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Comparative efficacy of UV screens in protecting the activity of a *Bacillus thuringiensis* formulation

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ABSTRACT: **UV inactivation of *Bacillus thuringiensis* var. *israelensis* (*B. t. i.*) toxin is a common problem encountered with any floating formulation against mosquito larvae. An alginate encapsulated, slow-release, floating formulation of *B. t. i.*, incorporated with UV screens such as acriflavin or methyl green or congo red was prepared and evaluated for larvicidal activity against *Culex quinquefasciatus*. The data indicated: i. Encapsulation of *B. t. i.* toxin in sodium alginate has prolonged its residual activity for 45 days, ii. all the UV screens used gave significant level ($F=21.66$, $df = 3716$, $P<0.0001$) of protection to *B. t. i.* toxin from inactivation by sunlight even after 180 h exposure to direct sunlight (4 hours exposed to direct sunlight daily till 45th day) and iii. compared to acriflavin or methyl green, congo red provided excellent protection.**

KEY WORDS: Acriflavin, alginate encapsulation, *Bacillus thuringiensis*, congo red, methyl green, photoprotection

Various formulations of *Bacillus thuringiensis* (*B. t.*) have been used for more than two decades as biological insecticides to control pests and vectors of a variety of human and animal diseases (Armstrong *et al.*, 1985). These formulations were not adequately stable under field conditions and rapidly lost their biological activity (Pinnock *et al.*, 1974). Photoinactivation is the major environmental factor affecting stability and efficacy of *B. t.* (Beegle *et al.*, 1981). A few preparations of *B. t.* var. *israelensis* (*B. t. i.*) have been reported to be ineffective against mosquito larvae in water due to inactivation on exposure to sunlight and /or UV rays (Ignoffo *et al.*, 1981; Pishokha *et al.*, 1986; Becker *et al.*, 1992; Liu *et al.*, 1993). The inactivation has been reported to be caused by the generation of peroxide radicals of amino acids (Ignoffo and Garcia, 1978). Efficacy of *B. t. i.*, mostly applied as wettable powder or emulsifiable concentrate formulations, was reported to be further reduced to 24 to 48h due to its disappearance from the water surfaces namely the feeding zone of mosquito larvae (WHO, 1982). *B. t. i.* toxin (Spore-crystal complex) will sink down to the bottom of the habitat due to its heaviness and hence several floating formulations have been attempted for effective control of mosquito larvae (Kuppusamy *et al.*, 1987). The problem of UV inactivation is presumed to be more with floating formulation as they are exposed to direct sunlight. Therefore, to overcome the problem of UV-inactivation of *B. t. i.* toxin and make the toxin available in the larval feeding zone for prolonged period, a slow-release floating formulation of *B. t. i.* incorporated with UV screens was prepared and same was evaluated for larvicidal

activity and the results are reported hereunder.

MATERIALS AND METHODS

An indigenous strain of *Bacillus thuringiensis* var. *israelensis (B. t. i.)*, VCRC B17 (Balaraman *et al.*, 1981) was grown in a pilot fermentor (100 L. capacity) at the Vector Control Research Centre (VCRC) and the spore-crystal complex (*B. t. i.* toxin) was harvested by centrifugation, lyophilized and its potency (ITU/mg) determined as per standard protocols (WHO, 1982). The *B. t. i.* toxin obtained through this method with an activity of 2000 ITU/mg was used as active ingredient (a. i.) of the formulation. The a. i. was mixed with a solution of sodium alginate (4%) at the ratio of 1:1, v/w (Durand and Na Varro, 1978) and divided into three lots. The UV-protectants (Sigma chemical company, USA) namely, acriflavin (AF), methyl green (MG) and congo red (CR), were added to these lots separately at 0.1 per cent level w/v and mixed to homogeneity. Using a Pasteur pipette of 6 mm diameter, these slurries were dropped separately to a 0.1 M solution of calcium chloride with constant stirring at 30 ± 2°C. The resultant spherical granules were allowed to harden by keeping them in the same solution for further one hour, then removed with a strainer, dried for 2 days in shade at 35 ± 2°C and stored at 28 ± 2° C. These formulations were tested for larvicidal activity against III instar larvae of *Culex quinquefasciatus* and the test procedure is as follows:

One hundred larvae were placed in 500-ml chlorine free tap water held in one litre beaker, along with 200mg of the formulation in quadruplicate. Beakers with larvae in water and with no formulation served as control. The larvae were fed with sterile dog biscuit and yeast daily. All the beakers were exposed to sunlight for 4 h and observations were recorded for larval mortality after 24 h. Then the formulations were transferred to fresh set of beakers containing water and larvae as mentioned above, the beakers were exposed to sunlight for 4 h and observed for larval mortality after 24 h. The experiment was terminated after 45 days because the beads start disintegrated one by one; the test was continued for 45 days following the above said procedure. From the data collected, percentage larval mortality was calculated after correcting to control mortality, if any (Abbott, 1984).

The data were subjected to one way (ANOVA) test after transforming the percentage values to arcsine values and Duncan's multiple range test was used to compare the mortality rates between formulations.

RESULTS AND DISCUSSION

The *B. t. i.* formulation with no UV-protectant (control) has lost 25 per cent of its activity by day 8, 50 per cent by day 16 and 75 percent by day 33 and the corresponding days for the formulations incorporated with the UV-protectants acriflavin, methyl green and congo red are 10, 21 and 38; 15, 27 and 38, and 23, 30 and 40, respectively (Fig.1). The *B. t. i.* formulation with UV protectant floated up to 45 days.

ANOVA test indicated that the efficacy of the three *B. t .i.* formulations differed significantly ($F=21.66$, $df = 3716$, $P<0.0001$). Further, Duncan's Multiple range test showed that the formulation containing the UV protectant congo red was more efficient than those containing methyl green or acriflavin.

Bacillus thuringiensis var israelensis has been reported to be highly toxic to mosquitoes (WHO, 1982). However, it has been observed that irradiation by UV rays at wavelengths of 250-380nm has a detrimental effect on the toxicity of *B. t. i.* (Ignoffo and Garcia, 1978). After exposure to a 1.34×10^5 J/m^2 of UV irradiation at 258 nm, the *B. t. i.* has been found to lose its toxicity completely (Liu *et al.*, 1993). Protection from UV-inactivation of *B. t.* toxins has been attempted by encapsulation technique, granular formulation and addition of UV screens to *B. t.* formulations (Dunkle and Shasha, 1989; Shapiro, 1989; Cohen *et al.*, 1991; Morris, 1983; Ahmed *et al.*, 1973)

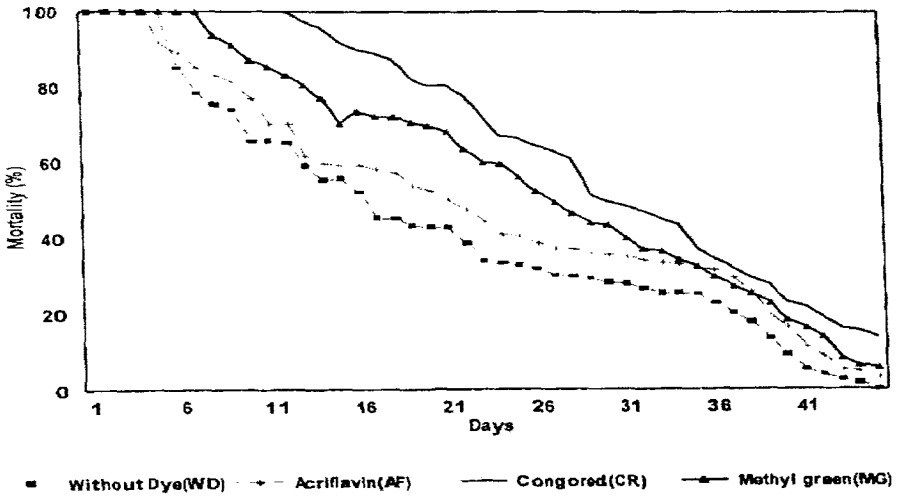

Fig 1. Results of *B. t.* encapsulated alginate beads with different dyes

Selective chromophores, exchange energy or electrons with an excited component of the *B . t.* toxin (Margulies *et al.*, 1985) and thus protect the delta-endotoxin from UV rays. The chromophores used were acriflavin, methyl green and rhodamine B (Cohen *et al.*, 1991). Congo red has also been found to absorb light intensely in the visible portion of the solar spectrum (Absorbance max 496 nm) and the ultraviolet (Gurr, 1971).

In the present study it has been demonstrated that the encapsulation of *B. t. i.* toxin in sodium alginate has prolonged its residual activity for 45 days and incorporation of UV screens has improved the larvicidal activity than the control. And among the UV screens, congo red provided excellent protection from UV rays.

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