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5. Objectives of the project (not more than 10 lines)

The work plan is framed to carried out research for **two consecutive years** (2007 -08 & 2008 – 09) with following major objectives:

A) Small scale production of biopesticides like *B.sphaericus* and *Bti* from cost-effective culture media (chicken–feathers) in the laboratory

B) Small-scale field trial (evaluation) in the urban areas of Puducherry to control the proliferation of mosquitoes responsible for causing vector-borne diseases.

C) To identify the possibility of other cost-effective culture media like chicken feathers

D) To explore the possibility of evaluating the Bs/Bti produced from cost-effective medium (feathers) from other agencies (depts. or institutions) either within or outside Puducherry (laboratory/field expts)

6. Likely benefits of the project

Background information

Mosquito borne diseases remain a serious global, public health problem. The three main species of mosquitoes responsible for causing filariasis, malaria and dengue are *Culex quinquefasciatus, Anopheles stephensi, Aedes aegypti* respectively. In our country, India, these mosquito vectors torment people's lives causing morbidity and mortality and is a serious health problem. It is a source of perpetual concern to the health authorities, environmentalists and administrators. The wisest approach to tackle this problem and save people's lives is by implementing mosquito control operations, on war-footing and large-scale, not merely at the onset of diseases but much prior to it and is to be continued throughout the year. Mosquito control is an essential component of disease control and relies on the use of chemical insecticides, though they are expensive and toxic to non-target organisms. The discovery of biopesticides (*Bs* and *Bti*) has revolutionized over conventional insecticides in mosquito eradication programs. The high cost of conventional media components, to produce these biopesticides on a large scale, make it necessary to utilize cheap and commonly available biological waste materials through simple fermentation technology.

Rationale

Human industrial activities, inevitably, generate industrial wastes, consisting of, inorganic and organic materials, discharged from factories, fisheries, poultries and food processing industries. Degrading or handling these wastes, as unused disposals, without acquiring any additional benefits has led to an idea to develop a suitable technology to utilize bio-organic wastes. **Biopesticide application in mosquito control** operations has gained

much importance, during the last two decades, in view of, environmental protection. However, production of *Bs* or *Bti* formulations using existing fermentation technology (using conventional culture media) incurs heavy expenditure. Therefore, the use of these biopesticides has limitations. Hence, it is imperative to develop a cheaper media for culturing *Bs* /*Bti* which would facilitate the production of biopesticides in a cost effective manner. Poopathi and co-workers have also reported **cost-effective culture media**, using potatoes, for the growth of mosquitocidal bacterial toxins (Poopathi et al., 2002b, 2003; Poopathi and Anupkumar 2004).

Chicken feathers have been discarded in bulk as waste from poultry processing industries, poultry farms and shops, globally. They normally accumulate structural proteins (keratins) that are resistant to biodegradation. Considering the abundant supply of these feather wastes, we have successfully produced the biopesticides by culturing *Bacillus sphaericus* (*Bs*) and *B. thuringiensis* serovar *israelensis* (*Bti*) strains to synthesize mosquitocidal toxins. These bacteria degrade keratin present in chicken feathers and produce endotoxins, which kill the mosquito larvae. This killing effect was confirmed by laboratory bioassays done against the mosquito vectors (*Culex quinquefasciatus, Anopheles stephensi, Aedes aegypti*) and the toxic effect was found to be high. The toxicity is due to the spore/crystal toxins produced by these bacteria during the sporulation period. Cost-effective analysis indicates that the use of chicken feather waste as culture medium is highly economical for the industrial production of these mosquito pathogenic bacilli. Recently, we have successfully developed a cost-effective culture medium from poultry industry waste, *i.e.,* chicken feathers, enabling the growth of *Bs* and *Bti* (Poopathi 2005, 2006). Utilization of chicken feathers as growth medium possesses the dual benefits of effective utilization of bio-organic waste materials from the environment and for the production of mosquitocidal biopesticides as well.

We have already standardized the methodology (simple fermentation technology) for the production of chicken–feather based biopesticides which is an outcome of a patent (Microbial fermentation process from bird feather for the production of bio-pesticides by S. Poopathi, Indian Patent Application No. 319/Del/2005). The outcome of background work done includes a paper publication in the journal: *Biological Control* (copy enclosed). Presently, the aim is to produce the biopesticides from feather waste on a small-scale basis for controlling the filariasis vectors in the field (UT of Puducherry), as a lab to land approach. The bio-organic materials (other than feathers) from the environment would also be screened for identifying suitable culture media.

7. Methodology

Production of bio- pesticides (laboratory scale)

Cultures of *Bacillus sphaericus* (IAB-59) and *B. thuringiensis* serovar *israelensis* (IPS-82) would be used for the present study.

Chicken feather waste would be collected from local poultry farm and brought to the laboratory, washed in tap water, air-dried and stored at room temperature. After making culture medium from these feather wastes, (Poopathi & Abidha, 2007, *BIOLOGICAL CONTROL,* 43:49 -55) they would be inoculated with the target mosquitocidal bacteria (*Bs/Bti)* and allowed to grow till the optimum growth is obtained. Subsequently, the bacterial spore/crystal toxins would be obtained by centrifugation from the culture medium. As these toxins need to be produced on small-scale basis for field evaluation, the growth medium also needs to be prepared in bulk quantities depending on the area for spraying application. Flask cultures of *Bs* and *Bti* would be carriedout to produce the bacterial toxins. The possibility of utilizing the biofermentor, available in the host laboratory would be assessed for the present

work. Laboratory bioassays would be carried out to find out the LC50 and LC90 values using these toxins. Other cost-effective organic materials from the environment would also be evaluated in the laboratory, whether they show bacterial growth or not.

Small-scale field trial (evaluation) in the urban areas of Puducherry to control the proliferation of mosquitoes responsible for causing vector-borne diseases.

Mosquito breeding habitats from Puducherry, which perennially supports heavy breeding by *Cx. quinquefasciatus* would be selected for the study. Topographical details of breeding places would be assessed with the help of local health authorities. Spraying operations and evaluation would be carried out subsequently using *Bs* and *Bti* (biopesticides). Bacterial spores and crystals produced from the chicken feather medium would be sprayed into canals/drains/cess-pits with hand-sprayers and pre and post treatment counts of immature stages of mosquito in the habitats would be done at regular intervals of time. The samples would be brought to the laboratory for identification. The residual activity of *Bs* and *Bti* toxins on the mosquito larvae and pupae would be studied based on microscopical and visual observations. Statistical analysis would be done to assess the efficacy of toxin produced from the culture media. The investigator has already exposure in conducting similar field trials using potato based culture medium against mosquitoes (Poopathi *et al.*, 2003, *BIOCONTROL SCIENCE & TECHNOLOGY* 13 (8): 743 – 748).