Israel Journal of Entomology

Vol. XXIII(1989) pp. 59-62

DEVELOPMENT OF MICROBIAL PESTICIDE FOR MOSQUITO CONTROL IN THE PHILIPPINES

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ABSTRACT

Bacillus thuringiensis subsp. *morrisoni,* serotype II 8a:8b (PG-14), has emerged as the most likely alternative to chemical Iarvicides against mosquito larvae in the Philippines. The selective and high toxicity ofPG-14 against mosquito larvae were determined after systematic screening of 686 isolates of *B. thuringiensis.*

This strain of *B. thuringiensis* was isolated from a soil sample taken from a canal in Cebu City, Philippines. Laboratory bioassay of PG-14 against several mosquito larvae species revealed a range of susceptibilities similar to, but not identical to, the susceptibility of these species to *B. thuringiensis* subp. *israelensis* (serotype H 14). PG-14 is not detrimental to other useful organisms preying on mosquito larvae like fish, tadpoles, copepods as well as aquatic insects. This is the first discovery from the tropics of a *B. thuringiensis* strain whose toxicity is about equal to B. *thuringiensis* subsp. *israelensis* but serologically different.

Earlier studies on PG-14 conducted at BIOTECH were concerned with its mass production utilizing locally available materials, particularly agro-industrial waste products. Among the material used were poultry manure decoction and molasses. However, mung bean, *Vigna radiata* L., decoction and flour proved to be the best fermentation media. The yield and toxicity of the produced bacterial cell was higher than that of the imported nutrient broth. Dead mosquito larvae were observed 30 minutes after application of PG-14 and 100% mortality was noted after 24 to 48 hours. The shelf life of the insecticide produced at BIOTECH is about 3 months under ordinary room conditions.

In a field trial of the formulated product of PG-14 applied against the larvae of malaria vector, about 90% reduction in the total larval population was observed in the study area.

INTRODUCTION

In the Philippines, like any developing country, control of mosquitoes has been based mainly on physical elimination of breeding habitats and on chemical insecticides. Initial biological control of the larvae was done using indigenous fish species, *Gambusia affinis* and *Poecilia reticulata.* The need for improved biological control agents led us to initiate the search for new methods. In search for safe, effective and economical mosquito larvicide, the bacterium PG-14, *Bacillus thuringiensis* subsp. *morrisoni* (serotype H 8a:8b) has emerged as the most likely alternative to chemical larvicides.

From a soil sample taken from a canal in Cebu City, Philippines, a bacterial pathogen designated as PG-14 was isolated (Padua et al., 1981). From tests, it was proven that the degree of toxicity of PG-14 against mosquito larvae is about equal to that reported for *Bacillus thuringiensis* subsp. *israelensis* (serotype H-14), which was isolated in Israel (Padua et al., 1984a; Ibarra and Federici, 1986). PG-14 was found to be highly toxic to several species of mosquitoes. This was the first lime such a variety was discovered from the tropics that is serologically different from *B. thuringiensis* subsp. *israelensis.*

PG-14 is an isolate whose H-antigenic structure is identical to that of the reference strain of *B. thuringiensis* subsp. *morrisoni* (serotype H 8a:8b). The selectivity and high toxicity of PG-14 against mosquito larvae is a product of systematic screening of 686 isolates of *B. thuringiensis*. PG-14 is now patented for use in the Philippines and the first patent granted for microbial control.

LABORATORY STUDIES

The mass production of insect pathogens for pest control is now a great concern. We found that poultry manure decoction (PMD) was a superior medium than nutrient broth (NB) in producing spores and crystals at different pH levels. Sporulation was already 100% in PMD after 48 hours, while in NB it was only 70%. The total sporulation of PG-14 in different pH levels of PMD was attained after 72 hours of fermentation. PG-14 in PMD produced more cells in any pH compared with NB. The activity of PG-14 in PMD was higher compared with NB based on number of spores per ml. The favorable growth of the bacterium was recorded between pH 7.2 to 7.8 in both media (Padua et al., 1984b).

In the production of any *B. thuringiensis*, the toxicity of the harvested cells can only be determined through bioassay or toxicity tests and cannot be predicted from serotype, fermentation medium or spore count. Difference between toxicity was observed in bacteria grown in PMD, while significant differences were observed from those grown in NB. LC_{50} values indicate the PG-14 grown in PMD are generally more toxic than those grown in NB. Bacterial cells grown at pH 7.4 in both media were found to be the most toxic (BIOTECH, 1983, 1984).

The addition of different carbon and nitrogen sources to PMD changes the toxicity of PG-14. It was also observed that poultry manure can be recycled and the toxicity of the bacteria was enhanced and maintained (BIOTECH, 1985). The efficient production and high toxicity of the organism must be both considered in producing a potential microbial insecticide.

Recently, in search for an alternate medium for PMD, mungbean (*Vigna radiata* L.) decoction and flour was found to be highly toxic. The LC_{50} value was 0.101 and 0.100 mg/l, respectively, which is higher than PG-14 grown in PMD. Considering the significant difference of the toxicity, the use of plain mungbean flour will save time and labor in the preparation of the fermentation medium. Furthermore, the yield and toxicity of PG-14 is stable when mungbean flour medium is used (Padua and Alcantara, in press; BIOTECH, 1986).

HOST RANGE AND SAFETY

PG-14 has been tested successfully against malaria vectors, particularly *Anopheles flavirostris* and *An. litoralis*, dengue vectors like *Ae. aegypti* and *Ae. albopictus* and filariasis vectors like *Ae. poecilus* and *Culex quinquefasciatus*. The bacterium is also toxic against *An. balabacensis*, *An. manyanus*, *An. indifinitus* and *Armegeris* sp. Recently, Lacey et al. (1988) reported the susceptibilities of the 8 species of mosquito larvae from temperate countries. The susceptibilities are similar to, but not identical to, the susceptibilities of these species to the toxin of *B. thuringiensis* subsp. *israelensis*.

The use of PG-14 is not detrimental to non-target organisms preying on mosquito larvae. No mortality was observed in all the non-target insect and animal species exposed to different concentrations of PG-14. The non-target organisms tested were two species of water bug, *Laccotrephes robustus* Stal., *Cercotmetus* sp., a damselfly, *Ischnura senegalensis* (Rambur), the larvivorous fish, *Gambusia affinis* and *Poecilia reticulata*, the frog, *Rana* sp., and 4 species of crustacea, *Cryptocyclops bicolor lijantus*, *Ectocyclops phaleratus*, *Phylognathopus viquieri* and *Cypricercus* sp. (BIOTECH, 1985).

Most of these toxicity tests have been conducted in the laboratory. These non-target organisms were also unaffected after ingesting the dead mosquito larvae infected with the pathogen.

SHELF LIFE STUDIES OF THE PRODUCT

A powder formulation of PG-14 had a long shelf life when stored in very low temperature (0–10°C). Formulation stored in ambient condition lost insecticidal activity after 8 weeks. The toxicity of the product in simulated field trials was found to be influenced more by the effect of formulation rather than the rate of application.

FIELD TRIALS

The effect of varying amounts of vegetation on the toxicity of PG-14 was evaluated using simulated stagnant water condition. In the present study, the different amounts of vegetation did not significantly affect the activity of the formulated product during fair weather regardless of the amount of insecticide applied. The presence of vegetation was found to be advantageous, as shown by the significant treatment differences.

An. flavirostris, the malaria vector in the Philippines, was the dominant species in the area (Palawan) where the field trial of PG-14 was conducted. *An. balabacensis, An. subpictus, An. franciscoi* and *An. vagus vagus* were the other species of mosquito larvae present. High larval density of *An. flavirostris* was recorded before the initial application of PG-14.

A highly significant treatment difference was obtained after PG-14 application. About 90% reduction in the total larval population exposed to the treatment was observed 2 days after application of the microbial insecticide. A marked decrease in pupal development from the early instar was also observed. On the other hand, there was 50% pupation when the stream was left untreated (control) for a week.

The favorable performance of the formulation was attributed to the high percentage of early instars in the treated stream and also the semi-stagnant water condition. Weekly application should be done during the early instar of the vector mosquito to reduce the probability of pupal development.

CONCLUSION

The most promising larvicide ever tried against mosquito larvae in the Philippines is PG-14. It possesses nearly every characteristic of an ideal microbial control agent. It is highly specific, effective, relatively inexpensive to produce and safe for most non-target species, including humans. PG-14 has a fairly good chance of replacing or at least complementing chemical insecticides in practical use. A potentially large market for the product also exists in developing countries where malaria and related diseases caused by mosquitoes are reported.

ACKNOWLEDGEMENTS

This research has been financed in part by the grant from UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, Department of Health (Malaria Eradication Service), Philippine Council for Health Research and Development, National Science and Technology Authority and the National Council on Integrated Area Development (Palawan Integrated Area Development Project) of the Philippines.

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