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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)

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ABSTRACT

This study was conducted to evaluate the virulence of 10 Iranian isolates of entomopathogenic fungi, *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metch) Sorok. and introduce the most virulent isolate for microbial control of the house fly *Musca domestica* L. under the laboratory conditions. Three bioassay methods were used: Topical, oral and bait method. Fungal isolates were first screened by immersing adults and medium sized larvae in a suspension containing 10^8 conidia mL^{-1}. Percentage mortalities ranged from 28-100% were recorded for adults and larvae and five isolates were found to be relatively more virulent. Data clearly show that there is a wide range in the response of the two stages (larvae and adult) of the house fly to the action of the tested isolated of *B. bassiana* and *M. anisopliae*. LC_{50} values in bait method for adults were 1.65×10^6, 1.7×10^6, 1.9×10^6, 2.9×10^6 and 3×10^6 conidia g^{-1} for the 5 highly virulent isolates designated Ma437C, Bb187C, Bb429C, Bb428C and Bb796C, respectively. Dose of 5×10^7 conidia g^{-1} bait resulted in up to 90% mortality within 3.5-6.5 day after exposure. Topical application for larvae resulted in LC_{50} values of 7.3×10^4, 1.1×10^6, 1.6×10^6, 2×10^6 and 2.9×10^6 conidia mL^{-1} for the isolates, respectively. Oral application of 10^9 conidia g^{-1} larval bedding resulted in larval mortalities of 98.4, 5 6 and 35.2% for Ma437C, Bb187C and Bb429C, respectively. Due to lower LC_{50} and LC_{90} values and shorter lethal time, Ma 437C was the most virulent isolate for house fly larvae and adult.

Key words: *Musca domestica*, *Beauveria bassiana*, *Metarhizium anisopliae*, fungi, bioassay

INTRODUCTION

The house fly, *Musca domestica* L. is one of the most important and cosmopolitan insect pests, from both a medical and a social standpoint (Axtell and Arends, 1990). It can affect livestock performance, cause irritation to human beings and possesses considerable potential for the mechanical transmission of 100 pathogens in human beings. The development of resistance to

pesticides by the house fly has increased the willingness of those involved in their production to seek alternative methods for fly management (Kaufman *et al*., 2005). The use of entomopathogenic fungi is the area of fly management that has garnered attention. Numerous studies are available documenting the attempted use of entomopathogenic fungi against house fly under laboratory and field conditions including *Entomophthora musca* (Cohn) Fresenius (Geden *et al*., 1993; Mullens, 1986), *Beauveria bassiana* (Bals.) Vuill (Barson *et al*., 1994; Geden *et al*., 1995; Watson *et al*., 1995,1996; Kaufman *et al*., 2005; Lecouna *et al*., 2005; Mwamburi *et al*., 2010) and *Metarhizium anisopliae* (Metch) Sorok. (Renn *et al*., 1999). *B. bassiana*, in particular, is an especially attractive candidate for use in the biological control of the housefly (Lecouna *et al*., 2005) because it is easily and economically produced and its conidia have a long storage life and can be formulated and applied in a variety of ways (Geden *et al*., 1995).

The objectives of the present study were (1) evaluate the effecacy of 10 isolates of *B. bassiana* and *M. anisopliae* against house fly adults and larvae, (2) determine the lethal doses and lethal times of selected isolates using different bioassay and (3) comparison between the efficiency of the pathogenicity of *B. bassiana* and *M. anisopliae* for control house fly.

MATERIALS AND METHODS

Insects: During 2007-2009 adult house flies were collected from a poultry house using sweeping net and were transferred to the laboratory where they were reared at 26±2°C, 50±5% RH. Adults were maintained in cages (40×40×40 cm^3) covered by gauze. Water and food in the form of sugar and powdered milk were provided and replenished every 24-48 h. Larval medium comprised: 55 g wheat bran, 3 g date extract and 2 g dried alfalfa suspended in 140 mL water. One cup (250 mL volume) of this medium was left in each cage for oviposition by adults and subsequent development of larvae. The food was replaced every 24-48 h.

Fungi: All fungal isolates were obtained from the Evin Plant Pests and Disease Research Institute of Iran (Table 1). After passage of the fungi through *M. domestica*, they were cultured on Sabouraud dextrose agar with yeast extract (SDAY) for 2 weeks at 27°C. Sporulating cultures (2-3 week old) were harvested by scraping the dry conidia from the surface of the culture plate with a scalpel and transferred to sterile distilled water containing 0.01% Tween-80. The concentration of the suspension was determined using a hemocytometer (Butt and Goettel, 2007).

Table 1: Fungal isolates investigated in the present study

Fungal species	Isolate	Designated abbreviation	Insect host or substrate	Site and date of origin
B. bassiana	Iran 187C	Bb 187C	*Leptinotarsa decemlineata*	Ardebil- 1995
B. bassiana	Iran 428C	Bb 428C	*Chilo suppressalis* Walker	Gilan-Rasht 2001
B. bassiana	Iran 429C	Bb 429C	*C. suppressalis* Walker	Hassan rud 2001
B. bassiana	Iran 440C	Bb 440C	Soil	Karaj-Atashgah 2001
B. bassiana	Iran 441C	Bb 441C	*Rhynchophorus ferrugineus* Olivier	Saravan 2001
B. bassiana	Iran 796C	Bb 796C	*Heterodera schachtii*	Urmia 2004
B. bassiana	Iran 797C	Bb 797C	*H. schachtii*	Urmia 2004
M. anisopliae	Iran 437C	Ma 437C	*C. suppressalis* Walker	Mazandaran
M. anisopliae	Iran715C	Ma 715C	Locust	
M. anisopliae	Iran1018C	Ma 1018C	*Parandra caspica* (Col.)	Rasht

Fungal viability: For each experiment, conidia viability was determined by enumerating the percentage of germinated conidia 24 h after spreading on fresh SDAY medium. A 0.01 mL of conidial suspension (10^7 conidia mL^{-1}) was spread on a 90 mm petri plate containing 15 mL of SDAY medium that was then incubated at 27°C for 24 h for germination (the formation of a distinct germ tube) to occur. Percentage germination was determined by first placing three 15 mm square cover slips directly on to the surface of the medium and counting the number of germinated conidia and total number of conidia per field of view at 250 X magnification. Three to four fields of view were observed per cover slip (Quesada-Moraga *et al*., 2006).

Screening fungal isolates virulence: An aqueous suspension of each fungal strain was prepared as described above and the concentration adjusted to 10^8 conidia mL^{-1} in sterile distilled water. In order to test the virulence of the fungal isolates on the larval stage of *M. domestica*, groups of 25 medium sized larvae were immersed in conidial suspension for 10 sec and were then placed on damp filter paper kept in a white disposable plastic container (100 mL volume). Containers were sealed with lids. Control larvae were either dipped in 0.01% Tween-80 solution. Each treatment was replicated four times. After 24 h, each group of larvae was transferred to the container together with 70 g larval medium. The tops of the dishes were covered with gauze and the dishes were incubated at 272°C, 75±5% RH and photoperiod of 14:10 (L: D). Emerged adults were recorded as the rate of survival and rate of mortality were calculated.

For testing the effect of fungal isolates on adults, 3-4 day old adults were anesthetized with CO_2 and batches of 25 individuals were dipped in a suspension containing 10^8 conidia mL^{-1} for 10 sec and each batch placed on the piece of filter paper in small cages (20×20×20 cm^3). They were provided with sugar and powdered milk as solid medium and water. Cages were kept at 26±2°C, 50±5% RH and 14:10 (L:D). Control flies were treated with 0.01% solution of Tween-80. All treatments were replicated 4 times. Mortality was recorded for 7 day at 24 h intervals.

Larval dishes and adult cages were checked daily and the cadavers were collected, surface-sterilized by immersing in a 10% sodium hypochlorite solution for 2-3 sec, thoroughly rinsed in sterile distilled water and transferred to sterile Petri dishes containing damp filter paper. True mortality was taken to occur for those cadavers on which sporulation were visible. Such cadavers were held at 4°C for use as subcultures (Lohmeyer and Miller, 2006).

Adult treatment: Six doses of conidia from most promising fungal strains were tested on cohorts of twenty-five 2-3 day old flies, feeding males and females housed in small cages (20×20×20 cm^3). Each cage contained a 9 cm diameter Petri dish lined with Whatman filter paper and 10 g bait (6 g sugar, 2 g powder milk and 2 mL distilled water). For better dispersion of fungus, 1 mL of the stock suspensions of the fungal strains under test was dispersed on the surface of bait to give doses of 5×10^7, 2.5×10^7, 5×10^6, 2.5×10^6, 5×10^5 and 2.5×10^5 conidia g^{-1}. Treated baits were left in the cages for 48 h before being removed and replaced with dry bait (sugar+powdered milk). Water was provided *ad libitum*. The experiments were run in a completely randomized design with four replicates per treatment. Cages were checked daily over a period of 7 days following presentation of fungus. Cadavers were removed daily and surface sterilized as mentioned above and transferred to a sterile Petri dish with damp filter paper. Sporulating cadavers were recorded as mortality due to fungal infection.

Larval treatment

Topical method: Aqueous suspensions of the most virulent strains for house fly larvae were prepared and serially diluted to give 10^8, 10^7, 10^6, 10^5 and 10^4 conidia mL^{-1}. Groups of 25 medium sized larvae were immersed simultaneously for 10 sec in the conidial suspension and each group placed on damp filter paper in a white disposable plastic container. Control larvae were dipped in 0.01% Tween-80 solution. Larvae were transferred to the larval medium after 24 h. There were four replicates per treatment. All containers were incubated at 27±1°C, 75±5% RH and a photoperiod of 14:10 (L:D). Mortality was recorded after treatments at intervals of 24 h until pupation occurred. Dead larva and pupae were held to monitor fungi-induced mortality. Externally sporulated cadavers were recorded as dead due to fungal infection.

Oral method: An experiment was designed to determine the effectiveness of Bb 187C, Bb 429C and Ma 437C for the control of house fly larvae in larval bedding. Three stock suspension of each strain were prepared as 5 ± 10^{10}, 5 ± 10^{11} and 5 ± 10^{12} conidia mL^{-1}. Plastic cups containing 50 g larval medium were treated with 1 mL of each stock solution to give doses of 10^9, 10^{10} and 10^{11} conidia g^{-1} and replicated five times. Each dose was thoroughly mixed with the larval medium. Each cup was inoculated with 25 medium size larvae, then maintained at constant condition of 27±1°C, 75±5% RH and a photoperiod of 14:10 L: D until pupation. Dead larva and pupa were held to monitor fungi-induced mortality.

Data analysis: Mortality rate were corrected using Abbott's (1925) formula. An arcsin transformation of the percent mortality was performed to stabilize variance. Lethal concentrations (LC_{50} and LC_{90}) and the mean lethal time (LT_{50}) required to killing one-half of the larva and adults was estimated using probit analysis procedure of SAS Software (SAS, 1998). Difference in lethal concentrations between strains were judged to be significant if their respective 95% confidence intervals (CIs) did not overlap. Yields of different strains were analyzed with one-way ANOVA and means were compared by Tukey's test ($p = 0.05$).

RESULTS AND DISCUSSION

Preliminary screening of fungal isolates by topical application of 10^8 conidia mL^{-1} to both adults and larvae of housefly showed that Bb187C, Bb428C, Bb429C, Bb796C and Ma437C were the most virulent isolates for adults and larvae and produced mortality rates that exceeded 80% (Table 2). In adults, there was no significant difference in virulence (mean percentage mortality at 7 days) among isolates Bb187C (99%), Bb429C (94%) and Ma 437C (95.8%). The virulence of strains Bb428C (82.6%) and Bb796C (81.6%) were also similar, but were significantly lower than the other isolates (Table 2). In larval bioassay, no significant differences in virulence were found among isolates Ma 437C (100%), Bb187C (94%) and Bb429C (92%). In addition to the greater virulence of the above mentioned isolates, their colony growth produced fast- growing cultures with compact, dense mycelia and large yields of conidia which were reasons why they were selected for bioassay tests.

Bait test for adults: Five strains were selected for further bioassay in small cages. Results of these experiments are presented in Table 3 and 4. Results of the test, in which conidia were dispersed on the surface of the bait, were quite encouraging. This method of application could prove a suitable method for deploying conidia in places where house fly populations are a problem. Green and white

Table 2: Mortality of *Beauveria bassiana* and *Metarhizium anisopliae* to larvae and adult house flies after exposure to a suspension of 10^8 conidia mL^{-1}

Isolate	% adult mortality (±SE)	% larval mortality (±SE)
Bb 187C	99.0±1.00A	94.0±1.48AB
Bb 428C	82.6±2.76BC	81.0±2.00BC
Bb 429C	94.0±2.94AB	86.0±2.03B
Bb 440C	62.8±2.20D	66.0±2.00D
Bb 441C	75.6±1.60DC	70.0±2.52DC
Bb 796C	81.6±8.59BC	92.0±4.64AB
Bb 797C	60.4±1.47D	50.4±4.60E
Ma 437C	95.8±2.08A	100.0±0.00A
Ma 715C	29.2±3.10F	28.0±2.19F
Ma 1018C	54.6±2.20E	52.0±2.19E

ANOVA for adult: F = 118.08; df = 10; p<0.0001 and for larvae: F = 65.31; df = 9 and p<0.0001; *Means followed by the same letter not significantly different (Tukey's test; α)

Table 3: Effect of selected isolates of *Beauveria bassiana* and *Metarhizium anisopliae* on mortality of adult of *M. domestica* using baiting method

Isolate	Concentration (conidia g^{-1})	% mortality	LT_{50} (CI)
Bb 187C	5×10^7	97.00±1.00	4.78 (4.40-5.17)
	2.5×10^7	81.00±1.15	5.22 (4.03-6.67)
	5×10^6	73.00±1.60	6.28 (5.65-7.13)
Bb 428C	5×10^7	94.00±2.23	5.32 (4.79-6.93)
	2.5×10^7	77.00±3.78	6.41 (5.12-7.12)
	5×10^6	59.00±2.38	7.13 (6.13-8.22)
Bb 429C	5×10^7	95.75±1.60	5.52 (4.71-6.96)
	2.5×10^7	75.5±1.89	6.23 (4.96-7.03)
	5×10^6	57.25±2.65	6.91 (6.26-8.23)
Bb 796C	5×10^7	89.0±3.57	6.47 (5.76- 7.11)
	2.5×10^7	73.0±2.1	7. 05 (6.67- 7.59)
	5×10^6	65.0±1.00	7. 89 (7.13- 8.43)
Ma 437C	5×10^7	98.0±1.15	3.5 (3.4-3.59)
	2.5×10^7	84.0±1.9	3.89 (3.55-4.37)
	5×10^6	71.0±1.00	4.15 (3.89-5.02)

muscardin on surface of the adult house fly cadavers caused by *M. anisopliae* and *B. bassiana* are shown in Fig. 4a and b. Bait containing 5×10^7 conidia g^{-1} of the isolates tested killed 80-100% of the flies within 3.5-6.5 days after exposure (Table 3) in particular Bb187C, Bb429C and Ma437C that killed the greatest number of individuals in the shortest period of time. Results of probit analysis indicated no significant difference between LC_{50} of Bb187C, Bb429C and Ma437C. The 95% CI of the LC_{50} values overlapped for these isolates (Table 4). Strains Bb187C and Ma437C exhibited the lowest LC_{50} values and the greatest slopes. This finding was consistent with the results of an earlier topical assay (Table 2) indicated that Bb187C and Ma437C were more virulent against adults than other isolates. The 95% CI of the LC_{50} values for Bb 428C and Bb796C overlapped.

Lecouna *et al.* (2005) reported that out of 19 fungal species and strains tested at 3×10^8 conidia/10 g sugar against the adult house fly, 5 only produced mortality rates that exceeded 85%. Geden *et al.* (1995) reported that baits containing 10^8 conidia/100 mg of two *B. bassiana* strains killed 78-88% of the adult house fly 5 days after exposure and 100% after 6 days. They observed

Table 4: Probit analysis of selected isolates of *Beauveria bassiana* and *Metarhizium anisopliae* against adult of *M. domestica* by the baiting method

Isolate	LC_{50} (%95CI)*	LC_{90} (%95CI)*	Slope (±SE)	χ (df)
Bb 187C	1.7×10^6 (1.4-2.5)	1.3×10^7 (9.6×10^6-2×10^7)	1.59±0.14	25.66(26)
Bb 428C	2.9×10^6 (2-3.9)	3.1×10^7 (2.1-5.1)	1.23±0.11	13.03(26)
Bb 429C	1.9×10^6 (1.2-2.4)	4.5×10^7 (2.9-5)	0.91±0.08	19.29(22)
Bb 796C	3×10^6 (2.7-3.8)	4.8×10^7 (3.3-5.2)	1.33±0.13	18.37(23)
Ma 437C	1.65×10^6 (1.2-2.6)	1.3×10^7 (9.8×10^6-1.7×10^7)	1.02±0.41	22.12(24)

*CI: Confidence intervals for LC_{50} and LC_{90}

that high levels of control (87-94%) were observed 6 days after exposure at a low concentration of 10^7 conidia/100 mg. Placing either one, two or four bait formulated with *M. anisopliae* on the floor of a 10 m^3 polythene cubicle and releasing 100 female and 50 male *M. domestica* had equal effect and between 95.2 and 100% of flies were killed after 10 days (Renn *et al*., 1999). Although, a 5-6 day interval between treatment and death is long compared with the rapid control that has been achieved with chemical insecticides, this long period may be acceptable to producers where flies have become too resistant to such chemicals that they can no longer be used as control agents. Utilizing entomopathogenic fungi in bait form as inundative release against the adult house fly would be attractive for several reasons: (1) It would lead to a reduction in the large volume of insecticides used universally for adult control, (2) Development of resistance to insecticides would be avoided, (3) The judicious timing and placement of bait would reduce the quantity and cost of innocula required compared with quantities required for broadcast premise or manure treatment, (4) The use of bait would provide a novel alternative to chemical insecticides and (5) It would minimize risks to other biological control agents such as parasitoids and predators deployed in the same habitat. Field trials of baiting system, together with the space sprays suggested by Barson *et al*. (1994) and Renn *et al*. (1999) will elucidate the full potential of *M. anisopliae* in controlling housefly infestations of animal rearing facilities.

Topical application of inoculum to larvae: Topical application of fungus to larvae showed a concentrations dependent response and caused 55-100% mortality, for all strains tested and with LT_{50} values varying between 3.84 (3.11-4.82) and 7.79 (5.89-8.12) days, for concentrations between 10^6 and 10^8 conidia mL^{-1} (Table 5). Results of probit analysis (Table 6) showed that Ma 437C was the most virulent isolate (LC_{50} =7.3×10^4 conidia mL, LC_{90} =2.6×10^6 conidia/mL and slope =0.86). Similarly, LT_{50} values of Ma 437C (3.84, 4.43 and 5.46 days) was the shortest one at 10^8, 10^7 and 10^6 conidia mL^{-1}, respectively. Bb 187C was the second virulent isolate (LC_{50} =1.6×10^6 conidia mL^{-1}, LC_{90} =1.2×10^7 conidia mL^{-1} and slope = 0.68). There were significant differences among strains due to non overlap in confidence intervals (CI) of LC_{50} values. Steinkraus *et al*. (1990) reported 35-52% mortality in third instars larvae of the house fly infected by *B. bassiana*. Likewise, Watson *et al*. (1995) obtained mortality rates between 48-56% in the second instar with high doses (10^{10} conidia mL^{-1}) while rates were negligible with lower doses. In contrast, Lecouna *et al*. (2005) were unable to infect larvae and pupae with any of the five *B. bassiana* strains that they tested, which was similar to the results reported by Geden *et al*. (1995). These differences may be due to differences in strain virulence, in assay method, conidial dose employed or in cultural methods. The colour of dead larvae was primarily light red which changed to dark violet (Fig. 1). After colour changing, white or green muscardin developed on larval cadavers (Fig. 2a, b). In some cases larvae changed to pupae, but these formed pupae were infected with white or green muscardin (Fig. 3).

Table 5: Efficacy of topical application of selected isolates of *Beauveria bassiana* and *Metarhizium anisopliae* on *Musca domestica* larval mortality

Isolate	Dose (conidia mL^{-1})	% mortality	LT_{50} (CI)
Bb 187C	10^8	89±1.66	4.94 (4.75-5.18)
	10^7	78±4.43	5.22 (4.03-6.67)
	10^6	57±1.60	6.28 (5.65-7.13)
Bb 428C	10^8	83±4.3	5.26 (4.66-6.84)
	10^7	67±3.8	5.67 (4.75-7.23)
	10^6	50±4.1	6.58 (6.1-7.41)
Bb 429C	10^8	86±2.24	4.94 (4.44-6.02)
	10^7	60±2.83	6.67 (6.1-7.78)
	10^6	53±3.57	7.33 (5.67-8.23)
Bb 796C	10^8	86±2.58	5.91 (4.56-6.12)
	10^7	55±3.42	6.37 (5.75-7.89)
	10^6	37±3.00	7.79 (5.89-8.12)
Ma 437C	10^8	100±0.00	3.84 (3.11-4.82)
	10^7	97±3	4.43 (3.91-5.26)
	10^6	79±3	5.46 (4.53-6.65)

Table 6: Probit analysis of the selected isolates of *Beauveria bassiana* and *Metarhizium anisopliae* against larvae of *M. domestica* by topical method

Isolate	LC_{50} (%95CI)*	LC_{90} (%95CI)*	Slope (±SE)	χ(df)
Bb 187C	1.1×10^6 (6.2×10^5-1.7×10^6)	1.2×10^7 (6.4×10^7-2.7×10^8)	0.68±0.057	14.68(18)
Bb 428C	2×10^6 (1.2-3.1)	9.8×10^7 (5.2×10^7-2.2×10^8)	0.65±0.056	12.72(18)
Bb 429C	1.6×10^6 (9.7×10^5-2.5×10^6)	1.9×10^7 (9.7×10^7-4.6×10^8)	0.67±0.055	20.9(18)
Bb 796C	2.9×10^6 (1.8×10^6-4.4×10^7)	2.4×10^8 (1.2×10^8- 5.6×10^8)	0.64±0.054	12.18(18)
Ma 437C	7.3×10^4 (4.6×10^4-1.1×10^5)	2.6×10^6 (1.5×10^6- 5.3×10^6)	0.86±0.071	16.65(18)

*CI: Confidence intervals for LC_{50} and LC_{90}

Fig. 1: Colour changing in dead larva of house fly, *M. domestica* due to entomopathogenic fungi

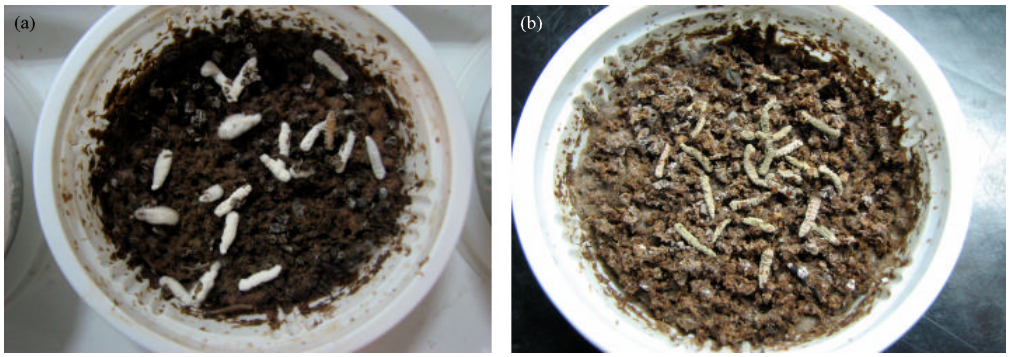

Fig. 2: (a) White muscardine on larval cadavers of *M. domestica* and (b) Green muscardine on larval cadavers of *M. domestica*

Fig. 3: Infected pupae of *M. domestica* due to *B. bassiana*

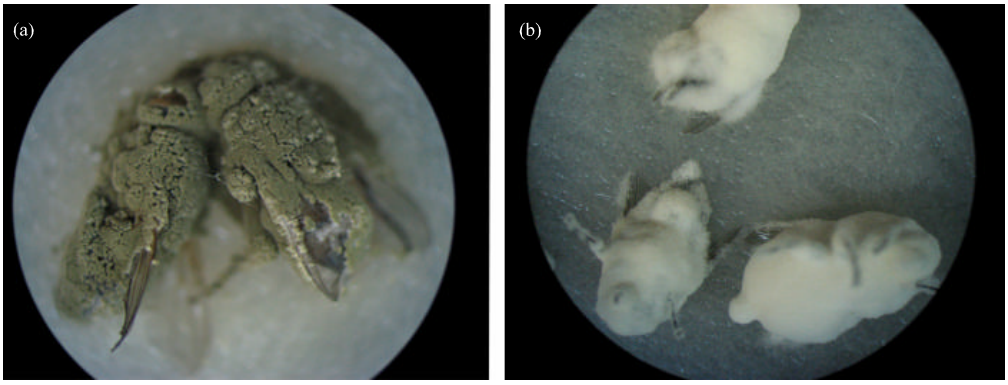

Fig. 4: (a) Green muscardine on adult cadavers of *M. domestica* and (b) White muscardine on adult cadavers of *M. domestica*

Oral application of fungus to larva: Analysis of variance indicated significant differences between in percent mortality of the strains at the different doses (Table 7). Ma437C produced the greatest mortality at the lowest dose tested (10^9 conidia g^{-1}: 98.4% mortality) and within a shorter time (4-5 days after exposure) than did other isolates. Accordingly, it is considered the most virulent of the strains and a good candidate for housefly larvae control. Data obtained following topical

Table 7: Larval dose response (percentage mortality) to 3 selected fungal isolates presented as conidia mixed in larval bedding

Isolate	Dose (conidia g^{-1})	% mortality (±SE)
Bb 187C	10^9	56±3.4D
	10^{10}	74.4±2.1C
	10^{11}	94±2.1B
Bb 429C	10^9	35.2±2.37E
	10^{10}	59±2.70D
	10^{11}	93±3.25) B
Ma 437C	10^9	98.4±0.81A
	10^{10}	100±0.00A
	10^{11}	100±0.00A

*ANOVAs among strains: F=231.21; df=2; P<0.0001, among Doses: F= 163.75; df = 2; p<0.0001 and among %Mortality: F = 43.24; df = 4; p<0.0001. Means followed by the same letter not significantly different (Tukey's test; $\alpha = 0.05$)

application of isolates (Table 5, 6) agree with this conclusion. Watson *et al.* (1995) reported that mortality was greatest for house fly larvae exposed to 10^{10} conidia/ cm^8 larval bedding averaging 56 and 48% for larvae treated with *B. bassiana* strain L90 and P89 respectively. Use of entomopathogenic fungi as a larvicide may be a convenient control strategy for the housefly, particularly if the pathogen persists in a bedding environment (Watson *et al.*, 1995). In a test on the adult fly, the sporulation of *B. bassiana* and *M. anisopliae* was encouraged by placing dead adult flies infected with the fungus on damp filter papers. Dead larvae infected with fungus were readily identified by the presence of white or green muscardin on the cadavers in the larval bedding. Because moisture and temperature of the bedding support the sporulation of entomopathogenic fungi, it is thought that dead larvae persisting in the bedding could serve as innocula for subsequent infection of larvae (Watson *et al.*, 1995).

Conidial exposure levels in the house fly larvae tests were much greater than those that readily controlled the adults. This result was similar to the findings of Watson *et al.* (1995).

In conclusion, the results of this research showed that entomopathogenic fungi such as *B. bassiana* and *M. anisopliae* were effective for control of *M. domestica* and could be recommended as biological control agents for the fly especially in inundative release of conidia. These results are supported by numerous studies of other researchers (Geden *et al.*,1993, 1995; Mullens, 1986; Renn *et al.*, 1999; Barson *et al.*, 1994; Watson *et al.*, 1995, 1996; Kaufman *et al.*, 2005; Lecouna *et al.*, 2005; Mwamburi *et al.*, 2010). *M. anisopliae* strain Iran 437C was the most virulent strain and has promise in future mycoinsecticidal development. However, because this study did not investigate the non target effects of these pathogens to other organisms such as parasitoids and pathogen, deploying entomopathogenic fungi in control programs should be done with caution to minimize effects on the environment. Also, because these tests were conducted in cage and room conditions designed to simulate natural conditions, it is necessary to evaluate the efficacy of their use on a large scale experiment such as in a poultry rearing facility or on a dairy farm.

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