PN-ABC-886

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 1/21/89

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. C 7-100
annual

Attachment

C 7-100

ANNUAL REPORT

US-Israel CDR grant No. DPE-5544-G-SS-7046-00

Title - Microbial Insecticides for Effective Control of Insect Pests: Improved Preparations by Inhibiting Bacterial Lysis and prolonging Field Stability

Principal Investigator: Professor Ephraim Cohen, Department of Entomology, Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot

Co-Investigator: Dr. Leodegario E. Padua, The University of the Philippines at Los Banos college, Laguna 3720, The Philippines

Co-Investigator: Dr. Leon Margulies, Department of soil and Water, Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot

Co-Investigator: Dr. Sergei Braun, The Biotechnology Unit, The Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem*

*Dr. Sergei Braun is replacing Dr. Zomer in the research project.

Rec'd in SCI: JAN 1 9 1989

The research project has been coordirated with the LDC co-investigator during a one week visit (on February 1988) to the Philippines. Dr. Padua arranged the visit and organized meetings with members of the Biotechnology Institute, where the research is to take place. Two Bacillus thuringiensis (B.t.) preparations (B.t. Kurstaki HD1 and HD284) which were brought by the principal investigator were the first to be examined against two major agricultural pests in the Philippines e.g. the diamondback moth and the Asiatic corn borer. The results of these preliminary experiments are included in this report. Other samples of B.t. preparations which were mailed from Israel, using the kind services of the Philippine Embassy, are currently under study using the above mentioned lepidopteran pests. As agreed while visiting the Philippines, additional B.t. formulations will be mailed from Israel on a continuous basis.

B.T. - FERMENTATION AND FORMULATION

Cultures and Harvest - B.t. strains were cultured in one litre shake-flasks containing 200ml of medium having the following composition: Glucose 0.1%, glycerol 0.1%, corn steep liquer 0.1%, yeast extract 0.5%, NZ-amine B (peptone) 0.1%, $MgCl_2$ 0.2%, $CaCO_3$ 0.2, pH 7.0. The flasks were incubated with shaking at 30°C for 40-48 hr, or until good sporulation and lysis of bacterial cells were evident. To prepare large samples, we used a New Brunswick Fermentor
with a capacity to contain 5 litres of medium. To suppress generation of foam, polypropylene glycol 2025 was added to the medium. The bacteria were grown in the fermentor at 30°C, with agitation (500 RPM) and air flow rate of 1 vvm. Spores, toxin crystals and cells were separated from the culture broth by centrifugation, washed once in distilled water and dried in a lyophilizer.

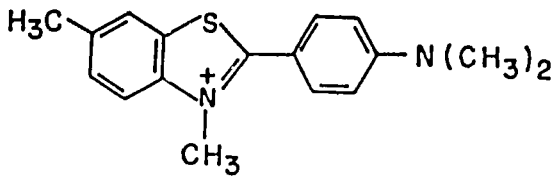
Strains and Formulations - The B.t. strains and formulation used in this research are the following:

1. B.t. Entomocidus (batch 57)
2. B.t. Entomocidus (batch 67)
3. B.t. Entomocidus (batch 27)
4. B.t. Entomocidus (batch 57, with polyoxin-D)
5. B.t. Entomocidus (batch 57, with polyoxin-B)
6. B.t. Kurstaki (HD1)
7. B.t. Kurstaki (HD284)
8. B.t. Kurstaki (HD1) with methyl acridinium (MA)*
9. B.t. Kurstaki (HD1) with thioflavin-T (TFT)*
10. B.t. Kurstaki (HD1) with methyl green (MG)*

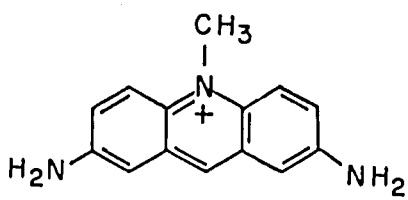
*Structures of the chromophores MA, TFT and MG are given in Figure 1.

The formulations of B.t. plus the chromophores were prepared by adding an aqueous solution of the dyes to a suspension of B.t. cells. 300 ml of B.t. suspension (37 OD at 650nm) at pH 7.3 were spun at 7,000RPM for 20 min, the supernatant was removed and the pellet suspended in 100 ml of distilled water (100 OD at 650nm). The suspension was divided into four vials (25ml in each) and 1.25ml of a 2% concentration of a given dye were added (final concentration of 0.1%). After 30 min at room temperature, the cell suspension was washed several times in distilled water followed by centrifugation, until clear supernatants were obtained. The chromophore-containing pellets were subsequently frozen and lyophilized.

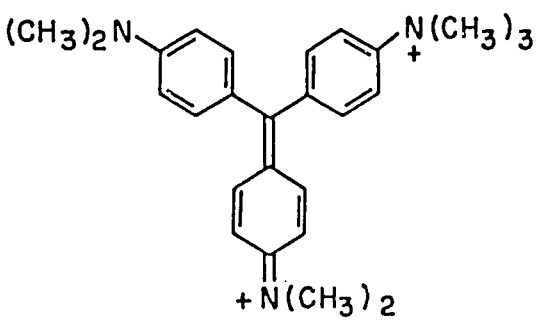
To find how much of the dye was absorbed by the B.t. preparation, a method proposed by Dr. Revithal Katzenelson from the Department of Microbial Ecology, the Hebrew University has been adapted. One gr of B.t. powder was suspended in 10 ml distilled water and mixed with an equal volume of a dye solution of a given concentration. The mixture was incubated overnight at room temperature followed by centrifugation and removal of the supernatant. The solid material was washed 10 times with X10 volumes



TFT



MA



MG

FIGURE 1. STRUCTURES OF THE CHROMOPHORES, THIOFLAVIN-T (TFT), METHYL ACRIDINIUM (MA) AND METHYL GREEN (MG)

of distilled water to remove all unbound dye. The tightly adsorbed dye was completely extracted by a 2.5% solution of sodium dodecyl sulfate (SDS) for 30 min at 80°C. This procedure was repeated twice and the combined extracts were measured spectrophotometrically. The amount of absorbed dye was calculated according to the extinction coefficient of a known concentration of a given dye dissolved in 2.5% SDS. It has been found that the amount od absorbed dye was independent of the B.t. strain used.

INSECT REARING

Our standard bioassay organism in Israel is the budworm Heliothis armigera, one of the most serious agricultural pests in the country. The insects have been raised on a large scale to provide continuous supply of larvae at the proper physiological age for the bioassay experiments. The insects are reared under controlled conditions of 27°C, 70% r.h. and a 14 hrs light photophase. The artificial diet for the larvae consists of water-soaked crushed beans, alfalfa pellet, dried yeast, sorbic acid, ascorbic acid, formaldehyde. p-hydroxybenzoic acid, chloramphenicol (or gentamycin sulfate) and agar. The ingredients after being thoroughly mixed in a blender are poured into shallow trays, and following solidification the diet is kept in the refrigerator.

For collecting eggs, ten pairs of adult moths are kept in a large 5 litre plastic container covered with cheese cloth. The insects are supplied with a sugar solution (10%) and the cheese cloth with newly layed eggs are removed daily into plastic cylinders (half litre volume). In an alternative method, the eggs are placed over several plastic Petri plates with artificial diet, and the hatched larvae reacheed the medium and start to feed. Initially, a large number of small larvae were allowed to feed on a sizable piece of artificial diet, and later individual insects were transferred to separate vials to avoid the cannibalistic behavior of

the pest. The larvae pupated inside the vials, and the pupae were removed and segregated according to sex.

In the Phillipines the sensitivities of two major insects pests to samples of B.t sent from Israel were bioassayed. The insect pests are the Asiatic corn borer Ostrinia furnacalis and the diamondback moth Plutella xylostella. Both insect pests were raised on agar-based artificial diet and continuously maintained following established procedures.

BIOASSAY

Various B.t. preparations were thoroughly mixed with the artificial diet. In the case of Heliothis, second instar larvae (3-5 mg) from stock cultures were removed and placed individually into a small Petri dish (5 cm in diameter) with about one gr of diet. For each treatment 20 larvae were bioassayed, and mortality was usually recorded six days later (in other experiments after 10 days). The LD_{50} values were calculated from computerized log dose - mortality curves. In the bioassayed carried out by the Phillipino collaborator, third instar larvae of either O. furnacalis and P. xylostella were used. The B.t. samples at various concentrations were mixed with the diet of the Asiatic corn borer (for a general scheme see Figure 2), or spread on fresh cabbage leaves and air dried prior to introduction of the diamondback moth larvae. In either assay, 6 concentrations of a given B.t sample was replicated 5 times and 5 larvae were used in each replicate. Mortality was recorded after 7 days and the data analyzed using a computerized Probit program.

UV IRRADIATION

To determine whether solar irradiation is an important factor in reducing efficacy of B.t. preparations under field conditions,

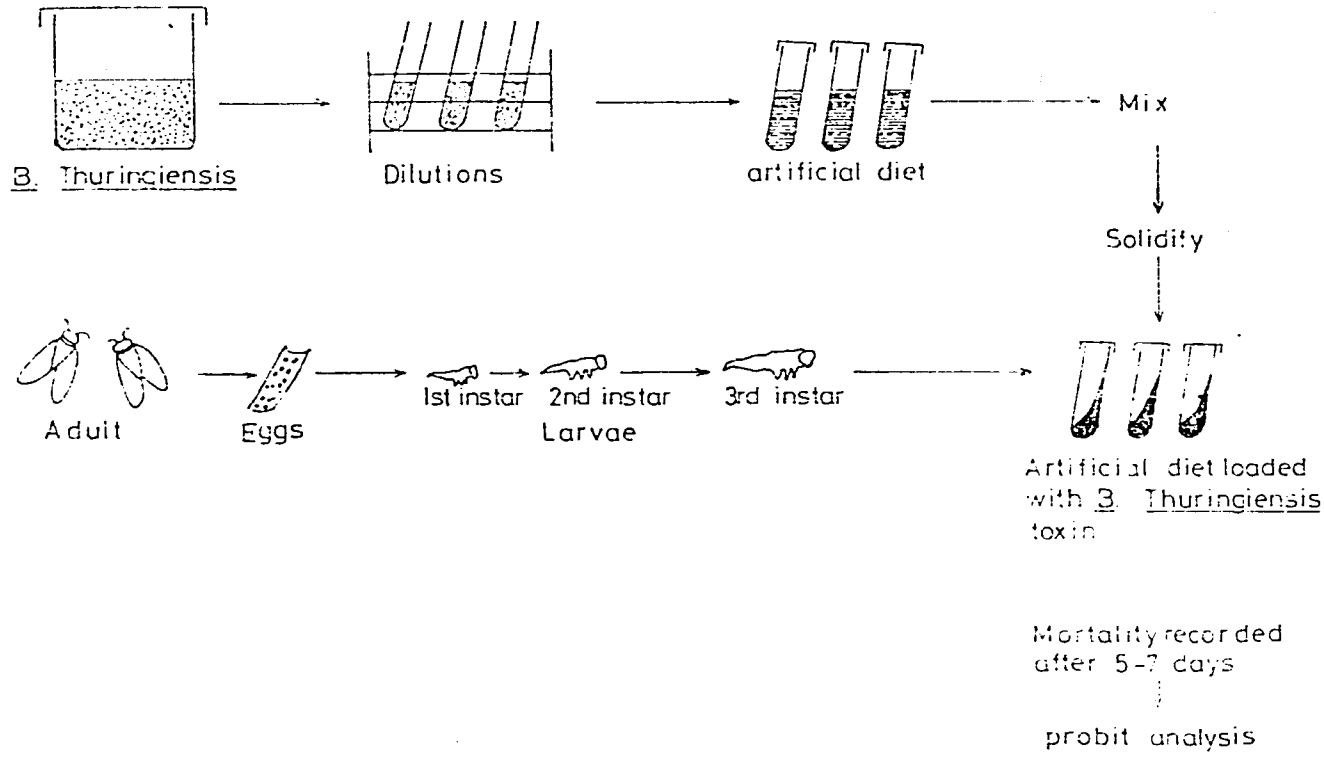


FIGURE 2. GENERAL BIOASSAY PROCEDURE FOR ESTIMATION OF INSECTICIDAL ACTIVITY (LC50) OF BACILLUS THURINGIENSIS

simulation experiments have been carried out. In a typical experiment samples containing 15 mg of B.t. Kurstaki HD1 (our standard B.t. preparation) were spread on the bottom of plastic Petri plates (5 cm inner diameter). The plates were covered with glass covers (to avoid short UV irradiation of less than 290nm) and irradiated for various time periods inside a Rayonet Photochemical Reactor at 300nm or 350nm and 1.1 Volt intensity. Irradiated and control B.t. samples were mixed with the Heliothis diet and bioassayed.

FTIR MEASUREMENTS

To measure spectra of B.t and B.t.-dye complexes, about 5 mg of a given sample was mixed with 100mg KBr and compressed to a small pellet. Fourier-transform infrared (FTIR) spectra of the pellets were measured using Nicolet MX-S spectrophotometer interfaced to an Elite Star 16-bit PC and a Goerz SE 284 digital plotter. To find whether an interaction exists between the dye and the B.t. sample, the spectra of B.t. was subtracted from that of the B.t.-dye complex. Such an interaction may indicate a possible energy transfer mecahnism upon irradiation.

DATA AND RESULTS

Bioassays of various B.t. formulations

Table 1. Effect of Various B.t. Preparations and Formulations on Mortality of *Heliothis armigera* Larvae

B.t.		Dose	Mortality (%)
Control		0	0
Kurstaki	(HD1	1mg/gr	100
Kurstaki	(HD1)	0.250mg/gr	100
Kurstaki	(HD1)	0.042mg/gr	90
Kurstaki	(HD1)	0.025mg/gr	60
Kurstaki	(HD1)	0.013mg/gr	30
Entomocidus	(57)	1mg/gr	50
Entomocidus	(67)	1mg/gr	0
Entomocidus	(29)	1mg/gr	90
Entomocidus	(57 + polyoxin-D)	1mg/gr	10
Entomocidus	(57 + polyoxin-D)	1mg/gr	69

In a preliminary experiment different B.t. strains and formulations were bioassayed using *Heliothis* larvae. B.t. Entomocidus had little effect on mortality of the lepidopteran larvae. Only batch #27 at a relatively high dose of 1mg/gr diet caused 90% mortality of the insects. Even the addition of the nucleoside peptide antibiotics polyoxin-D and polyoxin-B, known to interfere with chitin biosynthesis, did not improve the performance of this B.t. strain. B.t Kurstaki HD1 which is our standard preparation, is highly effective with 90% mortality at a relatively

low level of 0.042mg/gr artificial diet. Based on log dose - Probit analysis the LD_{50} value is 0.02mg/gr.

The experiments carried out in the Phillipines show that B.t. Kurstaki HD1 (#180) is about 18 times more toxic to the diamondback moth as compared to the Asiatic corn borer. The calculated LC_{50} for P. xylostella is 18mg/litre which is roughly 0.018mg/gr diet. This value is close to the LD_{50} value obtained for Heliothis. This B.t. strain is not toxic to the other lepidopteran species, for which the LC_{50} value is 329mg/litre diet. Similar results for both insects were obtained with a different B.t. sub-strain, the Kurstaki HD284 (#181). For detailed analysis see Table 2 and Figures

Table 2. The Effect of Bacillus thuringiensis Kurstaki on Mortality of Two Lepidopteran Pests in the Philippines

B.t. preparation	LC_{50}, mg/litre	Fiducial limit
B.t. Kurstaki HD1 (#180)		
Plutella xylostella	18.06	11.43 - 28.85
Ostrinia furnacalis	328.62	233.89 - 445.41
B.t. Kurstaki HD284 (#181)		
Plutella xylostella	19.26	11.42 - 28.85
Ostrinia furnacalis	254.53	123.79 - 381.99

Effect of UV irradiation

Data converging from many sources point to the fact that solar irradiation is a major environmental factor limiting the effective use of B.t. formulations in controlling insect pests in the field. To determine unequivocally that UV irradiation indeed affect the

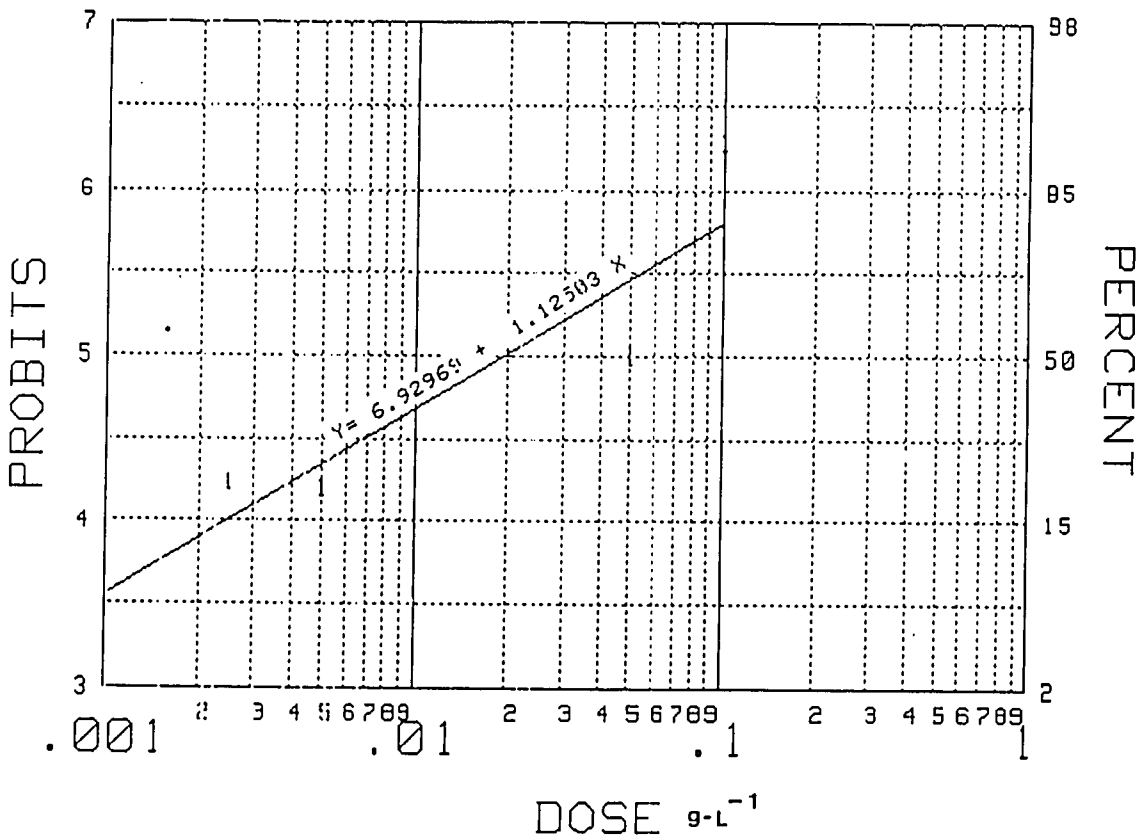


FIGURE 3A. TOXIC EFFECT OF BACILLUS THURINGIENSIS KURSTAKI HD1 ON PLUTELLA XYLOSTELLA LARVAE. A LOG DOSE - PROBIT CURVE

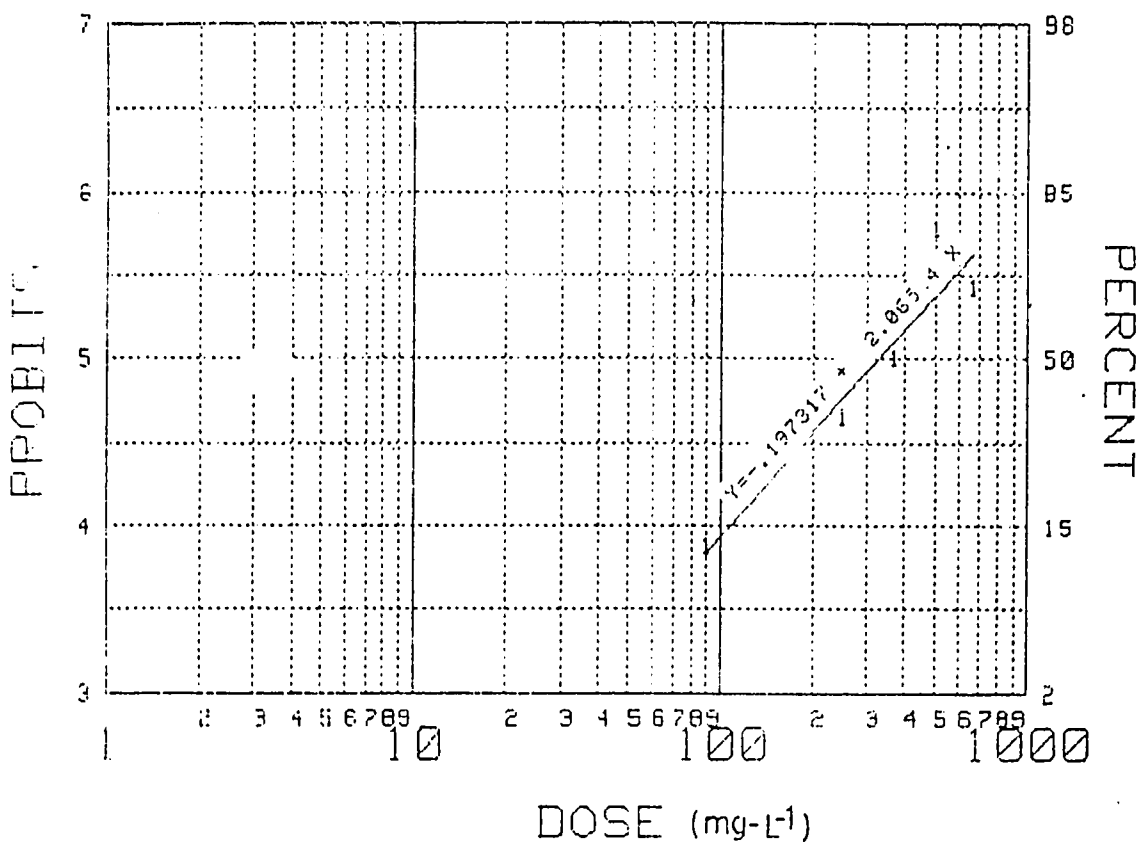


FIGURE 3B. TOXIC EFFECT OF BACILLUS THURINGIENSIS KURSTAKI HD1 ON OSTRINIA FURNACALIS LARVAE. A LOG DOSE - PROBIT CURVE

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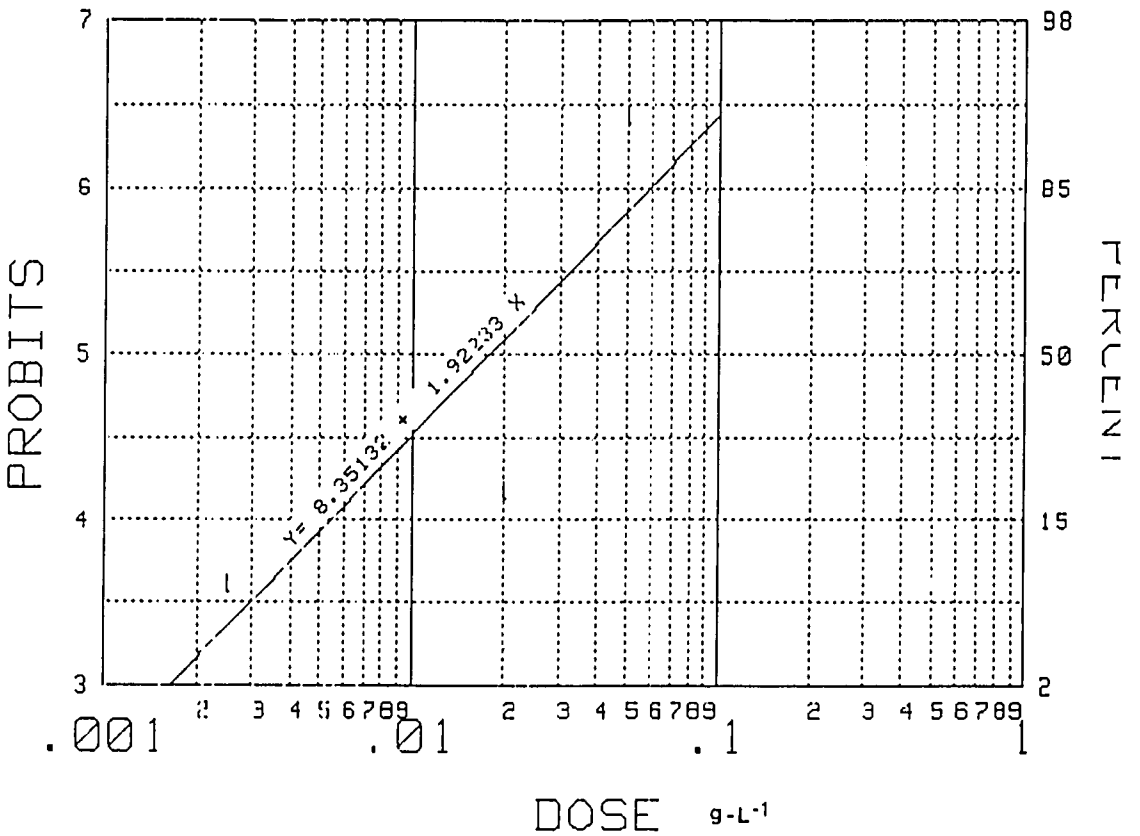

FIGURE 3C. TOXIC EFFECT OF BACILLUS THURINGIENSIS KURSTAKI HD284 ON PLUTELLA XYLOSTELLA LARVAE. A LOG DOSE - PROBIT CURVE

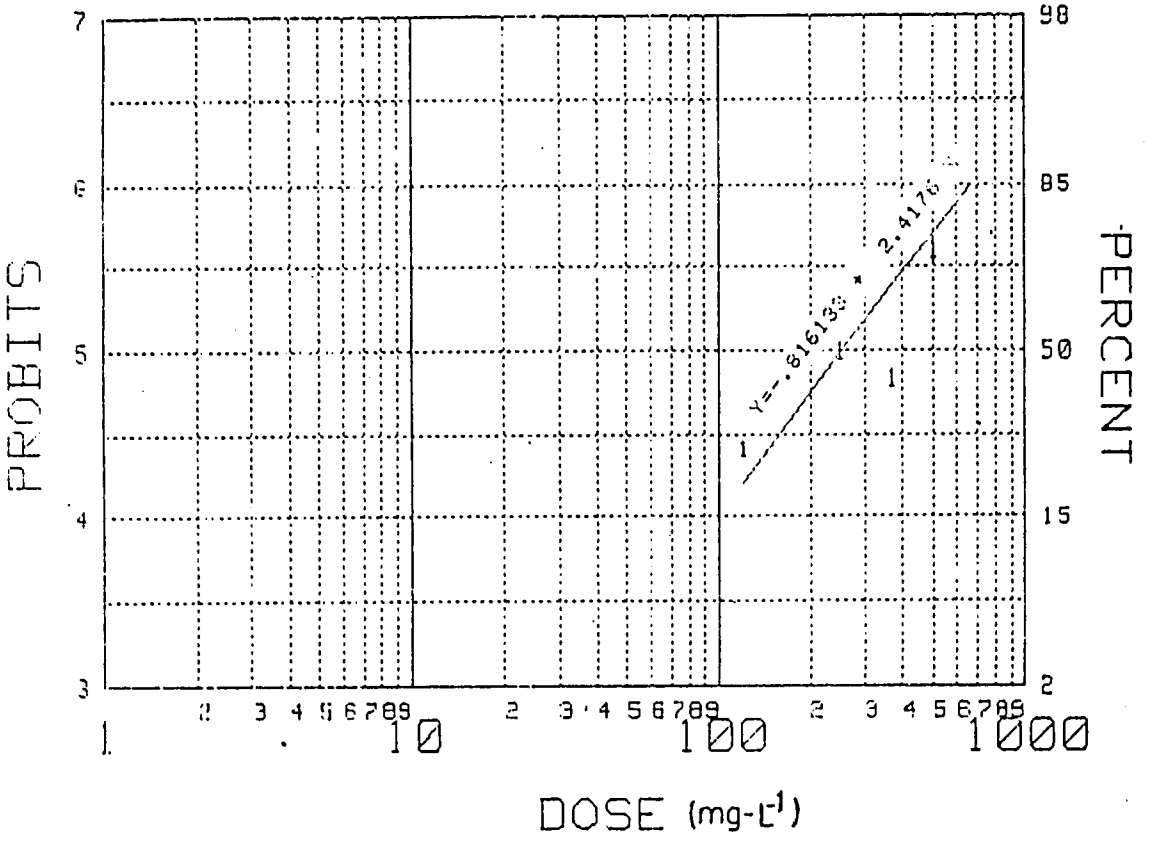

FIGURE 3D. TOXIC EFFECT OF BACILLUS THURINGIENSIS KURSTAKI HD284 ON OSTRINIA FURNACALIS LARVAE. ALOG DOSE - PROBIT CURVE

potency of B.t. to kill insects, simulation experiments were conducted. Plates with B.t. powder were exposed for various time periods to UV irradiation (300nm) in a solar simulator. Plates covered with aluminum foil were also placed inside the simulator and served as controls. The results (Figure 4) clearly show that prolonged exposure to 300nm UV irradiation completely destroyed the toxic effects of the B.t. Kurstaki. In less than 24 hr of exposure, 100% of the larvae survived, while after 5 hr of irradiation the mortality rate was about 50%. Even 1 hr at 300nm was shown to be effective, reducing the number of dead larvae by 30%.

In the previously described experiment the exposure of B.t. to short UV irradiation was examined. Table 3 summarizes results related to the effect of UV at a longer wavelength (350nm).

Table 3. Effect of Irradiation at 350nm on the Toxicity *Bacillus thuringiensis* Kurstaki HD1 to *Heliothis armigera*

Exposure Time (hr)	Mortality (%)
0	100
12	85
16	35
20	35
24	35
30	30
48 (covered)	80
Control (diet only)	0

Irradiation at a long UV range affect the potency of the B.t. preparation, yet prolonged exposure time was necessary to reduce the

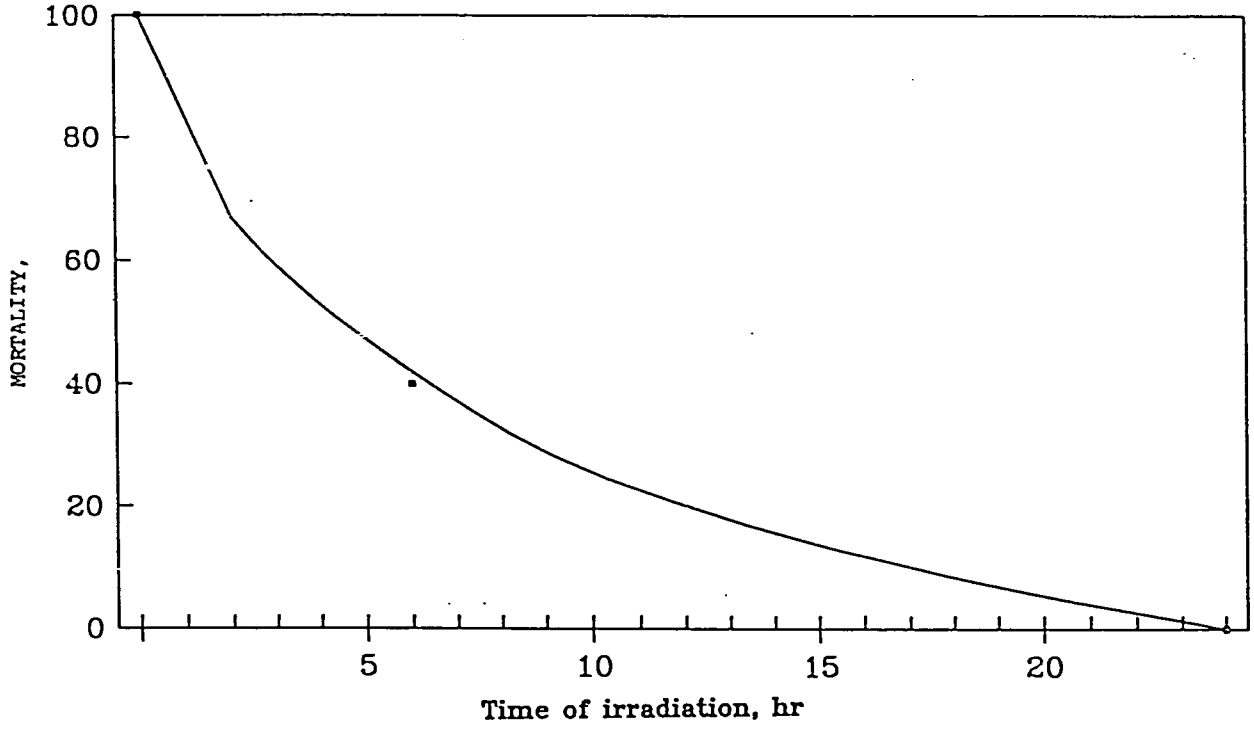

FIGURE 4. THE EFFECT OF UV IRRADIATION AT 300nm ON TOXICITY OF BACILLUS THURINGIENSIS KURSTAKI HD1 TO HELIOTHIS ARMIGERA LARVAE

toxicity. 30% mortality was recorded after 1 hr at 300nm irradiation (Figure 4) as compared to 30 hr at 350nm. Even after 48 hr of exposure still an appreciable number of Heliothis larvae died. The observation that 20% of the larvae survived in the control covered plates may indicate some effect of temperature on B.t. potency.

Chromophores and B.t.-chromophore complexes

To protect the B.t. toxin from photoinactivation, several formulations using different chromophores were prepared. The chromophores, which have been previously used to protect the pesticides bioresmethrin and NMH, from photodecomposition, are the monovalent dyes methyl acridinium (MA) and thioflavin-T (TFT), and the divalent dye methyl green (MG). The UV-visible absorbance spectra of the three dyes are shown in Figure 5. The MA has two peaks of absorbance, at 261nm and 449nm. The first peak may be relevant for competition and screening effects, while the second is appropriate for energy transfer mechanism. TFT has one major peak at 410nm, which could be effective for energy transfer. In contrast, MG has two small absorbance peaks at 310 and 420nm, and an irrelevant one as far as photoprotection is concerned, at 630nm.

B.t. - choromophore complexes were prepared as previously described. To determine the amount of the absorbed dye, the complex was extracted with 2.5% SDS at 80°C and the extract was measured spectrophotometrically using the corresponding molar extinction coefficients of the chromophores (see Table 4).

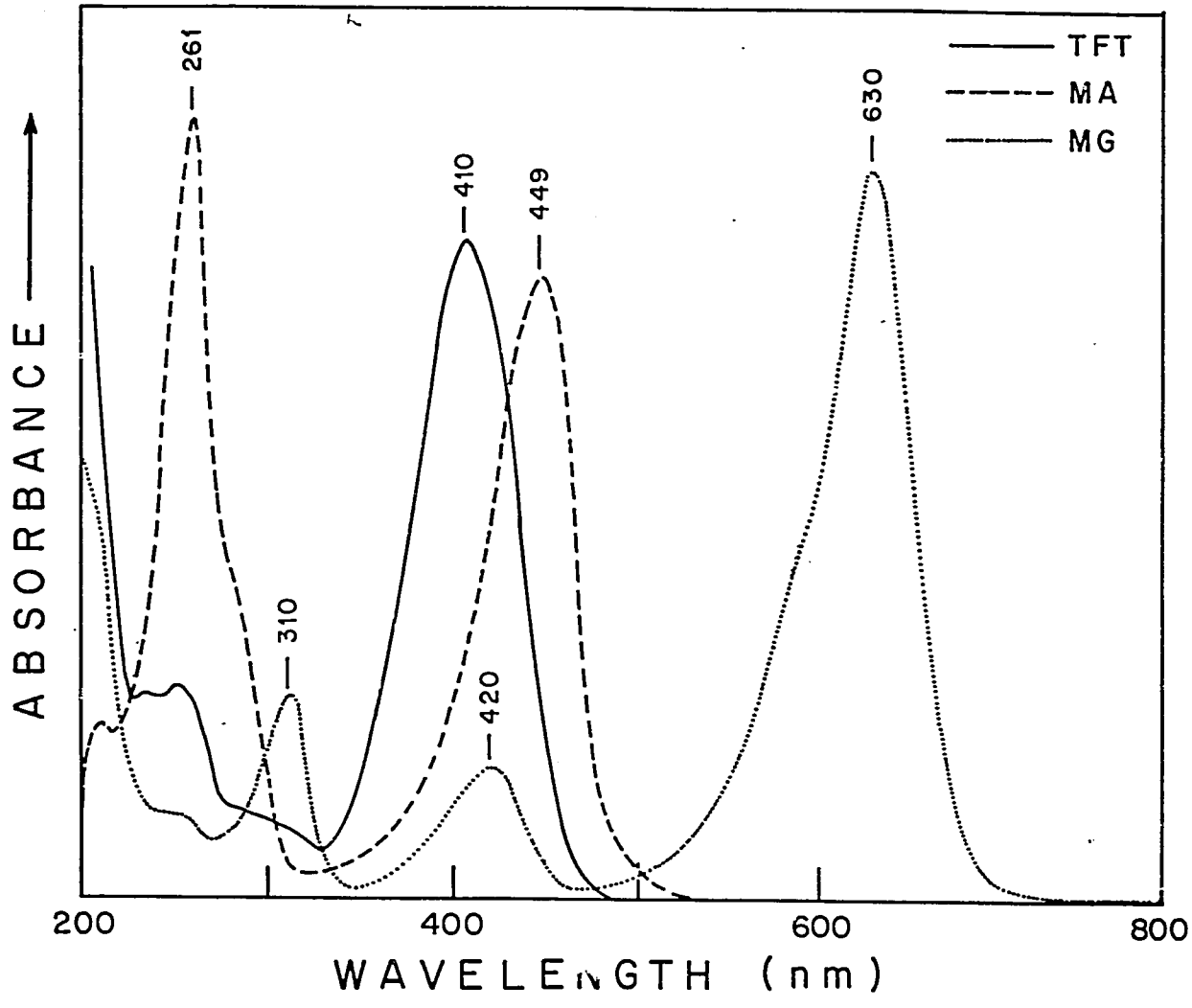

FIGURE 5. UV - VISIBLE SPECTRA OF THREE CHROMOPHORES
— TFT - thioflacin-T, -MA - methyl acridinium, --MG - methyl green

Table 4. Extinction Coefficients of Chromophores Used to Prepare B.t. - Dye Complexes

Dye	Wavelength, nm	Molar Ext., OD
Thioflavin-T	426	22900
Methyl Acridinium	454	26900
Methyl Green	640	12700

Using the curves in Figure 6, one can accurately calculate the amount of dye (in mmol per gr) adsorbed to the B.t. powder by knowing its initial concentration. It appears that methyl acridinium is strongly absorbed to the B.t. giving more than 0.1 mmol bound material per gr at the initial concentration of 10mM. At the same initial level, thioflavin-T and methyl green yielded 0.3 and 0.1 mmol per gr B.t. powder, respectively. Taking into consideration the fact that one of the absorbance peaks of methyl acridinium (at 449nm) is relevant for energy transfer mechanism, this dye is certainly a good candidate for photoprotection of B.t. preparations.

Absorbtion spectra of B.t. and B.t.-chromophore complexes

The absorbtion spectra at the UV, visible and Infrared of B.t., B.t.-dye complexes and subtraction spectra are given in Figures 7-10. In Figure 7 UV spectra of B.t. and three B.t.-dye complexes are illustrated. The B.t. spectrum has a characteristic peak at 261nm which does not change when a given dye is absorbed. The maximum absorbance peak of the dye in the complex is the same as in solution. The presence of these peaks indicates that the dyes are

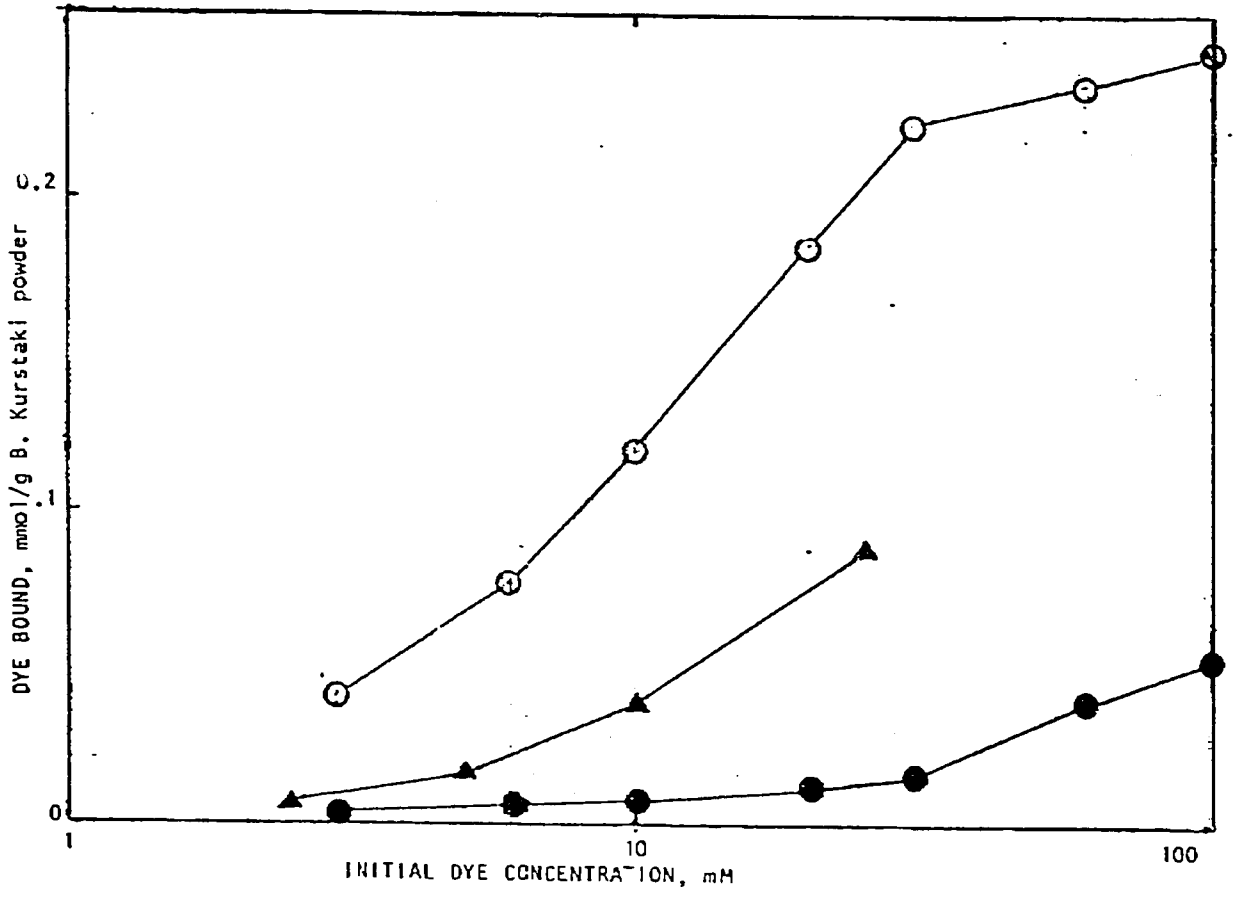

FIGURE 6. ADSORPTION OF CHROMOPHORES TO BACILLUS THURINGIENSIS KURSTAKI HD1

—O—methyl acridinium, —●— methyl green, — ▲ — thioflavin-T

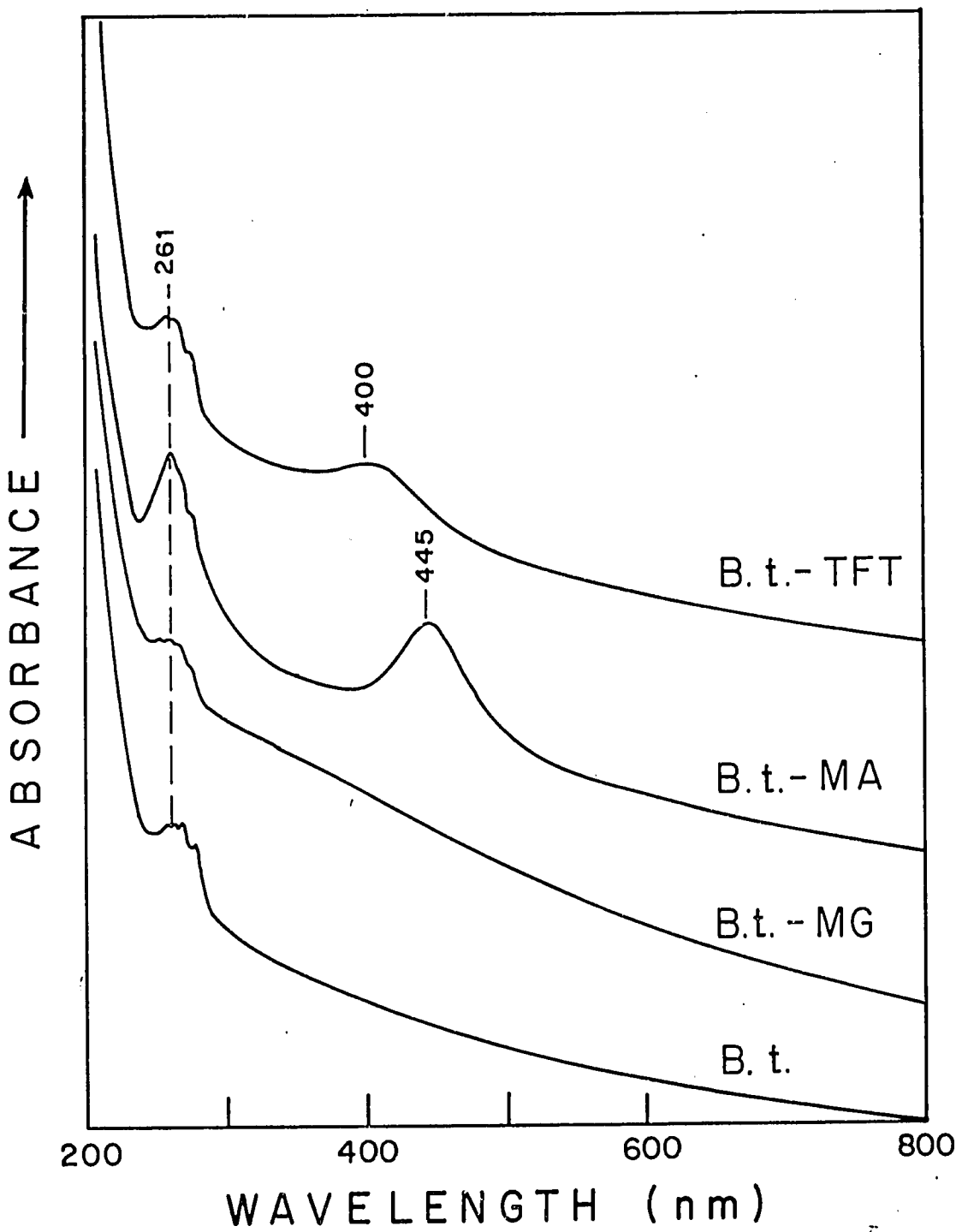

FIGURE 7. UV-VISIBLE SPECTRA OF B.t. AND B.t - CHROMOPHORE COMPLEXES
TFT- thioflavin-T, MA - methyl acridinium, MG - methyl green

adsorbed to the B.t. powder. The Infrared spectra of B.t. and B.t.-complexes is of great interest. In Figure 8 the Infrared spectra of B.t. and its complexes with three different choromophores are shown. Four major peaks can be observed at the region of 1800-1300cm^{-1}. It is evident that the peak of the B.t. at 1724cm^{-1} increased in intensity for the complexes with methyl green and methyl acridinium, indicating a strong interaction between the dye and the bacterial toxin. Such an interaction is absent in the case of thioflavin-T. Furthermore, by subtracting the b.t. spectrum from that of the b.t.-dye spectrum and comparing it to the dye powder per se, one can observe additional interactions. The above mentioned interaction at 1724cm^{-1}, is more emphasized after such subtraction (Figures 9 and 10). For methyl green a shift can be seen at 1481cm^{-1} and 1375cm^{-1} (Figure 9, a vs. b). For methyl acridinium, the shifts are at 1597cm^{-1} and 1385cm^{-1} (Figure 10, a vs. b). The B.t.-thioflavin-T complex does not show such interactions and therefore the corresponding difference spectrum is not included. For all the dye-complexes, the shifts in the absorbance peaks observed in the difference spectra, clearly indicate an interaction between the chromophore and the bacterial preparation. Such interactions coupled with UV absorbtion peaks at the relevant wavelength (see previous chapter), are promising as to protecting the B.t. toxin from solar irradiation by the energy transfer mechanism.

Protection of B.t. Preparation by Methyl Acridinium.

In a preliminary experiment to be followed by many others, the photoprotection of methyl acridinium adsorbed by B.t. was put into a test. The B.t alone and the B.t.-dye complex were irradiated at 300nm for 1, 24, and 48 hr. The B.t. preparations were mixed with the diet and bioassayed as described before. The data presented in Table 5 clearly show that when B.t. without a dye was subjected to irradiation for 48 hr, 80% of the Heliothis larvae survived, e.g. the treatment dramatically reduced the efficacy of the B.t. toxin.

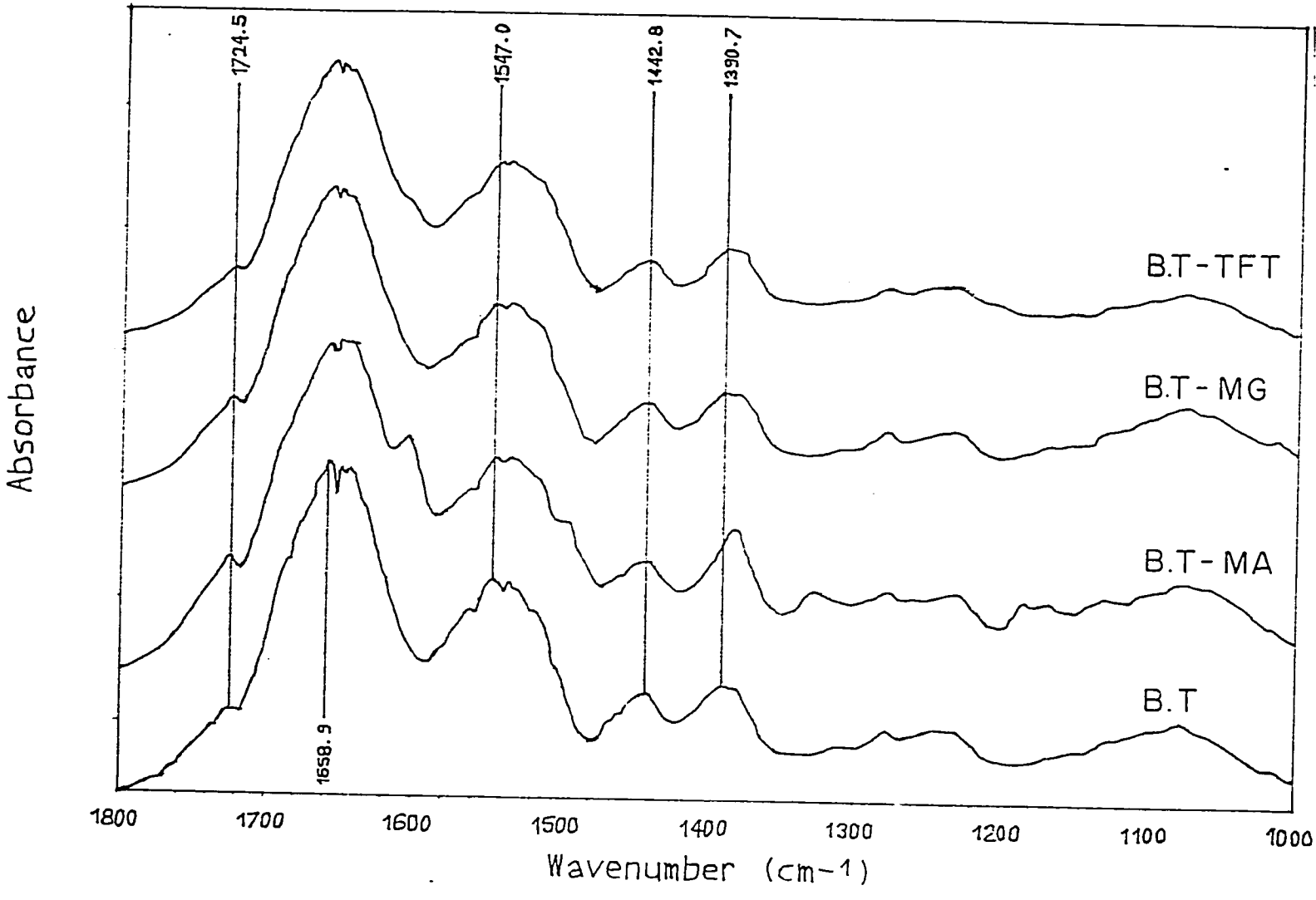

FIGURE 8. INFRARED SPECTRA OF B.t. AND B.t.- CHROMOPHORE COMPLEXES
TFT - thioflavin-T, MG - methyl green, MA - methyl acridinium

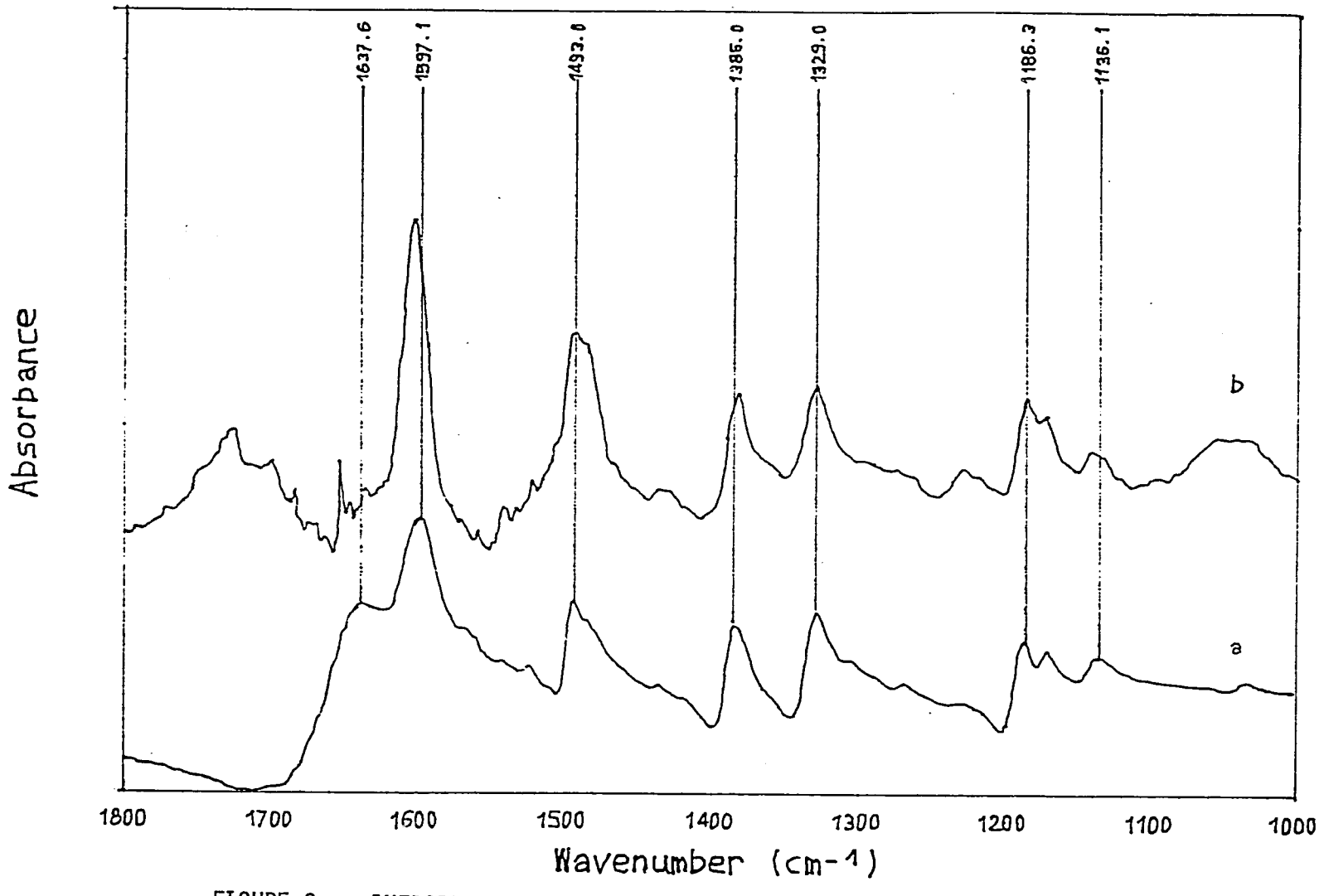

FIGURE 9. INFRARED ABSORBTION SPECTRA OF METHYL ACRIDINIUM AND THE DIFFERENCE SPECTRUM OF B.t. - METHYL ACRIDINIUM COMPLEX
a - methyl acridinium, b. methyl acridinium adsorbed on B.t.

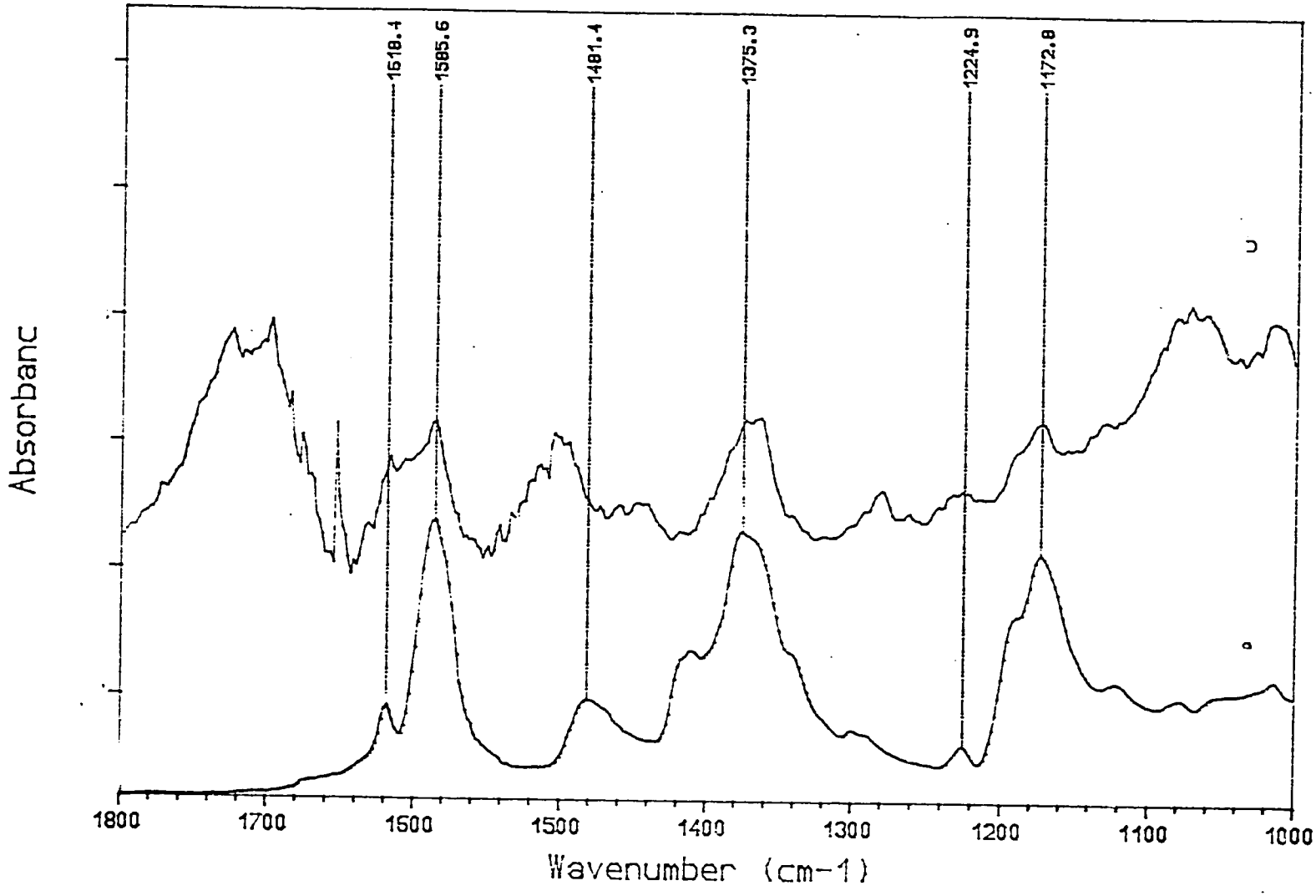

FIGURE 10. INFRARED ABSORBTION SPECTRA OF METHYL GREEN AND THE DIFFERENCE SPECTRUM OF B.T.-METHYL GREEN COMPLEX
a - methyl green, b - methyl green adsorbed on B.t.

However, with the same treatment but when a protective dye was adsorbed to the bacterial preparation, 100% of the larvae died. A similar phenomenon was observed even after 24 hr of irradiation.

Table 5. Photoprotection of B.t. by Adsorbed Methyl Acridinium

Irradiation* (hr)	% Mortality, days B.t. 6	8	10	B.t.-dye complex 6	8	10
1	80	80	100	75	75	100
24	80	80	80	60	60	100
48	20	20	20	95	95	100

*B.t. covered by aluminum foil at the time of irradiation retained their insecticidal activity, yielding 100% mortality.

It has been observed that larvae feeding on chromophore-protected B.t. were weak, sluggish and did not gain weight. Mortality recorded after 6 days did not give the full picture. When those larvae were checked after 10 days, most of them died. In future bioassays, the mortality will be recorded 10 days after the onset of the experiment and larval weight will be taken into consideration.

This report is based on experiments carried out in Israel by the principal investigator and the co-investigators as well as on research conducted by the collaborator in the Phillipines.

This report was prepared by:

Ephraim Cohen
Professor of Entomology and Toxicology

Rehovot
December 31, 1988

C7-100

INTERIM REPORT

US-Israel CDR grant No. DPE-5544-G-SS-7046-00

Title - Microbial Insecticides for Effective Control of Insect Pests: Improved Preparations by Inhibiting Bacterial Lysis and Prolonging Field Stability

Principal Investigator: Professor Ephraim Cohen, Department of Entomology, Faculty of Agriculture, The Hebrew University of Jerusalem.

Co-Investigator: Dr. Leon Margulies, Department of Soil and Water, Faculty of Agriculrure, The Hebrew University of Jerusalem.

Co-Investigator*: Dr. Sergei Braun, The Biotechnology Unit, Institute of Life Sciences, The Hebrew University of Jerusalem.

Co-Investigator: De. Leodegario E. Padua, University of the Philippines at Los Banos College, Laguna 3720, Philippines.

The research project has been coordinated with the LDC collaborator during a short visit (February 1988) to the Philippines. Dr. Padua arranged the visit which included meetings with the staff to be engaged in the above research. Two B.t. preparations (B.t. Kurstaki HD1 and HD284) which were brought along with me, will be tested against the Asiatic corn borer. Other samples to be assayed with this lepidopteran pest, will be mailed from Israel on a continuous basis, using the kind services of the Philippines Embassy.

* Dr. Sergei Braun is replacing Dr. Zomer in this research project.

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B.T. SAMPLES - B.t. strains were cultured in one litre shake-flasks containing 200ml of medium having the following composition: glucose 0.1%, glycerol 0.1%, corn steep liquer 0.1%, yeast extract 0.5%, NZ-amine B (peptone) 0.1%, $MgCl_2$ 0.2%, $CaCO_3$ 0.2%; pH7.0. The flasks were incubated with shaking at 30°C for 40-48 hrs, or until good sporulation and lysis of bacterial cells were evident. To prepare larger samples, we used a New Brunswick Fermentor, containing 5 litres of medium. To suppress generation of foam, polypropylene glycol 2025 was added to the medium. The bacteria were grown at 30°C, and with agitation (500 RPM) and air flow rate of 1 vvm. Spores, toxin crystals and cells were separated from the culture broth by centrifugation, washed once in distilled water and dried in a lyophilizer.

The B.t. strains and formulations used in the preliminary study were the following:

1. B.t. Entomocidus (batch 57)
2. B.t. Entomocidus (batch 67)
3. B.t. Entomocidus (batch 27)
4. B.t. Entomocidus (batch 57, with polyoxin-D)
5. B.t. Entomocidus (batch 57, with polyoxin-B)
6. B.t. Kurstaki (HD1)
7. B.t. Kurstaki (HD284)
8. B.t. Kurstaki (HD1) with methyl acridinium (MA)
9. B.t. Kurstaki (HD1) with thioflavine-T (TFT)
10. B.t. Kurstaki (HD1) with methyl green (MG)

The B.t. plus chromophores were prepared by adding an aqueous solution of the dyes to a suspension of B.t. cells. 300ml of B.t. suspension (37 OD at 650nm) at pH 7.3 were spun at 7,000RPM for 20 min, the supernatant was removed and the pellet suspended in 100ml distilled water (100 OD at 650nm). This suspension was divided into four vials (25ml each) and 1.25ml of a 2% concentration of a given dye were added (final concentration of 0.1%). After 30 min at room temperature, the cell suspension was washed several times in distilled water following by centrifugation, until clear supernatants were obtained. Subsequently, the pellets were frozen and lyophilized.

BIOASSAY - our standard bioassay in Israel employs the budworm Heliothis armigera, one of the most serious agricultural pests in the country. The insects are raised on a large scale to provide continuous supply of larvae at the proper physiological age for the bioassay experiments. The insects are reared under controlled conditions (27°C. 70% r.h.) on an artificial diet composed of beans, alfalfa pellets, dried yeast, sorbic acid, ascorbic acid, formaldehyde, p-hydroxybenzoic acid, chloramphenicol and agar. For the bioassays B.t. preparations and formulations are thoroughly blended with the artificial diet, and portions of about one gram each are placed in transparent small plastic vials. Larvae (8-10 mg) removed from the stock cultures are individually placed inside the vials, and mortality is recorded six days later. LD_{50} values will be calculated from computerized log dose - mortality curves.

Effect of Various B.t. Preparations and Formulations on Mortality of Heliothis armigera Larvae

B.t.		Dose	Mortality (%)
Control		0	0
Kurstaki	(HD1)	1mg/gr	100
Kurstaki	(HD1)	0.250mg/gr	100
Kurstaki	(HD1)	0.042mg/gr	90
Kurstaki	(HD1)	0.025mg/gr	60
Kurstaki	(HD1)	0.013mg/gr	30
Entomocidus	(57)	1mg/gr	50
Entomocidus	(67)	1mg/gr	0
Entomocidus	(27)	1mg/gr	90
Entomocidus	(57 + Polyoxin-D)	1mg/gr	10
Entomocidus	(57 + Polyoxin-B)	1mg/gr	69

B.t. Entomocidus has little effect on mortality of Heliothis larvae. Only Batch #27 at a very high dose of 1mg/gr diet caused 90% mortality of the insects. Even addition of the nucleoside peptide

antibiotics polyoxin-D and -B did not improve the effect. B.t Kurstaki HD1 which is our standard preparation, is effective with 90% mortality at a relatively low dose of 0.042mg/gr artificial diet. Based on log dose - Probit analysis the LD_{50} value is 0.02g/gr.

UV IRRADIATION - to determine whether solar irradiation is an important factor in reducing efficacy of B.t. preparations under field conditions, simulation experiments will be carried out. In a preliminary investigation, samples containing 15 mg of B.t. Kurstaki HD1 (our standard B.t. preparation) were spread on the bottom of plastic Petri plates (5cm in diameter). The plates were covered with glass covers (to avoid radiation of 250nm) and irradiated for various lengths of time inside a Rayonet Photochemical Reactor at 300nm and 1.1 Volt intensity. Irradiated and control B.t. samples were mixed with the Heliothis diet and bioassayed. The results (Figure 1) clearly show that prolonged exposure to 300nm UV irradiation completely destroyed the toxic effects of B.t. In less than 24 hr of exposure, 100% of the larvae survived, and after 5 hr of irradiation the mortality rate was about 50%. Even one hr was shown to be effective, reducing the number of dead insects by 30%.

To protect B.t. from photoinactivation, several formulations using different chromophores were prepared. The chromophores were the monovalent dyes methyl acridinium (MA) and thioflavin-T (TFT), and the divalent dye methyl green (MG). The UV-visible absorbance spectra are shown in Figure 2. MA has two peaks of absorbance, at 261nm and 449nm. The first peak may be relevant for competition and screening effects while the second is appropriate for energy transfer mechanisms. TFT has one major peak at 410nm, which could be effective for energy transfer. In contrast, MG has two small absorbance peaks at 310 and 420nm and an irrelevant one as far as photoprotection is concerned, at 630nm.

UV-visible and IR spectra of B.t. formulations containing the chromophores will be determined. The effect of irradiation for various periods of exposure at 300nm and 350nm on the above spectra

will be measured. Parallel bioassays will be performed to correlate wavelength, time of exposure and the presence of a given chromophore with effects on larval mortality.

This report was prepared by:

Ephraim Cohen
Prof. of Entomology and Toxicology

Rehovot
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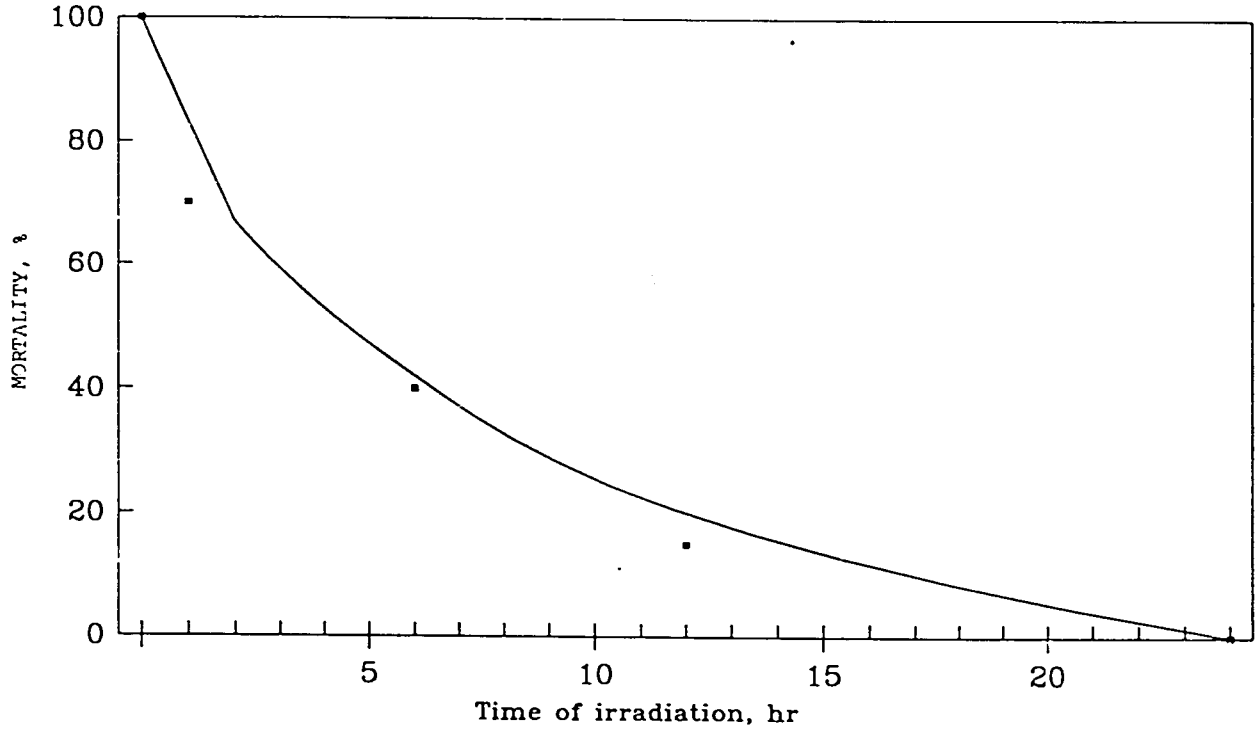
Figure 1. Effect of UV irradiation on B.t. Kurstaki HD1



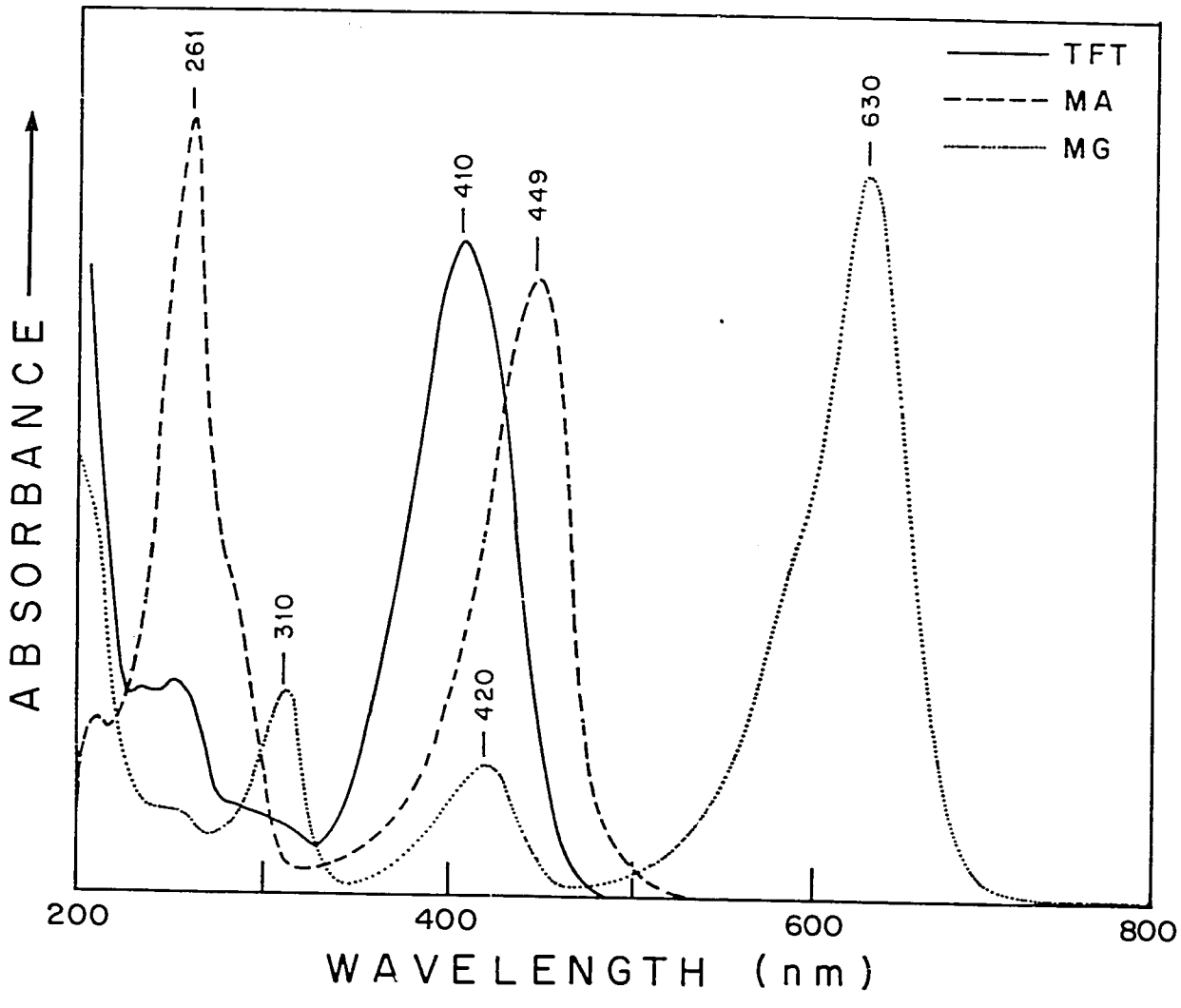


Figure 2. UV-Visible Spectra of Three Chromophores