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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.

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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

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