A Minor Research Project Proposal

on

Investigation on Impact of Entomopathogenic Fungi on Biochemical Parameters during Larval Developmental Stage of *Spodoptera Litura* (Fab)

Submitted

To

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RESEARCH PROMOTION COMMITTEE PROJECT

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5) Proposed Research Work

i) Project Title: Investigation on Impact of Entomopathogenic Fungi on Biochemical Parameters during Larval Developmental Stage of *Spodoptera Litura* (Fab)

ii) Introduction:

Spodoptera litura (Lepidoptera: Noctuidae) is an important and most detrimental pest of India and is widely dispersed throughout Asia. It is a polyphagus pest of large host range including cotton, groundnut, soybean, tomato, sweet potato, and many other crops [Balikai, et. Al. 2015]. *Spodoptera litura* has been reported to attack 150 plant species and causing 26 -100% yield loss under field conditions [Dhir et al., 1993]. Young larvae of *Spodoptera litura* eat entire leaves, and even flowers and fruits. The larvae are leaf eaters and they defoliate many economically important crops. On most crops, damage arises from extensive feeding by larvae. Therefore, there is a need to control these larvae. Generally, synthetic or chemical pesticides are used to control this pest but extensive use of these pesticides results in the development of resistance in this pest and synthetic pesticides adversely affect the non-targeted organisms, the yield of the crop, and human health. Controlling these larvae is essential to mitigate the economic impact on agriculture. Therefore, it is essential to find natural pesticide which is environmentally safe.

Therefore, it becomes imperative to search for alternative methods of control which are ecologically sound, reliable, economical and sustainable. Biological control offers a suitable alternative which includes the use of parasites, predators and microbial pathogens. Across the use of the different pesticide of entomogenous fungus, *Metarhizium rileyi* and *Metarhizium anisoplae* are an option for the management has several advantages over other traditional insecticides.

The protein metabolism is the important phenomenon during growth and development of insects. The different aspects of protein metabolism comprising quantitative changes in haemolymph protein synthesis and metabolic activity of specific enzymes have fascinated the interest of many insect biochemists. The observations from these biochemical studies indicate that protein metabolism is of immense importance in characterizing different stages of insect development (Chen, 1966). Proteases are key enzymes in the alimentary canal of insects that

are responsible for protein catabolism and release amino acids. Proteolysis plays a crucial role in insect physiology and food digestion (Chitgar *et al.,* 2013).

An entamopathogenic fungus causes infection to the host by contact mechanism of action. Once enter, the fungus grew and on response comprised formation of pathogenic structures like extracellular enzymes, production of toxins, secondary metabolites etc., which eventually caused mortality of insects (Sinha *et al.,* 2016). Changes are often expressed as metabolic changes with gradual changes in infected tissue and pathogenicity depends on the physiological state of the host. Thus, the separation and characterization of the insect proteins after infection of *N. rileyi* will be efficient in modulation of protein which can be utilized for the control of pests. Therefore, the current study examined the electrophoretic protein patterns in the 4th instar larvae of the *S. litura* treated with LC50 of *N. rileyi*. In addition, estimation of total protein and protease activity were carried out. Qualitative and quantitative evaluation of proteins in larval insect pests was of immense importance for the understanding of different physiological processes. Therefore, it is essential to study the effect of Entomopathogenic fungi that appeared on *S. litura* in terms of their toxicity and their impact on proteins and proteases that play a crucial role in relation to pathophysiology of the pest.

iii) Objectives:

1. To determine the *Spodoptera litura* feeding behaviour under field conditions. A marked feeding behaviour of on growing crop will be recorded.
2. Collection of different stages of life cycle of *Spodoptera* directly from fields and also

   their rearing in laboratory conditions.
3. To study normal proteins assay and proteases activity during larval development of *Spodoptera litura*.
4. Spraying of Entomopathogenic fungi biopestcide on the at different concentrations on larval developmental stage of *Spodoptera liyura* and **LC50** will be assayed.
5. To study proteins assay and proteases activity after spraying biopesticide during larval development of *Spodoptera litura.*

iv) Material and methods:

A) Insect

The larvae of *Spodoptera* from field will be collected and reared for maintenance of pure culture in laboratory will be maintained on natural diet.

B) Biopesticide–

M. rileyi and M. anisoplae are an entamopathogen it will be used as a biological controlling agent against *S. Litura.* .Commercial products of the fungus are available in market with known strength. The branded and quality product will be collected from Department of Entamology, University of Agriculture Sciences, Dharwad and will be used for experiment. LC-50 at every stage of pest will be determined and the relative toxicity of the entamopathogens will be studied at each instar of *Spodoptera litura.* The effective dose will be used for the present research work.

Methods

1. Estimation of proteins will be carried out by the method of Lowry (1951).By using standard graph of bovine albumin the amount of proteins will be estimated.
2. Estimation of some proteolytic enzymes of different developmental stages of *S. litura* will be carried out biochemically.
3. Spraying of biopestcide at different concentrations on the every stage of growth viz. eggs, larvae, pupae and adults and **LC50** will be assayed.
4. In this study, different developmental stages will be treated with biological controlling agent *M. rileyi* and *M. anisoplae* and its effect on protein as well as on proteolytic enzymes will be studied where it will be exactly inhibited.

v) Plan of proposed work-

Months	**Research work**
Month December	• Infested crops from local fields will be selected and observed continuously. • Morphological study of different developmental stages of *Spodoptera litura* will be conducted with photographic documentation. • The type of damage caused by *S. litura* will be recorded, and control measures used by farmers will be documented.

Month January	• Samples of *S. litura* will be collected from Sangli district fields, and laboratory culture will be maintained. • Seasonal environmental variations and their effects on developmental stages will be observed, photographed, and biochemically analyzed. • Study of normal proteins and protease activity during larval development of *S. litura*.
Month February	• Bio-pesticides (*M. rileyi* and *M. anisopliae*) will be applied to various developmental stages; subsequent protein and protease activity will be assessed.
Month March	• Total protein estimation will be performed using the Lowry (1951) method with BSA standard curve.
Month April	• Partial protein characterization and protease activity analysis will follow the method of Waghmare et al. (2015). • Data analysis
Month May	• Writing and submission of Project

vi) References:

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