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Evaluation of entomopathogenic fungal formulations *pacilomyces lilacinus* and *verticilium laccani* against larvae of *Musca domestica*

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Abstract
The house fly, *Musca domestica* (L.), is the major insect pest in poultry facilities and intensive animal units. Large populations of flies create problems for animal and public health. The commercially available two fungal formulations (*Verticilium laccani* and *Pacilomyces lilacinus*) were prepared with four different concentrations *viz.*10gm/L, 20gm/L, 40gm/L and 60gm/L with sterile distilled water. The third instar *M. domestica* larvae was evaluated and the observations were made at 24 h intervals up to pupation.The entomopathogenic fungal formulations, *V. laccani* and *P. lilacinus* seem to be effective in controlling the pest *Musca domestica*. The fungal formulation of *V. laccani* showed that the highest mortality 63.75% was observed in the 60g/l and *Pacilomyces lilacinus* seem to be effective in controlling the pest *Musca domestica*. The higher concentration 60g/l shows that the 15% to 72.5%.

Keywords: housefly, fungal formulation, entomopathpogenic fungi, larvicidal

Introduction
The house fly, *Musca domestica* (L.), is the major insect pest in poultry farms and intensive animal units. Large populations of flies create problems for animal and public health. Housefly management relies heavily on pesticide application (Chintalchere, *et al*., 2013) [15]. However, houseflies quickly develop resistance to these pesticides. Moreover, excessive use of synthetic pesticides results in enhanced pest resurgence as well as environmental/health problems (Raja and Suresh, 2015; Srinivasan, 2008 and Malik *et al*., 2007) [17, 4, 2]. As an alternate (Raja and Suresh, 2015 & 2017; Chavasse *et al*., 1999 and Emerson *et al*., 1999) [17, 1, 3]. biological control of housefly could be very promising being eco-friendly as well as cost-effective. Hence, efforts to control flies using Biological Control Agents (BCAs) are increasingly important. Entomopathogenic fungi infect their hosts by penetration through the exoskeleton, gaining access to the haemolymph, producing toxins and grow by utilizing nutrients present in the haemocoel and final death of host insect (Ambethgar, 2009) [22]. Fungal biotechnology has become an integral part of the human welfare (Raja *et al*., 2017 and Karthikeyan *et al.*, 2014) [19, 20]. Use of entomopathogenic fungi for housefly control could have a lot of potential due to their low mammalian toxicity and natural prevalence among flies population. The entomopathogenic fungal formulations are eco-friendly, economic, target-specific and easily biodegradable.

Materials and Methods
Collection and rearing of Experimental animal
The housefly larvae were collected from the poultry farm and brought to the laboratory and reared with poultry manure in the laboratory condition. The 3rd instar larvae were used for the experiment.
Collection of entomopathogenic fungi
The entomopathogenic fungi are an important natural pathogen of insects and it has been developed as a microbial insecticide for use against many major arthropod pests of *Musca domestica.*
The mycoinsecticides of entomopathogenic fungi is based on *Beauveria bassiana*, *Metarhizium anisopliae*, *Pacilomyces lilacinus* and *Verticelium laccani* were bought from Sun Agro Biotech Research Centre, Madanandapuram, Porur, Chennai followed by two entomopathogenic fungi isolated from the infected insect cadaver of *Musca domestica*

Maintenance of fungus culture
The pure stock culture of fungus was sub cultured at an interval of three months on Emerson's YPSs agar medium. Three weeks old culture was used in all the experiments by which time, the fungus sporulated abundantly. Fully sporulated fungal mat was taken out, thoroughly washed in water, filtered and used at required spore concentration.
Sources of fungal pathogens preparation of concentration
The fungal formulations were obtained from Sun Agro Biosystems Pvt. Ltd., Chennai and five different concentrations of the fungal formulations *viz.* 10gm/l, 20gm/l, 40gm/l and 60gm/l were prepared using sterile water each concentration having different spores.
Bioassay
Four fungal formulations *viz*., *B. bassiana*, *M. anisopliae, P. lilacinus* and *V.lecanii* were evaluated against *Musca domestica.* Five different concentrations of the fungal formulations *viz.*10gm/l, 20gm/l, 40gm/l and 60gm/l were prepared using sterile water. Third instar larvae were collected from the laboratory culture and were allowed to feed on the poultry manure @ 20 larvae/container. Pre-starving was avoided to encourage maximum crawling on treated surface and to avoid immediate settlement in a feeding spot. The mouth of the containers was covered with muslin cloth to prevent larval escape. Four replicates were maintained for each concentration as well as the control.

After 48 hours of exposure fresh untreated poultry manure were offered for all the treatment as well as control categories and the observations were made at 24 h intervals upto pupation (Altre *et al.*, 1999) [18].

Corrected mortality was calculated by Abbott's (1925) formulae as in the following formula:

$$\% \text{ corrected mortality} = \frac{\% T - \%C}{100\% - \%C} \times 100$$

Where % T= Percentage of dead test organisms.
% C = Percentage of dead control organisms.
For working mortality percentage mortalities were converted to correct per cent mortalities for control. Corrected mortality data were analyzed separately.

Confirmative test
The fungal treated dead larvae were incubated in a moist chamber for 3-4 days and randomly selected insects were surface sterilized in sodium hypochlorite (1%) for 1-2 minutes and washed in three serial changes of sterile water to eliminate saprobe growth. Larvae were placed on water agar (water + agar), then transferred to PDA media for pure culture. The presence of the fungus was confirmed through microscope examination (Perior *et al.*, 1995).
Statistical analyses
All experiments were repeated four times. Data were analyzed by analysis of variance using the ANOVA procedure of SPSS (SPSS software version 20) for a completely randomized design. When the effect was significant ($P < 0.05$), means were separated using Duncan's Multiple Range Test.

Results

Evaluation of entomopathogenic fungi *Verticilium laccani* against *M. domestica*

The entomopathogenic fungi *Verticilium laccani* seem to be effective in controlling the pest *Musca domestica*. Table 1 shows the percentage mortality rate of *M. domestica* larva when treated with different concentrations of *V. laccani* formulation, *viz* 10g, 20g, 40g and 60 g for duration of 8 days. There is a proportional increase in mortality with increase in incubation time as well as higher degree of mortality was reported at higher concentrations. The lower concentration of 10g/ml, the mortality on day1 was 12.5% and it shows a improved trend up to 42.5%. The higher concentration 60g/l shows that the 15% to 63.75%. The highest mortality 63.75% was observed in the 60g/l.
The figure.1 represents the larval mortality in the study sample group of 20 larva each for a period of 8 days when treated with 10g, 20g, 40g and 60g/l concentration of entomopathogenic fungi. It was identified that 60g/l concentration was the best effective concentration till the days of incubation reached 8 days. But 60g/l concentration reaches a comparative level only on day 7 and 8. All the four concentrations are effective but according to the data, the third concentration (40g/l) proves to be the best.
The two way Analysis of variance shows that the larval Mortality was highly significant at the given different concentration of entomopathogenic fungi against *Musca domestica* at ($P \leq 0.05$) 0.5% level of significance. The R squared value 0.98, indicates towards high degree of positive correlation between days of treatment and concentration of formulation (Table 2).

Table 1: Evaluation of entomopathnogenic fungi *Verticilium laccani* against *Musca domestica* larva

Day	Corrected percent mortality (%) Different concentration			
	10gm	20gm	40gm	60gm
1	12.5	6.25	25	15
2	18.75	28.75	41.25	21.25
3	20	30	46.25	28.75
4	26.25	36.25	52.5	36.25
5	31.25	36.25	52.5	43.75
6	35	40	57.5	47.5
7	37.5	45	57.5	52.5
8	42.5	45	61.25	63.75

Table 2: Analysis of variance shows the significant difference among the different concentration of *V. laccani*

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Model	2383.740[a]	11	216.704	17.209	.000
gms	211.443	3	70.481	5.597	.006
days	80.061	7	11.437	.908	.519
Error	264.447	21	12.593		
Total	2648.188	32			

a. R Squared =.900 (Adjusted R Squared =.848)

Fig 1: Experimental setup

Evaluation of entomopathogenic fungi *Pacilomyces lilacinus* against *M. domestica*

The table 3 shows that the entomopathogenic fungi *Pacilomyces lilacinus* seem to be effective in controlling the pest *Musca domestica*. Table7 shows the percentage mortality rate of *M. domestica* larva when treated with different concentrations of *V. laccani* formulation, *viz* 10g, 20g, 40g and 60 g for duration of 8 days. There is a proportional increase in mortality with increase in incubation time as well as higher degree of mortality was reported at higher concentrations. The lower concentration of 10g/ml, the mortality on day 1 was 7.5% and it shows a improved trend up to 36.5%. The higher concentration 60g/l shows that the 15% to 72.5%. The highest mortality 72.5% was observed in the 60g/l. The 40g/l and 60g/l are more effectively controlled the larvae by *P. lilacinus.*
The figure 2 represents the larval mortality in the study sample group of 20 larva each for a period of 8 days when treated with 10g, 20g, 40g and 60g/l concentration of entomopathogenic fungi. It was identified that 60g/l concentration was the best effective concentration till the days of incubation reached 8 days. But 60g/l concentration reaches a comparative level only on day 7 and 8. All the four concentrations are effective but according to the data,

the third concentration (40g/l) proves to be the best.

The two way Analysis of variance shows that the larval mortality was highly significant at the given different concentration of entomopathogenic fungi against *Musca domestica* at (P≤ 0.05) 0.5% level of significance. The R squared value 0.98, indicates towards high degree of positive correlation between days of treatment and concentration of formulation (Table 4).

Table 3: Evaluation of entomopathogenic fungi *Pacilomyces lilacinus* against *Musca domestica* larva

Day	Corrected percent mortality (%)			
	Different concentration			
	10gm	20gm	40gm	60gm
1	7.5	6.25	37.5	15
2	13.75	31.25	51.25	17.5
3	16.25	32.5	52.5	25
4	20	37.5	56.25	30
5	25	38.75	60	33.75
6	26.25	42.5	62.5	35
7	32.5	43.75	65	65
8	36.25	47.5	70	72.5

Table 4: Analysis of variance shows the significant difference among the different concentration of *P. lilacinus*

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Model	2198.350[a]	11	199.850	97.402	.000
gms	197.303	3	65.768	32.054	.000
days	182.248	7	26.035	12.689	.000
Error	43.088	21	2.052		
Total	2241.438	32			

a. R Squared =.981 (Adjusted R Squared =.971)

Fig 2: Dead larvae in the plastic container

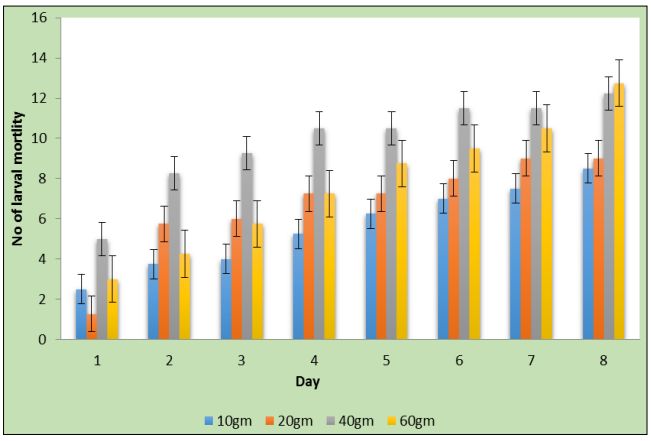

Fig 3: Evaluation of entomopathnogenic fungi *Verticilium laccani* against *Musca domestica* larva

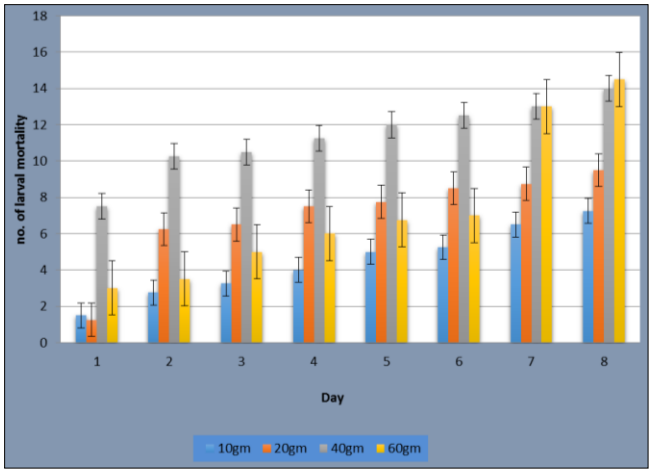

Fig 4: Evaluation of entomopathogenic fungi *Pacilomyces lilacinus* against *Musca domestica* larva

Discussion

Musca domestica has been reported to be susceptible to infection by several fungi (Barson *et al*.1994; Watson *et al*. 1996) [5, 13]. Some of these naturally infecting pathogens like *B. bassiana* and *M. anisopliae* are among the most virulent pathogens to adult houseflies (Sirietal, 2005).

In the present study, information on formulation of entomopathogenic fungi seem to be effective in controlling the domestic insect pest *M. domestica*. The percentage mortality rate of *Musca domestica* larva when treated with different concentrations of *Beaveria bassiana* formulation, *viz* 10g, 20g, 40g and 60 g for duration of 8 days. There is a proportional increase in mortality with increase in incubation time as well as higher degree of mortality was reported at higher concentrations. At a concentration of 10g/ml, the mortality on day1 was 1.25% and it shows a improved trend of up to 45.0% and the incubation time prolonged till day 8. The highest mortality 57.5 % was recorded in 60g/l. However, majority of the studies concerning entomopathogenic fungi deal with agricultural pests (as reviewed in Shah and Pell, 2003; Goettel *et al*., 2005) [12, 8].While among the vectors, many studies target mosquito (Mnyone *et al*. 2010; Leles *et al*., 2010) [11, 10] and relatively few studies have been conducted on housefly control.

Bellini *et al.* (1992) studied the dose mortality assay with *Entomophthora muscae* on housefly while Maitland (1994) reported its natural prevalence in parts of Europe and America. Similarly, *B. bassiana* and *M. anisopliae* have also been reported for housefly control studies (Steinkraussetal.1990; Geden *et al*., 1995; Watson *et al*.1995; Carswell *et al*., 1998; Lecuona *et al*., 2005) [13, 9]. These studies indicated absolute mortality of housefly populationin5–15daysperiod.However, in order to compete with the conventionally used chemical insecticides, 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.

Present study with *B. Bassiana, M. anisopliae P. lilacinus* and *V. laccani* gave absolute mortality of flies in 6 to 8 days at all the concentrations of fungal conidial suspension tested.

The result was in agreement with the data obtained by Geden *et al*. (1995) [7, 13] and Watson *et al*. (1995) [13] who reported absolute mortality of adult house flies in 5 and 7dayspost-exposure to *B. bassiana.* Barson *et al*. (1994) [5] reported complete mortality of adult houseflies within 6 days of exposure of *M. anisopliae*. The variance in time interval between exposure and kill may be attributed to difference in the strains of the pathogen used.

Conclusion

Use of entomopathogenic fungi for housefly control could have a lot of potential due to their low mammalian toxicity and natural prevalence among flies population. There is a proportional increase in mortality with increase in incubation time as well as higher degree of mortality was reported at higher concentrations. We conclude that the highest mortality 72.5% was observed in the 60g/L fungal formulation *P. lilacinus* are more effectively controlled the housefly larvae.

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