SUSCEPTIBILITY TESTS OF THE HOUSE FLY ADULTS, *Musca domestica* TO *Bacillus thuringiensis*

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ABSTRACT

Susceptibility tests of *Musca domestica* adults to *Bacillus thuringiensis* in a form of a biobacterial preparation, or insecticide "BIOTROL XK", proved that the LD_{50}, LD_{70} and LD_{90} after 24 hours were 5.35×10^9; 7.5×10^9 and 1.09×10^{10} viable spores/gm. After 48 hours, the same doses were 3.84×10^9; 5.54×10^9 and 7.75×10^9. After 72 hours, the doses were 3.31×10^9; 4.89×10^9 and 6.91×10^9. Finally, the same doses after 96 hours were 2.69×10^9; 3.86×10^9 and 5.62×10^9 viable spores/gm. respectively.

The adults were infected per os. The biological preparation could not be recommended in the biological control of this insect without studying the susceptibility of its larval instars to the same preparation.

INTRODUCTION

Bacillus thuringiensis is a significant pathogen for many insect species belonging to the Coleoptera, Diptera, Lepidoptera and Hymenoptera (STEINHAUS, 1957; MARTOURET & MILAIRE 1963, FEDORINDRIK, 1963 and HABIB, 1968). Sufficient exotoxin of *B. thuringiensis* appeared in animal droppings to kill larvae of the common house fly, *Musca domestica* (MILLAR, 1965 and GINGRICH, 1965).

HALL & ARAKAWA(1959) found that when preparations of *B. thuringiensis*, containing viable spores and protein crystals, were added to the larval medium of the house fly (*M. domestica*), the development of the larvae was markedly inhibited although there was little effect on the treated adults. The bacterial activity detected by McCONNELL & RICHARDS(1959) and that found by BURGERJON & BARJAC(1960) are often regarded as being identical despite differences in the effective routes of administration. Also, it is often taken for granted that the inhibition of development of house fly larvae caused by autoclaved *B. thuringiensis* supernatant results from the heat stable exotoxin.

In Brazil, FIGUEIREDO et alii(1960) made some tests in order to study the efficiency of *B. thuringiensis* in controlling some insect pests, and to determine the activity of this bacterium on house fly larvae and adults. They concluded that the adults showed less susceptibili

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ty than the larvae.

As a contribution to knowledge about the potentialities of bacterial preparations in reducing development and populations of *M. domestica*, the present work was undertaken, as the first of a series on the subject.

MATERIALS AND METHODS

A stock culture of house flies was obtained by rearing the larvae on pig ration at room temperature and relative humidity. The ration was formed by mixing parts of wheat, soybean, sun-flowers, sesame, rice, meat, fish, and lever powders; yeast, fat, calcium carbonate and phosphate, salt, and bone powder were added to the mixture.

A sample of "BIOTROL XK" from the Nutrilite products Inc., California, USA, containing 12 billion of viable spores per gram, as a wettable powder, was kept at 8^0C until use. Before using such preparation, it was necessary to evaluate its potency by estimating its content of the viable spores, using the following procedure:

Serial dilutions of "BIOTROL XK" from 1 : 10 down to $1:10^9$ were made. One cubic centimeter of each of the lower concentrations was pipetted into a petri dish; melted agar was poured into it (46^0C). The contents of each dish were stirred gently and then incubated for 24 hours at 28^0C. To avoid errors, only those dilutions which produced between 30 and 300 colonies per petri dish were considered. This process was repeated using several samples of each concentration. Average counts were taken and the number of viable spores per gram of the biobacterial preparation was easily calculated according to the following equation:

Viable spores/gm. = Average count in each plate x Reverse of dilution factor.

The different doses of "BIOTROL XK" were made, as suspensions in distilled water, ranging from 7×10^6 to 1.09×10^{10} viable spores per gram. A sugar solution (20%) was added to the dose to form the diet.

Fifty adults were treated by each dose and a similar number was used for the control. The tests were carried out at room temperature and relative humidity in sterile muslin-covered glass containers (6 cm in diameter and 13 cm in height), each containing 25 adults. The diet was given to each group in form of a piece of sterile cotton saturated with the diet suspension, and was renewed daily. In case of control, the cotton piece was saturated with only the 20% sugar solution.

At the end of each exposure time, the survivors were counted in every glass container. The percentage kill in the treated groups was calculated according to Abbott's formula (ABBOTT, 1925) and checked according to Haley's table of correction (HALEY, 1952).

RESULTS AND DISCUSSION

The results of plate counts of different samples of "BIOTROL XK"

are given in Table 1, which also includes the calculated number of viable spores per gram of the powder.

TABLE 1 - Results of plate counts of different samples of "BIOTROL XK", with averages taken from 10 plate counts per sample.

Dilution plated	Nº of cultures/cc Range	Average	Calculated Nº of viable spores/gm.
1: 3×10^8	32 - 185	36	1.08×10^{10}
1: 4×10^8	86 - 289	207	8.28×10^9
1: 5×10^8	101 - 297	269	1.345×10^{10}
1: 6×10^8	44 - 211	163	9.78×10^9
1: 7×10^8	71 - 268	209	1.46×10^{10}
1: 8×10^8	58 - 213	155	1.24×10^{10}

According to these data, the viability of spores in "BIOTROL XK" could be estimated by the range of 8.28 - 14.63 x 10^9, which was rated by the manufacturer as containing 1.2 x 10^{10} viable spores per gram.

The difference of data, found in the present work, concerning the total count of viable spores per gram in "BIOTROL XK", from those rated by the manufacturer, may be attributed to its storage at 8°C until it was used for such counting.

The present experiment proved that concentrations ranging from 1 x 10^9 to 8.09 x 10^9 viable spores per gram gave mortality which covered the range of 8 to 88.2% for the adult stage of *Musca domestica* within four days (Figure 1).

The relation between the number of viable spores per gram and the percentage of killed adults are shown in Figure 1 (Log./probit scale), from which the LD_{50}, LD_{70} and LD_{90} of the bacterial preparation can be easily obtained. The results showed that, theoretically, the dose 5.38 x 10^9 spores per gram which kills 50% of the population after 24 hours, is able to kill 69% of the same population after 48 hours, 76.5% after 72 hours and 89% after 96 hours. This decrease of tolerance with time, is due to the continuous development and growth of the bacterial cells, inside the body cavity of the host, and accordingly, to the increase of the exotoxin and analytic enzymes of this bacterium. Similarly, the concentration 7.5 x 10^9 viable spores per gram which kills 70% of the population after 24 hours, is able to control about 90% of the same population during 48 hours under the same conditions.

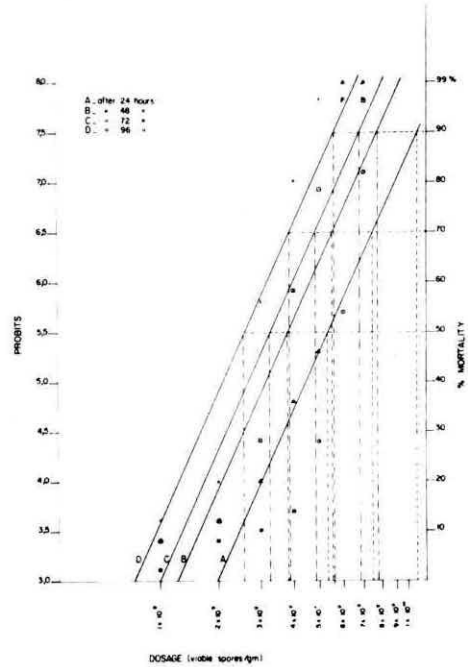

FIGURE 1 - Relationship between concentrations of *Bacillus thuringiensis* and adult mortality in *Musca domestica* (Log./probit; eye-fitted regression line).

The lethal doses of *Bacillus thuringiensis* which killed 50, 70 and 90% of the house fly adults, during four days, are shown in Table 2.

TABLE 2 - LD_{50}, LD_{70} and LD_{90} of "BIOTROL XK" during four days.

Dose	24 hours	48 hours	72 hours	96 hours
LD_{50}	5.35×10^9	3.84×10^9	3.31×10^9	2.69×10^9
LD_{70}	7.50×10^9	5.54×10^9	4.89×10^9	3.86×10^9
LD_{90}	1.09×10^{10}	7.75×10^9	6.91×10^9	5.62×10^9

It may be of interest to point out here that the 5th instar larvae of *Anagasta kuhniella* were mores susceptible to *Bacillus thuringiensis* (after 48 hours, LD_{50} was 7.0×10^8 of spores per gram, LD_{70} was 1.45×10^9 and LD_{90} was 3.5×10^9 of viable spores per gram) (HABIB, 1968) than the adults of the house fly in the present work.

From all the evidence at hand, however it may be concluded that the adults of *M. domestica* are susceptible to *B. thuringiensis*, but that possibility to use this bacterium as an agent of biological control of the same insect can be only determined after studying the susceptibility of the different larval instars to the same pathogen.

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RESUMO

Testes de susceptibilidade de adultos de *M. domestica* a *Bacillus thuringiensis* sob forma de preparação biobacteriana ou inseticida

"Biotrol XK", provaram que as DL_{50}, DL_{70} e DL_{90} após 24 horas foram $5,35 \times 10^9$; $7,50 \times 10^9$ e $1,09 \times 10^{10}$ esporos viáveis por grama. Após 48 horas, as mesmas doses foram $3,84 \times 10^9$; $5,54 \times 10^9$ e $7,75 \times 10^9$. Após 72 horas as doses foram $3,31 \times 10^9$; $4,89 \times 10^9$ e $6,91 \times 10^9$. Finalmente, as mesmas doses após 96 horas foram $2,69 \times 10^9$; $3,86 \times 10^9$ e $5,62 \times 10^9$ esporos viáveis por grama respectivamente.

Os adultos foram infectados por via oral. A preparação biobacteriana não pode ser recomendada no controle biológico deste inseto sem que se estude a susceptibilidade dos estádios larvais à mesma preparação.