

Survey and Isolation of natural incidence of different fungal pathogens against house flies in different urban habitats

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ABSTRACT

The investigation on survey of entomopathogenic fungi and isolation against house fly, *Musca domestica* L. (Muscidae: Diptera) were carried out during 2010-2011 at the Department of Entomology, UAS, Bengaluru and Bio Control Research Laboratory, Pest Control India Private Limited, Bengaluru. Surveys were carried out once in eight days during August to September 2010 in poultry farm, dairy farm, piggery farm and slaughter house in Bengaluru and surrounding areas for natural occurrence of fungal pathogens on house flies. House fly is a well known nuisance pest in all the visited farms and this species is also found in association with human activities, cattle units, poultry farms, and piggery farm. In all the visited farms, insecticides like Deltamethrin, Ektomin, Dichlorovas and pest-o-flash (light trap) are currently being used for managing the house fly. Soil baiting with larvae of *Galleria mellonella* yielded two isolates representing two genera, *Beauveria bassiana* and *Metarrizium anisopliae* from three different locations. No fungus was recovered from the UAS, GKVK Piggery farm. The survey revealed that incidence of fungal species in all farm sectors. House fly population was not widely prevalent in all the locations surveyed. Only two fungal species viz., *B. bassiana* and *Aspergillus flavus* were isolated during the survey and there was no epizootic incidence of fungal pathogens during this period. Future studies should be carried out by using different methods of isolation of fungus from naturally occurring house flies.

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INTRODUCTION

The house fly, *Musca domestica* L. (Diptera: Muscidae) is a major domestic, medical, and veterinary pest that causes irritation, spoils food, and acts as a vector of many medical and veterinary pathogenic organisms (Forester *et al.*, 2009; Sukontason *et al.*, 2000). It is a nuisance in human and livestock habitations and losses caused by *M. domestica* in poultry houses were reported to be in excess of 60 million US dollars per year in the United States (Anonymous, 1976). Moreover, they may be responsible for the transmission of over 100 different pathogens (Pospischil, 1996). It has been found to carry the etiological agents of typhus fever, dysentery, cholera, hematic carbuncle, bovine mastitis, conjunctivitis and poliomyelitis, protozoan cysts, and helminth eggs (Howard, 2001; Barin *et al.*, 2010). House fly reported as a vector of protozoan cysts of *Entomobea hystolitica*, *Escherichia coli*, *Giardia intestinalis* and bell moth eggs (Sennaet *et al.*, 2002) and transmit the deadly bacteria *Escherichia coli* O157:H7 (Iwasa *et al.*,

1999, Sasaki *et al.*, 2000), and are likely vectors for over sixty five human and animal intestinal diseases (Greenberg, 1965).

The most common method for the control of house flies is through the use of insecticides. Over the past seventy years, a variety of chemicals have been used to control houseflies including chlorinated hydrocarbons, organophosphates, carbamates and pyrethroids (Shono *et al.*, 2004). Today, commercial house fly control is limited to a few organophosphates, carbamate (Methomyl), pyrethrins and two pyrethroids. Unfortunately, house fly populations can rapidly evolve resistance to insecticides, limiting our ability to control them. Resistance in house flies has become a global problem (Pospischil *et al.*, 1996; Keiding, 1999; Cao *et al.*, 2006). Since, the pathogenic microorganisms are widespread in the hospital environment, there is abundant opportunity for flies to become contaminated and in turn, contaminate the patient environment (Fotedar *et al.*, 1992).

Several entomopathogenic fungal species have been used for *M. domestica* biological control, and emphasis has been focused on *Metarrizium anisopliae* (Cars Well *et al.*, 1998). Excessive use of synthetic pesticides results in enhanced pest resurgence as well as environmental/health problems. As an alternative, biological control of house fly could be very promising, it being ecofriendly as well as cost effective.

Uses of entomopathogenic fungi for house fly control potential to their low mammalian toxicity and natural prevalence among flies population (Malik *et al.*, 2007). However, it is desirable to investigate native entomopathogenic isolates, adaptable to local environment, and hence, more efficient for the control of pest population of the region. Houseflies are potential carriers of pathogenic microorganisms and an attempt was made to isolate and identify pathogenic bacteria, fungi and parasites from the house fly collected in the surgical ward of the All India Institute of Medical Sciences Hospital. A total of 113 showed positive results for *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Viridans streptococci*. Among there the isolation rate of *Staphylococcus aureus* was significantly higher in test houseflies than control. There was no significant difference in isolation of parasitic ova and cysts from test and control houseflies. Similar results were also found in *Candida* spp. (Fotedar, 1992).

Siriet *al.* (2005) reported that *M. domestica* adults were infected with fungus *B. bassiana*. They also observed that Natural prevalence of *B. bassiana* infection recorded between 0.4-1.45 per cent and pathogenicity assays under laboratory conditions showed 94 per cent adult mortality after 14 days of inoculation by *B. bassiana*. Lopez *et al.* (2006) reported the activity of an entomopathogenic fungus belonging to the *Muscae* sp. complex infecting *M. domestica* in Laplata, poultry houses at Argentina. The *Entomophthora* that caused natural infections between September 2001 and September 2003 fungus was identified as *E. ferdinandii*.

MATERIALS AND METHODS

Investigations were carried out on natural occurrence of fungal pathogens on the house fly

Musca domestica L. (Muscidae: Diptera) and its susceptibility to different entomopathogenic fungi such as *B. bassiana*, *Metarrizium anisopliae* and *Aspergillus flavus* during 2010-2011 at the Department of Entomology, UAS, Bengaluru and Bio Control Research Laboratory, Pest Control India PVT LTD, Bengaluru. The details of the experiments conducted on the above aspects are presented as follows.

Survey: Surveys were carried out once in eight days during August to September 2010 in poultry farm, dairy farm, piggery farm and slaughter house, in Bengaluru and surrounding areas for natural occurrence of fungal pathogens on houseflies. Maggots and naturally dead houseflies were collected using forceps and adult flies were captured with nylon net and afterwards reared in a wooden cage covered on the sides by nylon netting. Three collecting sessions were conducted at each location. After capture, the flies were separated in the laboratory and stored in test tubes (16 x 160 mm) in groups of ten specimens. Four tubes were separated out for each location and placed in a freezer for 5 min to anesthetize the flies. These tubes were then placed in a laminar flow chamber where the rest of the steps of isolation, purifications of fungal bioagents were carried out by following standard lab protocol. All the instruments and culture media used in this step were previously sterilized.

Soil sampling: Soil samples were collected from the base of the house fly breeding area in a poultry farm (7.5 cm diameter) at a depth of 10cm as described by Zimmerman (1986) and Parker *et al.* (1996). Six such samples were collected from each field and placed separately in plastic bags and labeled. Five such fields were sampled in a location and samples brought to the laboratory. All the samples collected from five fields at each location were pooled separately and mixed thoroughly. Sub samples were taken and kept in a refrigerator (4 degree centigrade) until use.

Isolation of fungus: The field or wild-caught flies were initially washed in a solution of 1 per cent sodium hypochlorite for 3 min and twice in sterile

distilled water for 1 min each (Seymour *et al.*, 1984). A group of ten flies in test tubes was transferred to a crucible containing a 0.85 per cent saline solution for maceration. In a laminar flow chamber, 0.1 ml of macerated preparation was retrieved and inoculated (spread using a loop) in Petri dishes (9 cm) containing the medium PDA at 2 per cent, supplemented with chloramphenicol to inhibit bacterial growth. The pH had been corrected previously to 7.0 by the addition of NaOH at 1N. Five dishes were inoculated for each replication. These inoculated petriplates were allowed for incubation at 25 ± 1 degree centigrade in BOD for a week. The cultured and identified colonies were then transferred to test tubes (16 x 160 mm) containing 10 ml of PDA, and cooled at an inclination of 30°C (Alves, 1998). The tubes were incubated in by keeping in an incubator chamber under 28 ± 1 degree centigrade and a relative humidity of 80 ± 5 per cent.

Identification of fungus: The plates were kept at room temperature for fungal growth. The fungi were identified using a light microscope. The samples stained with lactophenol and aniline blue for mounting between the slides and fungi were identified by studying their mycelia growth, spore characters and pigmentation on culture media.

Soil baiting with *Galleria mellonella*: We adopted a method reported by Zimmerman (1986) to isolate fungi from soil by using *G. mellonella* (Lepidoptera: Galleridae). Ten pre-heated larvae of *G. mellonella* (to avoid webbing by larvae) were released into the 100g sub samples of soil. The pure culture of *G. mellonella* was reared on artificial diet. *G. mellonella* are extremely susceptible to infection by entomopathogenic fungi and they quickly exhibit signs of infection. Because these larvae normally remain on the soil surface, boxes (20cm diameter) were inverted daily to force them to burrow through the soil surface, which maximized their opportunity to contact fungal inoculum. The treatments were replicated four times. Larvae were inspected on alternate days for three weeks and infected larvae were removed.

Mycosed *Galleria* larva exhibiting symptoms of infection was surface sterilized in 0.1 per cent

sodium hypochlorite (NaOCl) for 3-5 min, rinsed thrice in sterile distilled water to remove the traces of sodium hypochlorite, placed in humid chamber and held at $25 \pm 1^\circ\text{C}$ for at least 10 days. After fungal growth on the larva was evident, standard procedures were followed for isolation of fungal strain. The fungi were isolated from diseased cadavers, following the procedure of Lomer and Lomer (1995). The fungus developed on the diseased larva was subcultured and purified by hyphal tip method (Tuite, 1969). The fungus was identified based on spore structures.

RESULTS AND DISCUSSION

Status of house fly: Information gathered during survey has been summarized in Table 1. House fly is a well known nuisance pest in the all visited farms and this species is also found in association with human activities, cattle units, poultry farms, and piggery farms. In all the visited farms, insecticides like Deltamethrin, Ektomin, Dichlrovas and pest-o-flash (light trap) are currently being used for managing the house fly. In poultry farms, heavy fly infestations can mean more time spent cleaning eggs to remove fly specks, and possibly downgrading egg quality. In piggery, flies are especially attracted to pig farrowing units where they feed around sows' teats, eyes and open wounds. The results of the fungal species isolated from the soil as well as house fly are given below.

Soil baiting: Soil baiting with larvae of *Galleria mellonella* yielded two isolates representing two genera, *Beauveria bassania* and *Metarhizium anisopliae* from three different locations. No fungus was recovered from the UAS, GKVK Piggery farm. According to Steinkraus (1990) soil contains naturally distributed conidia or the corpses of contaminated arthropods, which can infect Muscidae pupae that come in direct contact with this medium after abandoning the larval substrate for pupation. These fungi can spread to newly emerged adult Muscidae through contact with the infected pupae. Hence, an attempt was made to isolate the potential mycopathogens from soil as described by Parker *et al.* (1996). In this present study Soil baiting with larvae of *Galleria mellonella* yielded two isolates representing two genera, *B. bassania* and *M. anisopliae* from three different

Table 1. Status of House fly in surveyed areas, infestation and their management practices adopted at different farms.

Place	Farming type	House fly infestation (Avg. House fly/ sweep)	Other insect pests	Management practices
UAS, GKVK, Bengaluru	Piggery farm	25	Blowfly	-
Hebbal Bengaluru	Dairy and poultry farm	50	Stable fly, blow fly, Mosquitoes, Cockroach, Mites, Ticks	Deltamethrin, Ektomin
KMF, Yelahanka	Dairy farm	25	Stable fly, Blow fly, Mosquitoes, Cockroach	Pest-o-flash (Light Trap)
Madhapana halli	Lotus Poultry farm	30	Stable fly, Blow fly, Mosquitoes, Cockroach	Dichlrovas Thiamethoxam+jag gary
Arakere	Poultry farm	20	Stable fly, Blow fly,	Ennova force
Yelahanka	Slaughter house	28	-	-
Yelahanka	Vegetable market	30	Stable fly,	-

location. This number is too small compared to otherfield explorations. Sarquis and Oliveira (1996) isolated and identified fungi from the soil in the State of Rio de Janeiro and found the genera *Aspergillus*, *Penicillium*, *Fusarium* and *Cladosporium* among the most common, once again showing that these species are present in soil that is also pathogenic to house fly population. Eyal *et al.*, 1994; Strasser *et al.*, 2000, reported *B. bassiana* isolates 7320, 7569 and 7771 isolated from the soil were identified as the most pathogenic isolates to adult houseflies, causing mortalities of 90 per cent.

Isolation of fungi from house fly: Surveys were undertaken in different farm sectors in Bengaluru district during August to September 2010 for natural occurrence of fungal pathogens on house fly population. The survey revealed that incidence of fungal species in all farm sectors house fly population was not widely prevalent in all the locations surveyed. Only two fungal species *viz.*, *B. bassiana* and *Aspergillus flavus* were isolated during the survey and there was no epizootic incidence of fungal pathogens during this period. Similarly, the natural infection of housefly by *B.*

bassiana (0.4- 1.45 %) was reported by Siri *et al.* (2005) and *Entomophthora ferdinandii* was reported by Claudia *et al.* (2005) in poultry houses. In another study by Senna Nunes *et al.* (2002) isolated genus *Aspergillus*, *Penicillium*, *Alternria*, *Curvularia*, *Mucorales*. In another study, Kaaya and Okech (1990) reported various species isolated pupae and adults on *Glossina pallidipes*, among which *A. flavus*, *A. niger*, *Penicillium* sp. and *Fusarium* sp. Our study demonstrated that house-fly is a carrier of fungal spores Majid *et al.* (2007) observed that fungi isolates recovered were mostly saprophytes. However, they isolated two pathogenic fungi which were Dermatophytes and 12 genera of fungi were detected in house flies. Among 12 genera *Aspergillus* sp. an important medical species was isolated.

Investigations were carried out on natural occurrence of fungal pathogens on the house fly *Musca domestica* L. (Muscidae: Diptera) and its susceptibility to different entomopathogenic fungi such as *B. bassiana*, *Metarrizium anisopliae* and *Aspergillus flavus*. House fly is a well known nuisance pest in all the visited farms and this species is also found in association with human activities, cattle units, poultry farms, and piggery farm. In all

the visited farms, insecticides like Deltamethrin, Ektomin, Dichlorvas and pest-o-flash (light trap) are currently being used for managing the house fly. Soil baiting with larvae of *Galleria mellonella* yielded two isolates representing two genera, *Beauveria bassiana* and *Metarrizium anisopliae* from three different locations. No fungus was recovered from the UAS, GKVK Piggery farm. The survey revealed incidence of fungal species in all farm sectors house fly population was not widely prevalent in all the locations surveyed. Only two fungal species viz., *B. bassiana* and *Aspergillus flavus* were isolated during the survey and here was no epizootic incidence of fungal pathogens during this period. Future studies should be carried out on the potential of these fungal bioagents to develop fungal infection in in-situ habitat of house fly.

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