

DEVELOPMENT OF FUNGAL FORMULATIONS FOR HOUSE FLY CONTROL

By

SAPNA

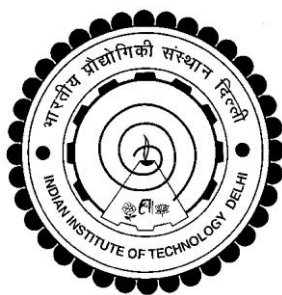
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To My Parents

CERTIFICATE

This is to certify that the thesis entitled “**Development of fungal formulation for housefly control**” being submitted by Ms. Sapna to the Indian Institute of Technology Delhi for the award of ‘Doctor of Philosophy’ is a record of bonafide research work carried out by her. She has worked under my guidance and supervision, and has fulfilled the requirements for the submission of this thesis. To the best of my knowledge the results contained in this thesis have not been submitted in part or full to any other university or institute for award of any degree or diploma.



(Dr. Anushree Malik)
Associate Professor

Centre for Rural Development & Technology
Indian Institute of Technology Delhi
New Delhi-110016

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ABSTRACT

The present study has attempted to deliver an efficient, stable and comprehensive strategy for biological control of housefly through native entomopathogenic fungal strains. The investigations involved have been isolation, selection, identification of native strain followed by process optimization, mechanism elucidation and product formulation. As a result various field applicable products with efficient housefly control potential were delivered. Out of several isolates obtained in the present study, Isolate '1' showing maximum insecticidal activity against housefly adults (100% mortality), larvae (72.3% mortality) and pupae (20.2% suppression in adult emergence) was selected for molecular identification through 18S-rRNA, and was found to be *Beauveria bassiana* HQ917687. Interestingly, the performance of this isolate was superior to twelve other native (Indian) isolates procured from culture collections.

With a view to understand the impact and range of environmental/nutritional factors on growth and infectivity of *B. bassiana*, optimization studies were conducted. Statistical optimization using temperature, yeast extract and pH for spore and biomass yield revealed 27.27 °C, 5.00 g/l and 6.75 as optimum value for temperature, yeast extract and pH respectively. Interaction between these factors showed temperature as dominant factor. In order to economize the production process, screening of various carbon and nitrogen sources as well as various agricultural by products was explored. Further optimization using glucose and NaNO₃ resulted in ≈ 16 fold increase in biomass production. Among the agricultural by products, rice husk (spore production, 4.3×10^8 conidia/g) was found to be best substrate. Virulence evaluation of selected *B. bassiana* isolate in relation to temperature and humidity showed 30 °C as the optimum temperature for infectivity in both, housefly larvae and adults, while the favourable humidity for pathogenicity was 90 and 100% RH. The study revealed temperature as the significant factor affecting housefly mortality, whereas relative humidity played prominent role in regulating the mortality at moderate temperature range.

The mechanism of action was elucidated through a combinational approach focusing on strain pathogenicity potential (via toxin and enzyme production) as well as host response (via microscopy and immune response assessment). Selected *B. bassiana* isolate depicted significant extracellular chitinase (5.24 U/ml), protease 1 (4.66 U/ml), protease 2 (5.48 U/ml) and lipase (2.26 U/ml) activity. The enzymes showed maximum activity in temperature range of 40-50 °C and pH 5-7. The toxin associated with *B. bassiana* was extracted by silica gel chromatography and characterized through HPLC, UV spectroscopy and NMR. The analysis of spectral data of ¹H and ¹³C NMR showed the principle active component of toxin sample to be beauvericin. The above results established that the *B. bassiana* strain carried a battery of enzymes and toxins known to mediate entomopathogenic action. The total haemocyte count (THCs) in the infected adult was found to be significantly higher (180%) as compared to the uninfected control, signifying the invasion. Subsequently, THCs declined with time indicating establishment of fungal infection over the insect's immune system. Further insight into relative haemocyte counts and area depicted a significant increase in prohaemocyte (PR) and decrease in granulocyte (GR) in infected housefly over uninfected ones. Comparison of haemolymph proteins between treated and untreated houseflies showed expression of 33 proteins in both the samples with down regulation of 17 proteins and

16 proteins with up regulation. Besides this, 35 protein spots showed expression only in the untreated sample, while other 21 protein spots were expressed in the treated samples only. The above observations formed a pioneering report on fundamental cellular and humoral responses in adult housefly against *B. bassiana* infection.

In order to optimize the application of selected fungal isolate, four formulations; bait, dust, encapsulation and emulsion were prepared and evaluated for their insecticidal activity in simulated field and field settings. Tea waste based bait formulation of *B. bassiana* showed 100% mortality (within 72 h) in lab assay against housefly adults. In field assay using traps, 65% relative entrapment and 100% mortality (within 60 h) of entrapped flies was observed. Dust (CaCO₃ based) formulation of *B. bassiana* resulted in 43% housefly larval mortality in a simulated field condition of decaying waste matrix. Although the bait and dust formulations were low cost and easy to prepare and transport, their storage ability was limited. Hence, more advanced formulations in form of encapsulation and emulsion was attempted. Encapsulated *B. bassiana* conidia (using skimmed milk powder, polyvinyl pyrrolidone K-90 and sugar additives) showed 100% conidial germination and retained 78% conidial viability, even after storage for 12 months at 30 °C. Encapsulated product showed 54.8% (freshly prepared) and 30.6% (after 12 month storage) mortality to housefly larvae in a simulated field condition. Emulsion formulation was prepared by initial selection of suitable surfactant with respect to effect on conidial viability, germination and germination kinetics. Tween 20 selected as best surfactant was chosen for emulsion preparation using seven vegetable oils; soybean, rapeseed, sunflower, olive, castor, til and linseed. Emulsion with linseed oil showing maximum conidial germination (94%) was evaluated for shelf life analysis and pathogenicity against housefly larvae. Shelf life analysis of emulsion revealed 28% conidial viability and 19.9% housefly larval mortality after 12 months of storage as opposed to 94% conidial viability and 51.7% of larval mortality with fresh product. Significant increase in shelf life (particularly in case of encapsulation), bioefficacy and site targeted application of *B. bassiana* formulation is expected to increase its mass applicability for housefly control. Also, the variability among products presents diverse opportunities for commercialization. While dust and bait formulations can suit local production and consumption cycle through cottage industries; advanced emulsion and encapsulation formulations can provide entrepreneurship models for organized sector.

In view of the paucity of research investigations using entomopathogenic fungi for housefly control in hot and humid climatic condition of tropical region, the present study provides a suitable option with native *B. bassiana* isolate. Academically, results constitute pioneering reports on housefly immune response against fungal infection, triggering fundamental proteomic research. On the practical side, the study delivers novel products tested in simulated field conditions for housefly control. It is expected that findings of this study would go a long way in devising mitigation measures against the neglected but challenging vector that swarms the rural/slum populations in India. Multi-dimensional approach that exerts control on all the life stages of housefly but simultaneously preserves the fly's natural enemies could be an ecologically sustainable way of maintaining the fly populations below maximally acceptable limits.

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