bassiana* (Bb-01) caused the maximum mortality (89.29 ± 3.57%) of *M. domestica* adults with an LC_{50} of 6.58×10^6 spores/mL within 4.10 days (Table 3). Overall the fungal isolates (Bb-01, Bb-08, Bb-10, Ma-2.3, Ma-4.1 and If-2.3) caused 67.00–89.00% mortality within 4–6 days, while the results showed the least significant difference among the LC_{50} values of Bb-08 and Ma-2.3.

Immersion method for larvae

The data showed the mortality of housefly larvae to be concentration-dependent for all isolates. The mortality varied between 18.00% and 86.00% with LT_{50} values ranging from 5.24 (4.63–5.58) to 7.05 (6.42–7.75) (Table 5). The results of probit analysis designated (Bb-01) as the most virulent isolate with an $LC_{50} = 7.42 \times 10^6$ spores/mL and LT_{50} values of 5.21, 5.61 and 5.91 days at 3×10^8, 2×10^8, and 1×10^8 spores/mL,

Table 2 – LC_{50} (spores/mL) values of entomopathogenic fungi against adults of *M. domestica* by immersion and bait methods.

Fungi	Isolate(s)	Immersion method				Bait method			
		LC_{50}	FD[a]	Slope	χ^2	LC_{50}	FD[a]	Slope	χ^2
B. bassiana	Bb-01	3.79×10^6	1.27×10^6–1.13×10^7	0.65 ± 0.13	2.31	6.58×10^6	1.70×10^6–2.54×10^7	0.46 ± 0.12	3.05
	Bb-08	9.05×10^6	2.20×10^6–3.72×10^7	0.41 ± 0.12	1.55	2.15×10^7	6.29×10^6–7.36×10^7	0.41 ± 0.12	0.57
	Bb-10	1.96×10^7	5.11×10^6–7.57×10^7	0.38 ± 0.12	2.11	5.88×10^7	$1.50^7 \times 10$–2.30×10^8	0.36 ± 0.12	0.39
M. anisopliae	Ma-2.3	3.38×10^7	1.06×10^7–1.07×10^8	0.46 ± 0.09	1.72	2.41×10^7	5.57×10^6–1.05×10^8	0.34 ± 0.12	1.54
	Ma-4.1	1.30×10^7	4.05×10^6–4.16×10^7	0.47 ± 0.12	1.71	1.14×10^7	2.23×10^6–5.88×10^7	0.34 ± 0.12	1.76
	Ma-11.1	2.68×10^7	6.78×10^6–1.07×10^8	0.36 ± 0.12	1.08	6.03×10^7	1.23×10^7–2.96×10^8	0.31 ± 0.12	0.47
I. fumosorosea	If-02	1.57×10^8	2.61×10^7 –9.45×10^8	0.32 ± 0.12	0.97	1.52×10^8	3.65×10^7–6.36×10^8	0.40 ± 0.12	0.21
	If-2.3	2.88×10^7	7.97×10^6 –1.04×10^8	0.38 ± 0.12	1.62	3.46×10^7	8.34×10^6–6.63×10^7	0.38 ± 0.12	2.11
	If-03	3.42×10^7	8.45×10^6–1.38×10^8	0.35 ± 0.12	0.70	1.50×10^8	3.17×10^7–7.08×10^8	0.37 ± 0.12	0.60

[a] Fiducial limit.

respectively. The second most virulent isolate was Ma-2.3, causing 78.00% mortality with $LC_{50} = 2.29 \times 10^7$ spores/mL, and LT_{50} varied from 5.50, 6.27 and 7.04 days at 3×10^8, 2×10^8, and 1×10^8 spores/mL, respectively.

Bait method for larvae

The results of applying different concentrations of fungal isolates to housefly larvae by the bait method are listed in Table 4. The results of probit analysis showed results similar to those resulting from the immersion method. In this method Bb-01 proved to be the most virulent isolate with an $LC_{50} = 1.70 \times 10^7$ spores/mL which caused 79.00%, 65.00% and 57.00% mortality with LT_{50} values of 5.50, 6.10 and 6.47 days at 3×10^8, 2×10^8, and 1×10^8 spores/mL, respectively (Table 5).

Effect of entomopathogenic fungi on the longevity and fecundity of M. domestica

The fungal infections reduced the survival of houseflies regardless of their sex and also tended to decrease egg production in females. The mean number of eggs/female after application of sublethal doses of entomopathogenic fungi ranged from 120.45 to 212.7 which was far less compared to the control group (462.68 ± 10.57) ($F = 212.0$, $p = 0.000$). In addition, the mean longevity after application of different isolates of the entomopathogenic fungi ranged from 8.90 to 16.21 days for males ($F = 93.0$, $p = 0.000$) and from 10.21 to 17.21 days for females ($F = 129.0$, $p = 0.000$), which showed the capability of sublethal doses of different fungi to considerably reduce the longevity of both male and female flies (Table 6).

Discussion

Insect entomopathogenic fungi are microbial control agents that can play an important role in integrated pest management. These fungi are used as biological control agents for a broad range of insects including gregarious pests. The current study was planned to evaluate the virulence of local isolates of *B. bassiana*, *M. anisopliae* and *I. fumosorosea* against larvae and adults of *M. domestica*. The results showed that entomopathogenic fungi have great potential to control both larvae and adults of *M. domestica*. The results of the current study are supported by a number of previous trials and numerous preceding studies.[1,19,21–23,30–32] In the current study, the Bb-01 fungal strain resulted in 96.00% mortality, which is close to the absolute mortality of houseflies from entomopathogenic fungi applied by the immersion method. This result is in agreement with the findings of Watson et al.[21]

The application of entomopathogenic fungi as bait against the housefly also showed promising results. In the present study, the maximum mortality (89.29%) was caused by the fungal strain Bb-01, which confirms the findings of Sharififard et al.,[23] who evaluated different isolates of *B. bassiana* and *M. anisopliae* against houseflies. Baits with doses of 5×10^7 conidia g^{-1} showed up to 90.00% mortality within 3.5–6.5 days after exposure. Moreover, the findings of the current study regarding the application of entomopathogenic fungi as a bait against *M. domestica* is supported by Lecuona et al.[1] who evaluated 19 fungal species and strains at a concentration of 3×10^8 conidia/10 g in sugar bait against housefly adults. The results showed five strains caused mortality higher than 85.00%. Similarly, Geden et al.[33] reported two strains of *B. bassiana* when applied as bait at concentrations of 10^8 conidia/100 mg killed 78.00–88.00% of adult houseflies after 5 days and caused 100% mortality after 6 days of bait application. In addition, 87.00–94.00% mortality was observed at low-dose concentrations of 10^7 conidia/100 mg six days after exposure.

Even though a 5–6-days interval between treatment and death is extended when compared with the quick control achieved by chemicals, this longer period may possibly be acceptable where houseflies have developed resistance against the chemical insecticides such that the chemicals can no longer be used to control housefly populations. Employing entomopathogenic fungi in bait form as inundative releases against housefly adults may possibly be attractive for many reasons. First, a large quantity of insecticides is needed to control adult insects. Second, development of resistance against chemical insecticides would be avoided. Third, careful timing and placement of bait can reduce the quantity and cost of inocula required compared with the quantities required for broadcast or manure treatments.[23] Additionally, infecting insects with entomopathogenic fungi by the immersion method in the field is quite impossible;[34] therefore bait methods are more interesting in that regard. The present study

Table 3 – LT_{50} values (days) of entomopathogenic fungi against adults of *M. domestica* by immersion and bait methods.

Fungi	Isolate(s)	Immersion method						Bait method				
		Concentration	%Mortality	LT_{50}	FD[a]	Slope	χ^2	%Mortality	LT_{50}	FD[a]	Slope	χ^2
B. bassiana	Bb-01	3×10^8	96.43 ± 3.57a	4.20	3.84–4.59	5.88 ± 0.79	4.85	89.29 ± 3.57a	4.10	3.63–4.62	3.91 ± 0.56	3.09
		2×10^8	85.71 ± 5.83ab	4.65	4.24–5.10	5.50 ± 0.79	0.82	75.00 ± 3.57ab	5.34	4.79–5.96	4.87 ± 0.79	1.11
		1×10^8	78.57 ± 7.14b	4.99	4.55–5.48	5.61 ± 0.84	1.09	67.86 ± 6.84b	5.83	4.95–6.87	3.45 ± 0.62	1.23
		1×10^7	60.71 ± 6.84c	5.99	5.24–6.83	4.48 ± 0.80	1.27	48.33 ± 1.45c	–	–	–	–
		1×10^6	42.86 ± 5.83d	–	–	–	–	40.43 ± 3.57cd	–	–	–	–
	Bb-08	3×10^8	82.14 ± 3.57ab	5.05	4.58–5.58	5.27 ± 0.81	1.51	75.00 ± 3.57ab	5.60	5.04–6.22	5.31 ± 0.88	1.29
		2×10^8	71.43 ± 5.83b	5.70	5.13–6.33	5.43 ± 0.91	1.55	67.86 ± 6.84b	5.98	5.34–6.69	5.38 ± 0.95	2.29
		1×10^8	64.29 ± 9.22bc	6.08	5.33–6.94	4.68 ± 0.85	0.74	64.29 ± 9.22b	6.24	5.50–7.09	5.06 ± 0.94	1.51
		1×10^7	46.75 ± 3.54cd	–	–	–	–	40.00 ± 2.11cd		–	–	–
		1×10^6	38.21 ± 2.11d	–	–	–	–	32.14 ± 2.45d		–	–	–
	Bb-10	3×10^8	78.57 ± 4.12b	4.90	4.46–5.40	5.35 ± 0.80	1.06	67.86 ± 3.57b	5.96	5.25–6.77	4.69 ± 0.83	1.32
		2×10^8	64.29 ± 9.22bc	5.43	4.80–6.15	4.29 ± 0.71	2.02	60.71 ± 3.57bc	6.51	5.66–7.49	4.94 ± 0.95	1.35
		1×10^8	57.14 ± 10.10bc	6.16	5.38–7.08	4.42 ± 0.81	1.23	57.14 ± 5.83bc	6.74	5.77–7.87	4.75 ± 0.95	0.89
		1×10^7	42.32 ± 2.56d	–	–	–	–	36.54 ± 3.11cd	–	–	–	–
		1×10^6	35.12 ± 4.31de	–	–	–	–	28.50 ± 1.34cd	–	–	–	–
M. anisopliae	Ma-2.3	3×10^8	89.29 ± 6.84a	4.59	4.20–5.01	5.88 ± 0.83	1.54	75.00 ± 6.84ab	5.45	4.89–6.07	5.02 ± 0.82	0.54
		2×10^8	75.00 ± 3.57b	5.53	4.99–6.11	5.43 ± 0.89	2.28	60.71 ± 3.57bc	6.35	5.49–734	4.53 ± 0.85	1.12
		1×10^8	64.29 ± 4.12bc	6.28	5.54–7.10	5.27 ± 0.98	1.54	57.14 ± 5.83cd	6.70	5.67–7.92	4.25 ± 0.84	1.16
		1×10^7	40.56 ± 3.56cd	–	–	–	–	42.43 ± 1.67cd	–	–	–	–
		1×10^6	24.45 ± 1.67e	–	–	–	–	35.67 ± 3.78cde	–	–	–	–
	Ma-4.1	3×10^8	82.14 ± 6.84ab	5.01	4.56–5.51	5.44 ± 0.82	1.39	75.00 ± 3.57ab	5.14	4.57–5.79	4.32 ± 0.69	1.03
		2×10^8	71.43 ± 0.00b	5.65	5.03–6.35	4.78 ± 0.81	2.13	67.86 ± 3.57b	5.84	5.12–6.68	4.34 ± 0.76	0.67
		1×10^8	60.71 ± 8.99c	6.47	5.53–7.57	4.28 ± 0.82	1.23	53.57 ± 8.99bc	6.99	5.71–8.57	3.65 ± 0.74	0.67
		1×10^7	44.65 ± 3.56cd	–	–	–	–	46.54 ± 2.67cd	–	–	–	–
		1×10^6	33.77 ± 1.67d	–	–	–	–	39.11 ± 1.89cd	–	–	–	–
	Ma-11.1	3×10^8	71.43 ± 5.83bc	5.57	4.99–6.22	4.98 ± 0.83	0.96	64.29 ± 4.12b	6.03	5.27–6.89	4.53 ± 0.81	0.84
		2×10^8	64.29 ± 4.12bc	6.14	5.37–7.02	4.64 ± 0.85	1.31	57.14 ± 0.00bc	6.58	5.63–7.69	4.44 ± 0.86	0.56
		1×10^8	53.57 ± 6.84cd	6.84	5.78–8.10	4.42 ± 0.89	0.59	48.57 ± 3.77bc	–	–	–	–
		1×10^7	41.45 ± 5.21d	–	–	–	–	41.11 ± 4.21bc	–	–	–	–
		1×10^6	33.56 ± 3.24de	–	–	–	–	30.56 ± 2.56d	–	–	–	–
I. fumosorosea	If-02	3×10^8	60.71 ± 10.71c	6.31	5.39–7.38	4.08 ± 0.77	1.48	57.14 ± 5.83bc	6.50	5.50–7.67	4.01 ± 0.77	0.96
		2×10^8	53.57 ± 6.84cd	6.89	5.79–8.20	4.31 ± 0.88	0.64	48.31 ± 4.65c	–	–	–	–
		1×10^8	41.43 ± 2.11d	–	–	–	–	37.54 ± 4.21cd	–	–	–	–
		1×10^7	33.57 ± 2.56de	–	–	–	–	30.21 ± 3.89d	–	–	–	–
		1×10^6	26.12 ± 1.67de	–	–	–	–	22.10 ± 3.21de	–	–	–	–
	If-2.3	3×10^8	75.00 ± 6.84b	5.33	4.80–5.90	5.14 ± 0.82	0.81	67.86 ± 3.57b	5.83	5.18–6.55	4.97 ± 0.86	0.76
		2×10^8	64.29 ± 4.12bc	6.12	5.38–6.97	4.82 ± 0.87	0.65	42.31 ± 4.65cd	–	–	–	–
		1×10^8	50.15 ± 3.76cd	–	–	–	–	35.00 ± 3.86cd	–	–	–	–
		1×10^7	42.54 ± 4.31d	–	–	–	–	27.43 ± 1.67de	–	–	–	–

Table 3 – (*Continued*)

Fungi	Isolate(s)	Immersion method						Bait method				
		Concentration	%Mortality	LT_{50}	FD[a]	Slope	χ^2	%Mortality	LT_{50}	FD[a]	Slope	χ^2
	If-03	1×10^6	31.59 ± 4.28de	–	–	–	–	23.21 ± 2.54de	–	–	–	–
		3×10^8	71.43 ± 5.83b	5.77	5.14–6.47	4.99 ± 0.86	1.12	60.71 ± 6.84bc	6.23	5.40–7.20	4.40 ± 0.82	1.19
		2×10^8	64.29 ± 4.12bc	6.26	5.54–7.10	5.27 ± 0.98	2.28	57.14 ± 0.00bc	6.70	5.67–7.92	4.25 ± 0.84	0.67
		1×10^8	57.14 ± 5.83c	6.90	5.85–8.13	4.65 ± 0.95	0.93	42.67 ± 3.22cd	–	–	–	–
		1×10^7	40.42 ± 4.67d	–	–	–	–	31.21 ± 3.65d	–	–	–	–
		1×10^6	32.19 ± 3.56de	–	–	–	–	23.32 ± 1.65de	–	–	–	–
		F-value	63.00					43.20				
		p-value	0.001					0.000				
		LSD-value	17.70					18.94				

[a] Fiducial limit.
Means followed by the same letters in columns are not significantly different at the 5% level.

Table 4 – LC_{50} values (spores/mL) of entomopathogenic fungi against larvae of *M. domestica* by immersion and bait methods.

Fungi	Isolate(s)	Immersion method				Bait method			
		LC_{50}	FD[a]	Slope	χ^2	LC_{50}	FD[a]	Slope	χ^2
B. bassiana	Bb-01	7.42×10^6	3.87×10^6–1.43×10^7	0.49 ± 0.06	4.91	1.70×10^7	8.63×10^6–3.33×10^7	0.41 ± 0.06	7.37
	Bb-08	3.51×10^7	1.82×10^7–6.77×10^7	0.40 ± 0.06	3.87	4.81×10^7	2.40×10^7–9.65×10^7	0.37 ± 0.06	1.39
	Bb 10	6.49×10^7	3.13×10^7–1.34×10^8	0.37 ± 0.06	5.23	1.19×10^8	5.37×10^7–2.64×10^8	0.36 ± 0.06	3.96
M. anisopliae	Ma2.3	2.29×10^7	2.53×10^6–2.06×10^8	0.39 ± 0.11	9.66	6.22×10^7	2.76×10^7–1.40×10^8	0.32 ± 0.06	4.78
	Ma-4.1	2.80×10^7	1.37×10^7–5.73×10^7	0.36 ± 0.06	4.28	3.18×10^7	1.39×10^7–7.26×10^7	0.31 ± 0.06	3.21
	Ma-11.1	1.40×10^8	6.30×10^7–3.11×10^8	0.38 ± 0.06	3.27	1.97×10^8	9.76×10^7–3.96×10^8	0.46 ± 0.07	2.35
I. fumosorosea	If-02	6.21×10^8	4.78×10^8–8.73×10^8	0.34 ± 0.07	7.33	4.75×10^8	1.47×10^8–6.23×10^8	0.34 ± 0.07	2.36
	If-2.3	6.43×10^7	3.20×10^7–1.29×10^8	0.38 ± 0.06	4.65	2.80×10^8	8.31×10^7–9.39×10^8	0.29 ± 0.06	3.88
	If-03	1.07×10^8	4.42×10^7–2.58×10^8	0.32 ± 0.06	1.49	4.21×10^8	1.41×10^8–7.23×10^8	0.33 ± 0.06	2.04

[a] Fiducial limit.

Table 5 – LT_{50} (days) values of *B. bassiana*, *M. anisopliae* and *I. fumosorosea* against larvae of *M. domestica* by immersion and bait methods.

Fungi	Isolate	Immersion method						Bait method				
		Concentration (spores/mL)	%Mortality	LT_{50}	FD[a]	Slope	χ^2	%Mortality	LT_{50}	FD[a]	Slope	χ^2
B. bassiana	Bb-01	3×10^8	86.00 ± 1.15a	5.24	4.63–5.58	6.52 ± 2.09	18.09	79.00 ± 5.26a	5.50	4.62–6.53	5.87 ± 0.93	17.33
		2×10^8	75.00 ± 3.00ab	5.61	5.31–5.92	5.48 ± 0.48	15.96	65.00 ± 3.00ab	6.10	5.73–6.49	5.38 ± 0.51	8.94
		1×10^8	68.00 ± 3.65ab	5.91	5.13–6.81	5.59 ± 0.81	12.49	57.00 ± 1.00b	6.47	6.04–6.93	5.28 ± 0.54	11.06
		1×10^7	49.00 ± 1.45c	–	–	–	–	42.00 ± 3.21c	–	–	–	–
		1×10^6	36.00 ± 2.45cd	–	–	–	–	35.21 ± 2.00 cd	–	–	–	–
	Bb-08	3×10^8	72.00 ± 4.32ab	5.88	5.15–6.73	6.37 ± 3.77	21.16	67.00 ± 2.52a	6.36	5.98–6.92	5.64 ± 1.64	12.34
		2×10^8	61.00 ± 5.26ab	6.28	5.17–7.55	5.46 ± 0.93	15.03	58.00 ± 3.46b	6.59	6.09–7.12	4.73 ± 0.49	2.96
		1×10^8	48.00 ± 2.11c	–	–	–	–	55.00 ± 1.00b	6.60	6.13–7.10	5.15 ± 0.53	8.66
		1×10^7	38.00 ± 1.45cd	–	–	–	–	37.00 ± 3.45c	–	–	–	–
		1×10^6	30.00 ± 2.11d	–	–	–	–	27.00 ± 1.56 cd	–	–	–	–
	Bb-10	3×10^8	68.00 ± 4.32ab	6.04	5.67–6.44	5.10 ± 0.48	7.26	63.00 ± 4.43ab	6.26	5.84–6.71	4.95 ± 0.49	6.01
		2×10^8	54.00 ± 1.15b	6.80	6.22–7.44	4.32 ± 0.46	0.85	51.00 ± 2.52bc	7.06	6.41–7.79	4.25 ± 0.47	0.68
		1×10^8	47.00 ± 2.34bc	–	–	–	–	44.00 ± 3.54bc	–	–	–	–
		1×10^7	36.00 ± 3.56cd	–	–	–	–	32.00 ± 1.56bc	–	–	–	–
		1×10^6	28.00 ± 3.67d	–	–	–	–	25.00 ± 3.21cde	–	–	–	–
M. anisopliae	Ma-2.3	3×10^8	78.00 ± 2.58ab	5.50	5.19–5.81	5.08 ± 0.44	7.54	67.00 ± 1.91ab	6.28	4.83–8.17	5.48 ± 1.10	7.76
		2×10^8	63.00 ± 4.12b	6.27	5.97–6.77	4.29 ± 0.42	2.95	56.00 ± 2.83b	6.65	6.15–7.19	4.89 ± 0.51	3.79
		1×10^8	53.00 ± 3.00bc	7.04	6.38–7.79	4.13 ± 0.45	1.59	46.00 ± 3.45bc	–	–	–	–
		1×10^7	40.00 ± 2.45bc	–	–	–	–	38.00 ± 4.11c	–	–	–	–
		1×10^6	34.00 ± 2.11cd	–	–	–	–	31.00 ± 3.54c	–	–	–	–
	Ma-4.1	3×10^8	72.00 ± 3.65ab	5.66	5.33–6.04	4.74 ± 0.43	3.50	69.00 ± 4.12ab	5.88	5.56–6.22	5.53 ± 0.51	10.35
		2×10^8	61.00 ± 3.42b	6.42	5.90–6.99	4.15 ± 0.42	2.48	60.00 ± 3.65b	6.48	5.99–6.99	4.70 ± 0.48	1.83
		1×10^8	52.00 ± 2.31bc	7.05	6.42–7.75	4.44 ± 0.49	1.46	52.00 ± 2.31bc	7.00	6.42–7.62	4.88 ± 0.54	5.38
		1×10^7	45.00 ± 3.11c	–	–	–	–	42.00 ± 3.21bc	–	–	–	–
		1×10^6	31.00 ± 2.45cd	–	–	–	–	34.00 ± 3.67c	–	–	–	–
	Ma-11.1	3×10^8	61.00 ± 3.42b	6.48	6.03–6.96	5.07 ± 0.52	6.96	59.00 ± 4.73b	6.62	6.13–7.15	4.92 ± 0.51	5.26
		2×10^8	54.00 ± 3.83bc	6.94	6.26–7.69	3.81 ± 0.41	1.41	51.00 ± 4.43bc	7.09	6.44–7.81	4.38 ± 0.48	1.08
		1×10^8	42.00 ± 3.21c	–	–	–	–	39.00 ± 2.00bc	–	–	–	–
		1×10^7	31.00 ± 3.27cd	–	–	–	–	28.00 ± 3.11d	–	–	–	–
		1×10^6	23.00 ± 2.45d	–	–	–	–	15.00 ± 1.56de	–	–	–	–
I. fumosorosea	If-02	3×10^8	56.00 ± 2.31bc	6.88	5.77–7.89	5.65 ± 0.42	9.47	52.00 ± 4.32bc	7.49	6.71–8.36	4.24 ± 0.50	6.08
		2×10^8	39.00 ± 2.45cd	–	–	–	–	45.00 ± 2.54bc	–	–	–	–
		1×10^8	31.00 ± 1.45cd	–	–	–	–	36.00 ± 1.34cd	–	–	–	–
		1×10^7	27.00 ± 1.54d	–	–	–	–	25.00 ± 2.32de	–	–	–	–
		1×10^6	18.00 ± 2.67de	–	–	–	–	20.00 ± 1.32d	–	–	–	–
	If-2.3	3×10^8	68.00 ± 4.32ab	6.03	4.99–7.30	5.57 ± 0.95	6.31	57.00 ± 6.19bc	6.62	5.44–8.05	5.27 ± 0.90	13.52
		2×10^8	57.00 ± 2.52bc	6.64	6.15–7.16	5.01 ± 0.52	5.91	47.00 ± 4.32bc	–	–	–	–
		1×10^8	47.00 ± 3.45c	–	–	–	–	40.00 ± 1.42c	–	–	–	–
		1×10^7	35.00 ± 3.11cd	–	–	–	–	29.00 ± 3.11cd	–	–	–	–
		1×10^6	28.00 ± 4.21d	–	–	–	–	27.00 ± 2.67cd	–	–	–	–
	If-03	3×10^8	61.00 ± 3.42b	6.57	5.59–7.43	5.60 ± 1.73	14.32	53.00 ± 3.00bc	6.88	6.33–7.48	4.89 ± 0.53	5.04

Table 5 – (Continued)

Fungi	Isolate	Immersion method					Bait method				
	Concentration (spores/mL)	%Mortality	LT_{50}	FD[a]	Slope	χ^2	%Mortality	LT_{50}	FD[a]	Slope	χ^2
	2×10^8	53.00 ± 1.00bc	7.02	5.01–9.43	5.31 ± 1.45	9.62	45.00 ± 2.54bc	–	–	–	–
	1×10^8	46.00 ± 2.87c	–	–	–	–	36.00 ± 3.11cd	–	–	–	–
	1×10^7	37.00 ± 2.00cd	–	–	–	–	30.00 ± 1.76cd	–	–	–	–
	1×10^6	27.00 ± 3.21d	–	–	–	–	17.00 ± 2.15de	–	–	–	–
	F-value	15.3					25.1				
	p-value	<0.0001					<0.0001				
	LSD-value	18.35					18.17				

[a] Fiducial limit.
Means followed by the same letters in columns are not significantly different at the 5% level.

showed that *B. bassiana* is much more efficient in controlling houseflies than *M. anisopliae* or *I. fumosorosea*. Similar observations were made by Mishra et al.[35] who reported that *B. bassiana* is more efficacious compared to *M. anisopliae*. However, Dimbi et al.[36] reported contradictory results, finding *M. anisopliae* to be more efficient compared to *B. bassiana*. In this study, post-mortem fungal activity was monitored by keeping the dead insects on damp filter paper. Examinations of external fungal sporulation showed fungi-induced mortality. The pathogenicity of the local isolates of entomopathogenic fungi was highly variable; however, Bb-01 was highly pathogenic to *M. domestica*. Prior studies have also shown differences in pathogenicity among strains. These differences may be attributable to various fungi-related causes such as strain origin, species, exposure method and dosage, as well as humidity and temperature factors.[19,21,33,37,38]

For a comprehensive pest management strategy, the effectiveness of entomopathogenic fungi has been evaluated for different stages of insect pests.[35] The larvicidal action of entomopathogenic fungi has been reported for other insects,[39] but only a few reports describe the effects on *M. domestica* larvae. These reports include Steinkraus et al.[18] who reported 35.00–52.00% mortality in third instar housefly larvae by *B. bassiana*, while Watson et al.[21] reported 48.00–56.00% mortality at 10^{10} conidia mL^{-1} in housefly larvae. Conversely, Lecuona et al.[1] observed no effect on housefly larvae and pupae with any of five tested strains of *B. bassiana* a result that was similar to an earlier report by Geden et al.[33] Regardless of these earlier reports, the current study demonstrates significant mortality (51.00–86.00%). In addition, noteworthy mortality up to 79.00% was observed when entomopathogenic fungi were applied as bait on larval food. The use of entomopathogenic fungi as larvicide may be a suitable control approach against housefly, particularly if the pathogen survives in bedding environments.[23] Dead larvae infected with fungus were identified by presence of white or green muscardine on cadavers. The moisture and temperature of bedding support the sporulation of fungi and dead larvae in bedding could serve as inocula for further infection of larvae.[21] Moreover, *B. bassiana*, when applied in the manure pits where the flies were breeding and emerging, aids in controlling houseflies prior to mating and egg laying.[22]

In the current study *B. bassiana* and *M. anisopliae* resulted in the maximum mortality of housefly populations at both adult and larval stages. Comparing these results with reports from other studies, absolute mortality has been obtained within 5–15 days[1,35] in the laboratory, providing support for the findings of the present study.

Various physiological characteristics of insects including age, sex and nutritional status can be influenced by their susceptibility to fungal infection. For example, fungal infection reduces the survival and inhibits blood feeding behaviour of mosquitoes.[40] Moreover, decreased survival and fecundity with increasing doses of entomopathogenic fungi have been reported.[41,42] The results are similar in the current research; housefly longevity and fecundity were both reduced after exposure to a sublethal dose (1×10^6 spores/mL) of different entomopathogenic fungi compared to controls. The differences in housefly longevity and fecundity might be due to the

Table 6 – Effect of sublethal dose (1×10^6 spores/mL) of entomopathogenic fungi on the longevity and fecundity of *M. domestica*.

Fungi	Isolate(s)	Mean number of eggs/female	Longevity (days)	
			Male	Female
B. bassiana	Bb-01	120.45 ± 1.09G	8.90 ± 0.20F	10.21 ± 0.16D
	Bb-08	144.58 ± 3.47F	12.3 ± 0.36E	13.1 ± 0.42C
	Bb-10	176.3 ± 2.82DE	12.6 ± 0.30E	14.19 ± 0.39C
M. anisopliae	Ma-2.3	212.7 ± 4.30B	14.21 ± 0.15D	13.61 ± 0.38C
	Ma-4.1	185.15 ± 1.35CD	10.12 ± 0.27F	11.22 ± 0.50D
	Ma-11.1	202.85 ± 13.32BC	14.32 ± 0.41CD	16.5 ± 0.29B
I. fumosorosea	If-02	177.5 ± 4.66DE	15.53 ± 0.31BC	16.1 ± 0.43B
	If-2.3	161.88 ± 8.69EF	16.21 ± 0.24B	17.21 ± 0.60B
	If-03	183.88 ± 3.13D	13.21 ± 0.68DE	10.23 ± 0.44D
Control		462.68 ± 10.57A	24.33 ± 0.86A	26.7 ± 0.50A
F		212.0	93.0	129.0
p		<0.0001	<0.0001	<0.0001
LSD value		18.87	1.25	1.23

Means followed by the same capital letters in columns are not significantly different at the 5% level.

virulence potential of the fungus and/or the susceptibility of houseflies.

In conclusion, the results of the current research showed that *B. bassiana*, *M. anisopliae* and *I. fumosorosea* are effective biological controls against houseflies and can be used as biological control agents against *M. domestica* particularly through inundative releases of conidia. The *B. bassiana* isolate (Bb-01) proved to be the most virulent and could be promising in future mycoinsecticidal development. However, its field efficacy, especially in poultry and dairy farms, still needs to be evaluated.

Conflict of interest

The authors declare no conflicts of interest.

REFERENCES

1. Lecuona RE, Turica M, Tarocco F, Crespo DC. Microbial control of *Musca domestica* (Diptera: Musciadae) with selected strains of *Beauveria bassiana*. *J Med Entomol*. 2005;42:332–336.
2. Förster M, Klimpel S, Sievert K. The house fly (*Musca domestica*) as a potential vector of metazoan parasites caught in a pig-pen in Germany. *Vet Parasitol*. 2009;160:163–167.
3. Ugbogu OC, Nwachukwu NC, Ogbuagu UN. Isolation of Salmonella and Shigella species from house flies (*Musca domestica* L.) in Uturu, Nigeria. *Afr J Biotech*. 2006;5:1090–1091.
4. Azzam S, Hussein E. Toxicities of several insecticides to the house fly *Musca domestica* from different regions in Jordon. *Sarhad J Agric*. 2002;18:69–75.
5. Cao XM, Song FL, Zhao TY, Dong YD, Sun CX, Lu BL. Survey of Deltamethrin resistance in houseflies (*Musca domestica*) from urban garbage dumps in Northern China. *Environ Entomol*. 2006;35:1–9.
6. Malik A, Singh N, Satya S. Housefly (*Musca domestica*): a review of control strategies for a challenging pest. *J Environ Sci Health B*. 2007;42:453–469.
7. Yadav SK. Pesticide applications-threat to ecosystems. *J Hum Ecol*. 2010;32:37–45.
8. Scott JG, Alefantis TG, Kaufman PE, Rutz DA. Insecticide resistance in house flies from caged-layer poultry facilities. *Pest Manage Sci*. 2000;56:147–153.
9. Harris CR, Turnbull SA, Whistlecraft JW, Surgeoner GA. Multiple resistance shown by the field strains of house fly, *Musca domestica* (Diptera: Muscidae) to organochlorine, organophosphorus, carbamates and pyrethroid insecticides. *Can Entomol*. 1982;114:447–454.
10. Ozaki K, Kassai T. The insecticidal activity of pyrethroids against insecticide resistant strain of plant hoppers, leaf hopper and the house fly. *J Pestic Sci*. 1984;9:61–66.
11. Malinowski H. Studies on the insect resistance to the pyrethroid insecticides used in forest protection. *Sylwan*. 1993;137:45–54.
12. Gebara AB, Ferreira CS, Miguel O. Efficacy of seven pyrethroids against *Musca domestica* Linn. (Diptera: Muscidae). *Arquivos-do-Instituto-Biologico-Sao-Paulo*. 1997;64:111–113.
13. Mostafa AA, Zayed AB. Resistance of *Musca domestica* (L) (Diptera: Muscidae) from Gamsa City to some insecticides. *J Egypt Soc Parasitol*. 1999;29:193–201.
14. Kocisova A. The stability of resistance in a field house fly population, *Musca domestica*, over 60 generations is following the interruption of insecticides selection pressure. *Czech J Anim Sci*. 2001;46:281–288.
15. Geden CJ. Status of biopesticides for control of house flies. *J Biopest*. 2012;5:1–11.
16. Khan S, Guo LH, Maimaiti Y, Mijit M, Qiu DW. Entomopathogenic fungi as microbial biocontrol agent. *Mol Plant Breed*. 2012;3:63–79.
17. Skovgård H, Steenberg T. 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. *Bio Control*. 2002;47:45–60.
18. Steinkraus DC, Geden CJ, Rutz DA, Kramer JP. First report of the natural occurrence of *Beauveria bassiana* (Moillales: Moniliaceae) in *Musca domestica* (Diptera: Musciadae). *J Med Entomol*. 1990;27:309–312.
19. Barson G, Renn N, Bywater AF. Laboratory evaluation of six species of entomopathogenic fungi for control of house fly,

Musca domestica L., a pest of intensive animal units. *J Invertebr Pathol*. 1994;64:107–113.
20. Bywater AF, Barson G, Renn N. The potential of oil-based suspension of *Metarhizium anisopliae* conidia for the control of the housefly (*Musca domestica*), a pest of intensive animal units. *Proc Brighton Crop Prot Conf*. 1994;3:1097–1102.
21. Watson DW, Geden CJ, Long SJ, Rutz DA. Efficacy of *Beauveria bassiana* for controlling the house fly and stable fly (Diptera: Musciadae). *Biol Control*. 1995;5:405–411.
22. Kaufman PE, Reasor C, Rutz DA, Ketzis JK, Arends JJ. Evaluation of *Beauveria bassiana* applications against adult house fly, *Musca domestica*, in commercial caged-layer poultry facilities in New York State. *Biol Control*. 2005;33:360–367.
23. Sharififard M, Mossadegh MS, Vazirianzadeh B, Zarei Mahmoudabadi A. 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*. 2011;4:128–137.
24. Bell HA, Robinson KA, Weaver RJ. First report of cyromazine resistance in a population of UK house fly (*Musca domestica*) associated with intensive livestock production. *Pest Manage Sci*. 2010;66:693–695.
25. Freed S, Jin FL, Ren SX. Determination of genetic variability among the isolates of *Metarhizium anisopliae* var. *anisopliae* from different geographical origins. *World J Microbiol Biotechnol*. 2011;27:359–370.
26. Freed S, Jin FL, Ren SX. Phylogenetics of entomopathogenic fungi isolated from the soils of different ecosystems. *Pak J Zool*. 2011;43:417–425.
27. Quesada-Moraga E, Maranhao EAA, Valverde-GarcÍa P, Santiago-Álvarez C. 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 Control*. 2006;36:274–287.
28. Fletcher MG, Axtell RC, Stinner RE. Longevity and fecundity of *Musca domestica* (Diptera: Muscidae) as a function of temperature. *J Med Entomol*. 1990;27:922–926.
29. Abbott WS. A method for computing the effectiveness of an insecticide. *J Econ Entomol*. 1925;18:265–267.
30. Geden CJ, Steinkraus DC, Rutz DA. Evaluation of two methods for release of *Entomopthora muscae* (Entomopthorales: Entomopthoraceae) to infect housefly (Diptera: Muscidae) on dairy farms. *J Environ Entomol*. 1993;20:1201–1208.
31. Watson DW, Rutz DA, Long SJ. *Beauveria bassiana* and Sawdust bedding for management of housefly *Musca domestica* (Diptera: Musciadae) in calf hatches. *Biol Control*. 1996;7:221–227.
32. Mwamburi LA, Laing MD, Miller RM. Laboratory screening of insecticidal activities of *Beauveria bassiana* and *Paecilomyces lilacinus* against larval and adult housefly (*Musca domestica* L.). *Afr Entomol*. 2010;18:38–46.
33. Geden CJ, Rutz DA, Steinkraus DC. Virulence of different isolates and formulation of *Beauveria bassiana* for house flies and the Parasitoid *Muscidofurax raptor*. *Biol Control*. 1995;5:615–621.
34. Sedighi N, Abbasipour H, Askary H, Gorjan AS, Karimi J. Pathogenicity of the entomopathogenic fungus *Metarhizium anisopliae* var. *major* on different stages of the sunn pest *Eurygaster integriceps*. *J Insect Sci*. 2013;13:150.
35. Mishra S, Kumar P, Malik A, Satya S. Adulticidal and larvicidal activity of *Beauveria bassiana* and *Metarhizium anisopliae* against housefly, *Musca domestica* (Diptera: Muscidae), in laboratory and simulated field bioassays. *Parasitol Res*. 2011;108:1483–1492.
36. Dimbi S, Maniania NK, Lux SA, Ekesi S, Mueke JK. Pathogenicity of *Metarhizium anisopliae* (Metsch.) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin, to three adult fruit fly species: *Ceratitis capitata* (Weidemann), *C. rosa* var. *fasciventris* Karsch and *C. cosyra* (Walker) (Diptera: Tephritidae). *Mycopathology*. 2003;156:375–382.
37. Angel-Sahagún CA, Lezama-Gutiérrez R, Molina-Ochoa J, et al. Susceptibility of biological stages of the horn fly, *Haematobia irritans*, to entomopathogenic Fungi (Hyphomycetes). *J Insect Sci*. 2005;5:50.
38. Anderson RD, Bell AS, Blanford S, Paaijmans KP, Thomas MB. Comparative growth kinetics and virulence of four different isolates of entomopathogenic fungi in the house fly (*Musca domestica* L.). *J Invertebr Pathol*. 2011;107:179–184.
39. Chernaki-Leffer AM, Sosa-Gòmez DR, Almeida LM. Selection for entomopathogenic fungi and LD_{50} of *Metarhizium anisopliae* (Metsch.) Sorok. for the Lesser Mealworm *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae). *Rev Bras Cienc Avic*. 2007;9:187–191.
40. Mnyone LL, Kirby MJ, Mpingwa MW, Lwetoijera DW, Bart GKJ, Takken W. Infection of mosquitoes *Anopheles gambiae* with entomopathogenic fungi: effect of host age and blood-feeding status. *Parasitol Res*. 2011;108:317–322.
41. Flores AE, Garcia GP, Badil MH, Tovar LR, Salas IF. Effects of sublethal concentrations of vectovac® on biological parameters of *Aedes aegypti*. *J Am Mosq Control Assoc*. 2004;20:412–417.
42. Pelizza SA, Scorsetti AC, Tranchida MC. The sublethal effects of the entomopathic fungus *Leptolegnia chapmanii* on some biological parameters of the dengue vector *Aedes aegypti*. *J Insect Sci*. 2013;13:22.