FINAL REPORT

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Title - Photostabilization of *Bacillus thuringiensis* Formulations
for Effective Pest Control

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Executive Summary

The purpose of the research project was to provide safe, stabilized and environmentally-acceptable pest control agents. *Bacillus thuringiensis* (B.t.) is certainly unique by being highly selective and safe from any toxicological and ecological point of view. However, its performance under field conditions has been compromised by instability to sunlight irradiation, and as a consequence, by performance inconsistency. The main goal of this research project is to produce photostabilized B.t. formulations. Basically, this end will be accomplished by using selected novel photostabilizing organic chromophores, which will be included as the principal components in the formulations.

This final report covers collaborative research activities carried out by the research teams in Israel and the Republic of Georgia. It contains also details on collaboration, mutual visits of the collaborating Israeli the Georgian PIs, training periods for the Georgian scientists, and participation in International Conferences. Ultimately, the above was essential for coordinating and harmonizing the research project, the careful planning of experiments, and eventually contributed and facilitated the overall success of the entire joint research project. Moreover, the visits and training periods were of utmost benefit for the Georgian Institute as related to transfer of technical skills, access to modern methodologies and awareness of scientific approaches in the research area. In the future, this combined with the purchased equipment should help in conducting similar or related research projects. Attending an International Conference exposed the Georgian team to the international scientific community.

Most of the planned experiments outlined in the research proposal were carried out and the information gathered is included and analyzed in this final report. Steps in conducting the studies included the following: a) preparation of B.t. complexes using clays and protecting dyes; b) establishing rearing and application methodologies; c) exposure regimens of solar and simulated irradiation; d) application of B.t. complexes and formulations and carrying out relevant bioassays with the experimental insect pests. The overall photostabilizing effects bestowed by the experimental chromophores (acriflavin, palmatine, bereberine and Calcofluor White) and by a Chinese bark powder were highly significant. Inclusion of clay in the formulations increased the overall photostabilization of B.t. following prolonged exposure to UV irradiation. Obtaining stabilized formulations of B.t. for effective pest control as the major objective of the

project has been properly addressed and clearly achieved. Such formulations are immensely important in the context of environmentally-based pest control strategies aimed at reducing and replacing chemical control agents.

Research Objectives

Resistance of pests to conventional pesticides as well as environmental and toxicological problems associated with massive use and often abuse of such chemicals are of widespread concern in many countries, particularly in countries like Georgia, where the agricultural sector is very large. Microbial insecticides like B.t. offer a desired IPM-compatible alternative to broad spectrum, environmentally-unfriendly pest control agents. However, B.t. formulations lack stability under field conditions largely due to solar irradiation, and as a result, lose their potency for controlling agricultural pests. The overall research goal is to develop photoprotected B.t. formulations that will perform longer and in a more consistent manner under stressful field conditions.
The report includes the following topics and information:

(a) B.t.-chromophore complexes and formulations.

(b) Baseline data on sensitivity of lepidopteran pests in Georgia to B.t. *Kurstaki* HD1.

(c) Establishing rearing, spraying and bioassay procedures for a variety of lepidopteran pests.

(d) Assessment of the photostabilzing capacity of various chromophores and additives following exposure to irradiation (simulated and solar).

(e) Details on the mutual visits of the collaborating scientists

(f) Equipment purchased in Israel for Georgia.

Methods and Results

The Israeli PI (Prof. Ephraim Cohen) provided the collaborating team in Georgia with the following B.t. complexes and formulations:

(1) B.t. sample (267,000 units/gr) serving as control

(2) B.t.-acriflavin complex (264,000 units/gr)

(3) B.t.-berberine complex (255,000 units/gr)

(4) B.t.- Chinese bark mixture (214,000 units/gr)

Table 1 summarizes the effect of various B.t. concentrations on larval mortality of four lepidopteran species. The species were almost equal in sensitivity to the microbial toxin, as levels of 1 and 2 gr B.t./lit (or 270 or 540 units/ml) already resulted in 86-98% mortality. Significant mortality occurred after 3 days, and by the 7th day more than 90% of larvae died. Mortality in the relevant control did not exceed the 5% value.

Exposure of B.t. to solar irradiation decreased its insecticidal capacity. Figure 1 summarizes results from experiments in which leaves treated with B.t. were exposed for various periods of time to solar irradiation, fed to larvae of 4 different insect pests, and mortality recorded 10 days later. Based on the mortality curves, it appears that compared to the other insect species, the Gypsi moth *Ocneria dispar* is less sensitive to B.t. Mortality is higher for the ermine moth and the European cabbage butterfly fed B.t. treated leaves which were exposed to sunlight. The difference between the species was evident throughout the whole range of the irradiation exposures.

Photostabilization bestowed by the various photoprotectants is apparent and summarized in Figures 2-7 . Acriflavin, berberine and the Chinese bark powder were effective in the photostabilization of B.t. as there adsorption on B.t. or inclusion in the B.t. formulations proved to protect the microbial toxin from inactivation as a result of exposure to solar irradiation. The acriflavin chromophore gave significant protection after 72 hrs of irradiation as over 80% of larvae of *P. brassicae*, *Y. malinellus* and *O. dispar* died as compared to about 60% in the corresponding controls. After prolonged exposure of 144 hrs the protection by this chromophore was notable, as mortality in the control was less than 40% as compared to over 60% kill of larvae treated with the photostabilized B.t.-acriflavin complex. The cationic chromophore berberine was slightly less effective than acriflavin after the similar exposure to solar irradiation (i.e. 72 and 144 hrs). Except for the gypsi moth, the Chinese bark powder was as effective as acriflavin towards the two other insect larvae following prolonged exposure time. Data involving the fourth insect pest (Figures 5 and 7), *H. cunea*, display again the stabilizing effect of acriflavin. After prolonged exposures of 72 and 144 hrs mortality rate of the pest larvae was approximately 90% and 45%, respectively.

Berberine *per se*, berberine mixed with B.t. and berberine adsorbed to B.t. were bioassayed for their toxicity or photostabilizing capacity using 2nd instar larvae of *Spodoptera littoralis* and *Helicoverpa armigera*. Irradiation experiments were carried

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out using a UV chamber for simulating solar irradiation. Inside this chamber a UV lamp (254 nm) and a lamp (120 cm) emitting light energy at 290-400 nm were placed. Part of the UV lamp was covered with aluminum foil exposing only 32 cm of its original length. Samples were placed at a 10 cm distance from the irradiation source. Berberine mixed with B.t. or berberine adsorbed on B.t. were applied on glass slide and irradiated for various time periods. Slides covered with aluminum foil to mask irradiation, served as controls. B.t. mixed or complexed with the chromophore berberine was suspended in distilled water as a level of 0.5gr per ml. 20 μl of this suspension were applied on an agar-based artificial diet (0.5 gr). The treated diet was transferred to 1.5 ml conical Eppendorf tubes and the relevant noctuid larvae were introduced. Larval mortality was recorded 7 days later and the toxic or photostabilizing effects were evaluated.

Results summarized in Figure 8 show that 10μg of berberine is quite toxic to both *S. littoralis* and *H. armigera* larvae causing about 30% mortality. Obviously, the toxicity to larvae decreased in correspondence with lower levels of the chromophore. Irradiation reduced the insecticidal activity of B.t. (Figure 9) as 43 and 52 hrs of exposure yielded about 40% and 25% mortality of *S. littoralis* larvae, respectively. Berberine mixed with B.t. however, photostabilized the insecticidal component as after similar irradiation exposure time, the mortality was about 80% and 75%, correspondingly (40%-50% photoprotection). The results presented in Figure 10A demonstrates that berberine adsorbed on B.t. was effective in protecting the bacterial toxin from photoinactivation. Following 72 hrs of irradiation, B.t. alone lost 80% of its insecticidal potency against *S. littoralis*, versus a mere loss of 40% of the B.t.-berberine complex. A similar photoprotection potency is also evident when *H. armigera* was bioassayed (Figure 10B). Zero mortality was evident when larvae fed untreated diet, while 100% of insects died feeding on diet containing irradiated aluminum-covered B.t.

TABLE 1. The insecticidal activity of B.t. on four lepidopteran pests

A. The gypsi moth - *Lymantria dispar*

B.t. concentration gr/l	Mortality of larvae, days 3	5	7	10	Mortality of larvae %	Corrected* mortality, %
0 (control)	0	0	0	0	0	-
1	17	29	36	6	88	88
2	19	32	37	3	91	91
3	20	34	39	2	95	95
4	27	39	30	4	100	100
5	35	48	17	0	100	100

B. The ermine moth - *Yponomeuta malinellus*

0	0	0	3	2	5	-
1	19	33	32	7	91	90.5
2	23	34	35	3	95	94.7
3	28	36	30	5	99	98.9
4	37	41	22	0	100	100
5	43	52	5	0	100	100

C. The European cabbage butterfly - *Pieris brassicae*

0	0	2	0	2	4	-
1	21	26	31	8	86	85.4
2	35	30	22	9	96	95.8
3	32	35	25	6	98	97.9
4	38	34	26	2	100	100
5	44	36	20	0	100	100

D. The fall webworm - *Hyphantria cunea*

0	0	0	0	1	1	-
1	22	41	23	5	91	90.9
2	31	50	15	2	98	97. 9
3	52	48	0	0	100	100
4	82	16	0	0	100	100
5	100	0	0	0	100	100

*Mortality of larvae was corrected using to the Abbot's formula.

The bioassays were conducted using 2nd or 3rd instar larvae, and a B.t. batch containing 267,000 units per gr. Apple leaves previously sprayed with different B.t. suspensions (1-5 gr/l) were fed to larvae. Leaves sprayed with distilled water served for control bioassays.

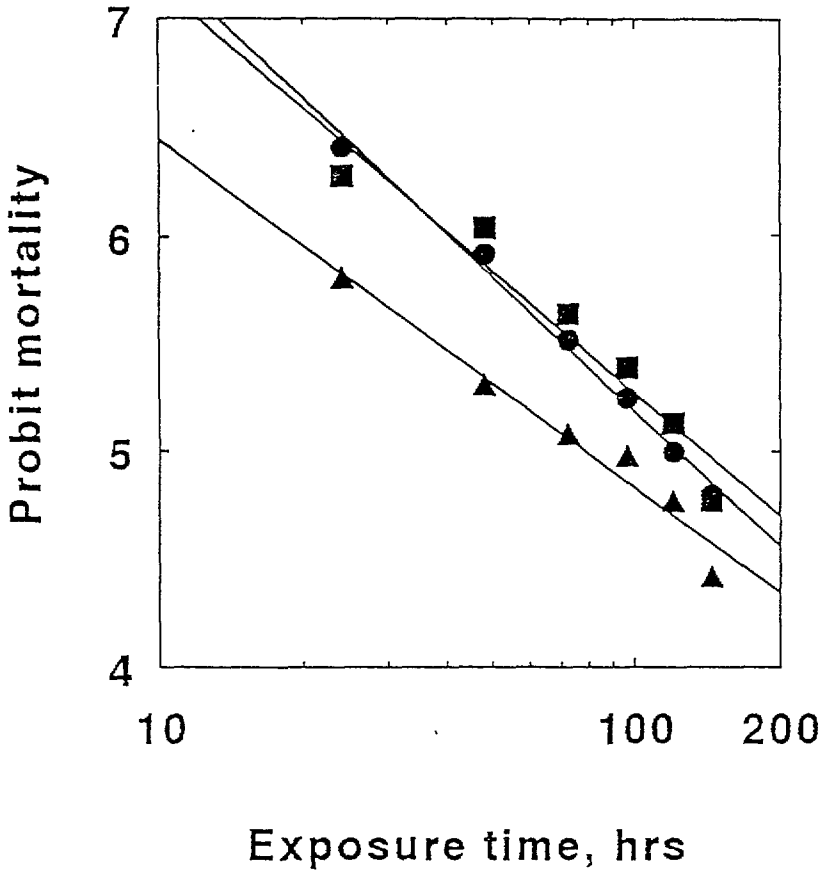


Figure 1. Effect of solar irradiation of B.t. on its insecticidal activity. Leaves treated with a B.t. formulation was exposed to solar irradiation for the time specified. Mortality of larvae feeding on treated leaves were monitored 10 days later. ▲ *O. dispar;* ● *Y. malinellus;* ■ *P. brassicae.*

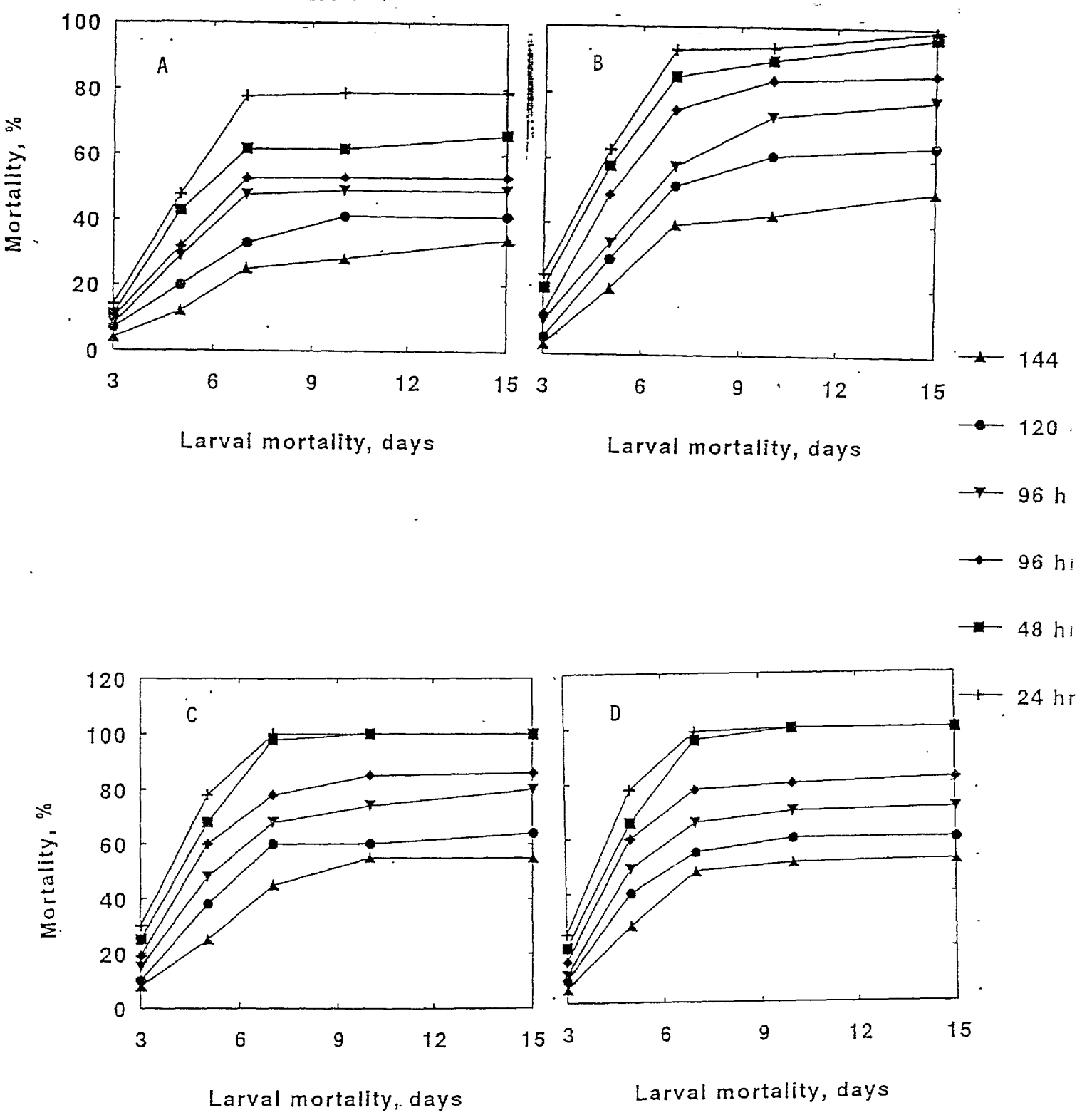


Figure 2. The effect of B.t. and B.t. complexes exposed to solar irradiation on mortality of *O. dispar*. Leaves treated with B.t. were exposed to solar irradiation for the time specified. A. control; B. B.t.-berberine; C. B.t.-acriflavin; D. B.t.-Chinese bark powder.

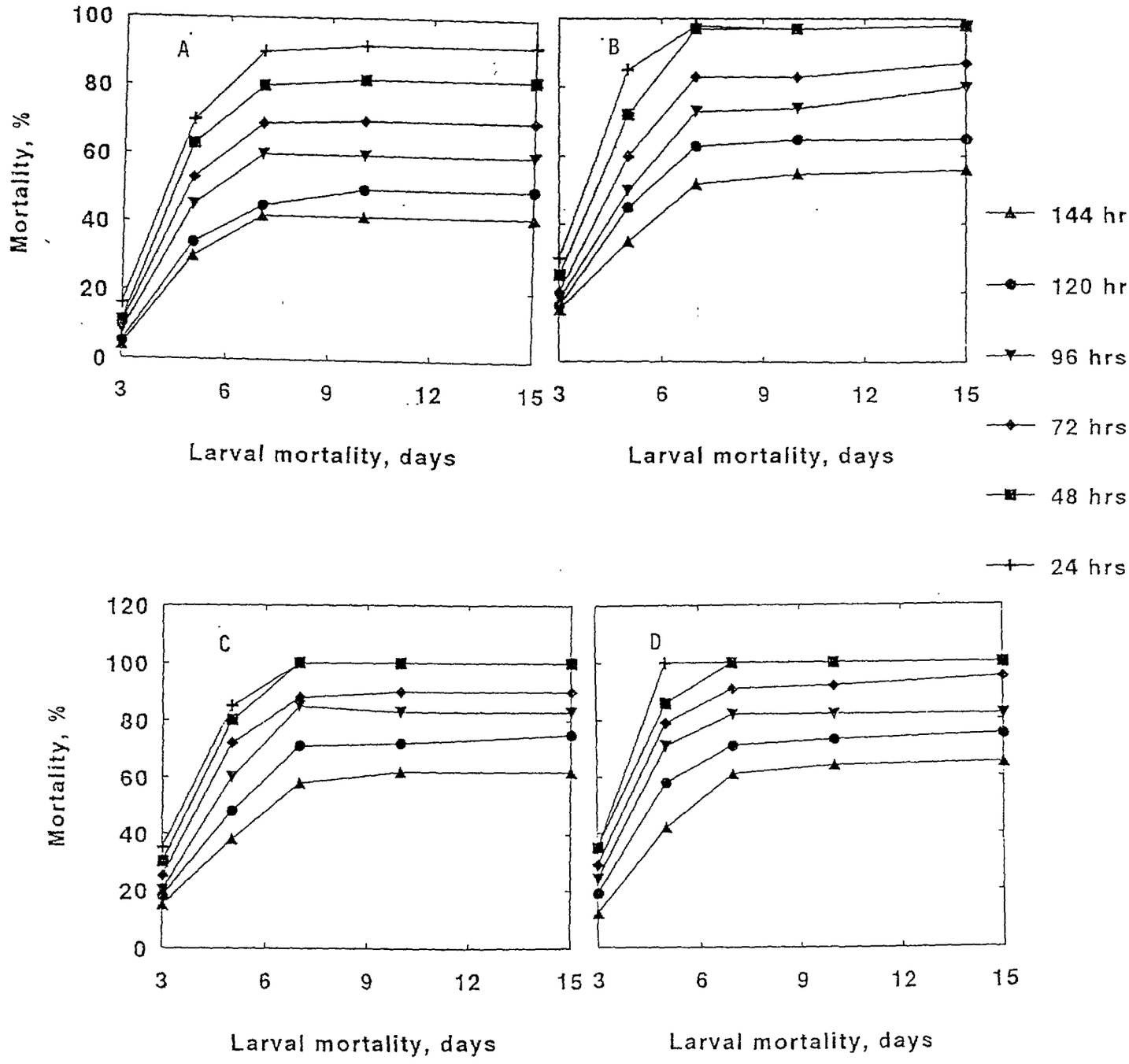


Figure 3 The effect of B.t. and B.t. complexes exposed to solar irradiation on mortality of *Y. malinellus*. Leaves treated with B.t. were exposed to solar irradiation for the time specified. A. control; B. B.t.-berberine; C. B.t.-acriflavin; D. B.t.-Chinese bark powder

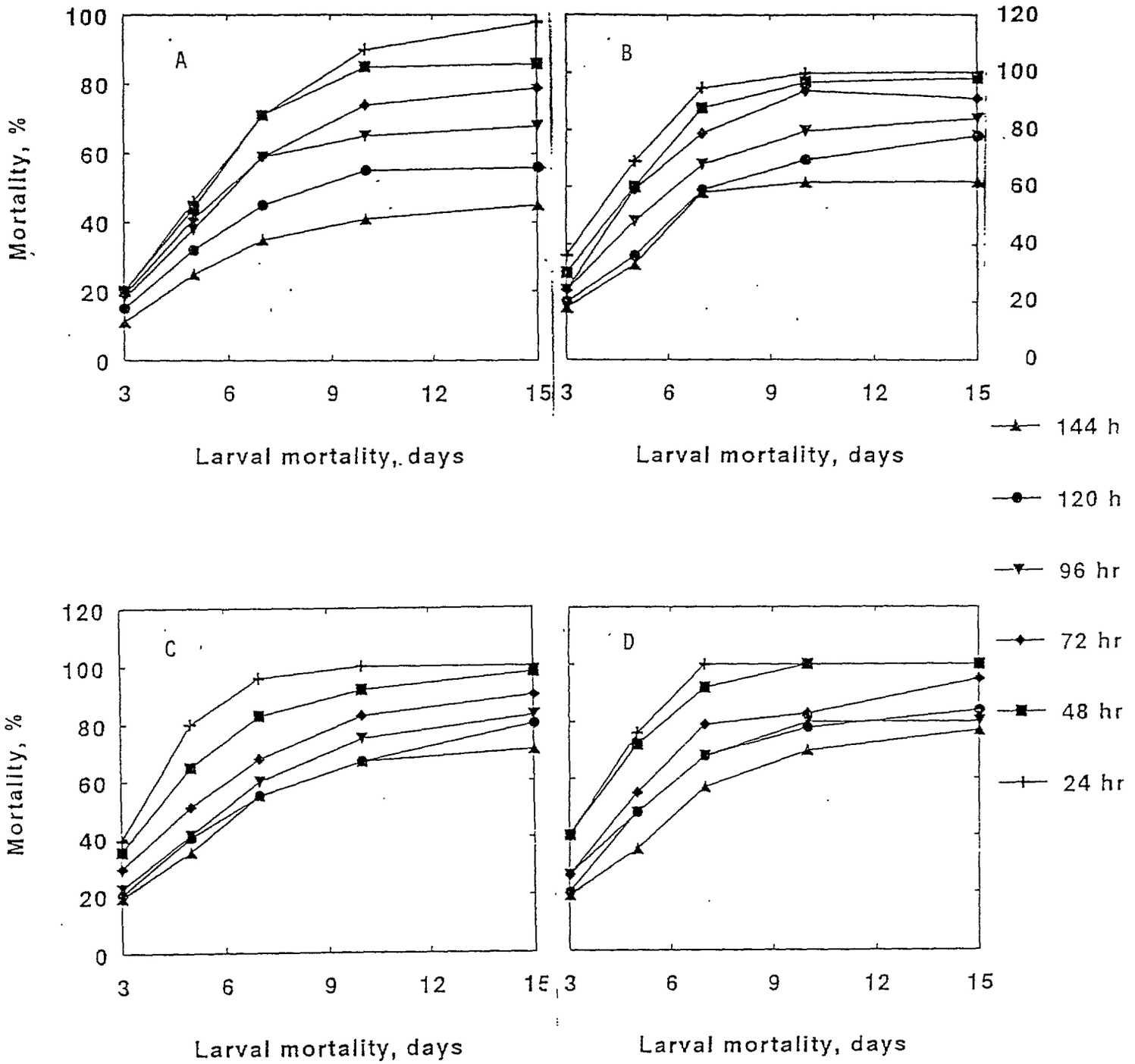


Figure 4. The effect of B.t. and B.t. complexes exposed to solar irradiation on mortality of *P. brassicae*. Leaves treated with B.t. were exposed to solar irradiation for the time specified. A. control; B. B.t.-berberine. C. B.t.-acriflavin; D. B.t.-Chinese bark powder.

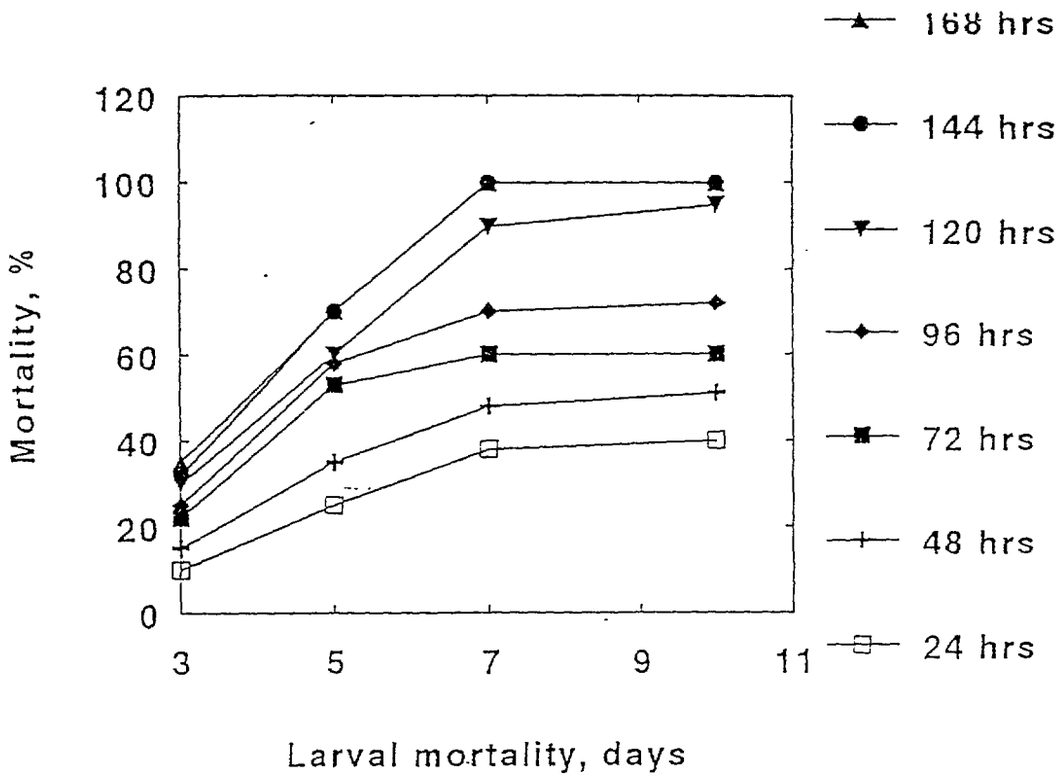


Figure 5. The effect of B.t.-acriflavin complex exposed to solar irradiation on mortality of *H. cunea*. Leaves treated with B.t.-acriflavin complex were exposed to solar irradiation for the time specified.

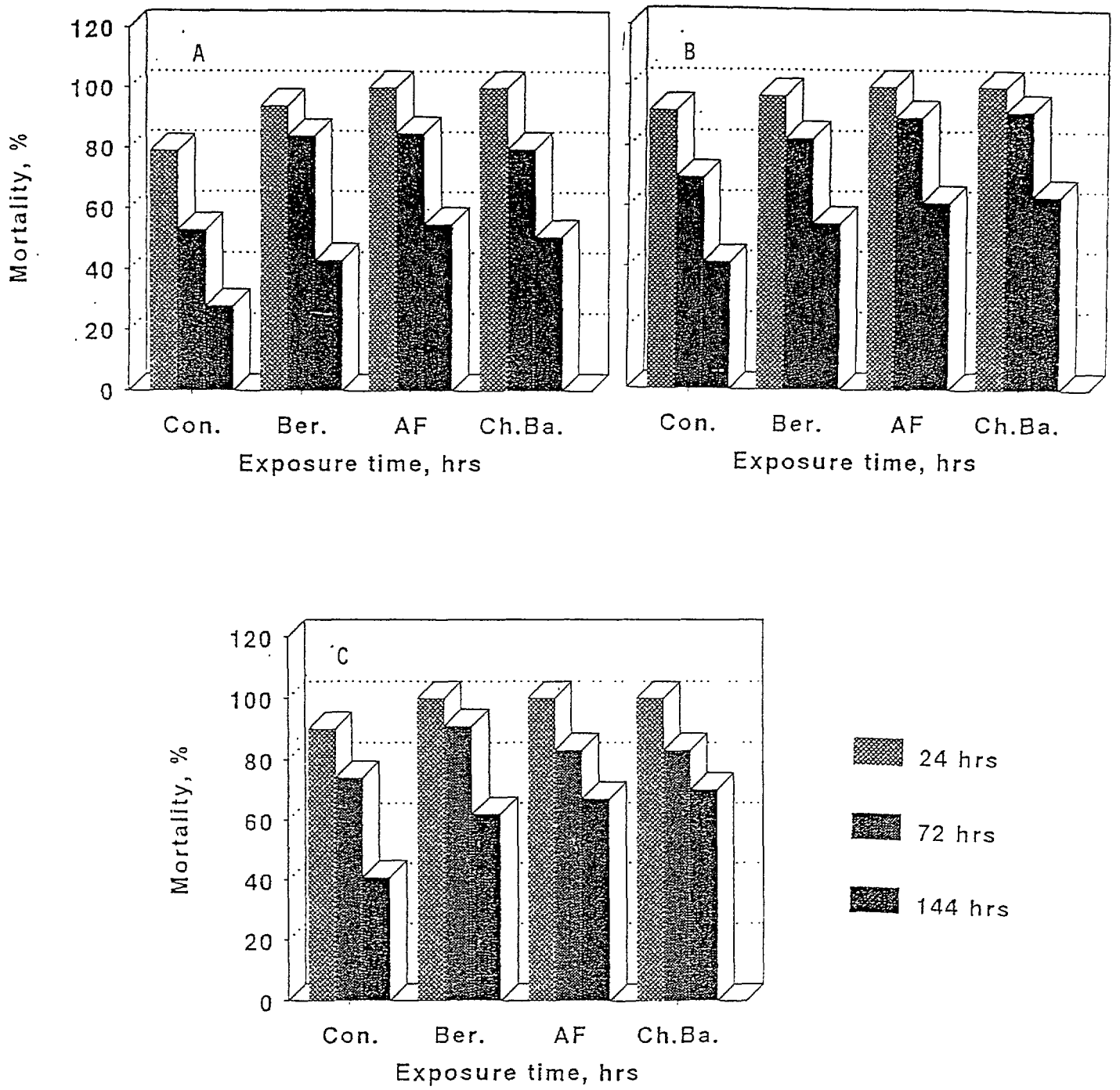


Figure 6. Photostabilization of B.t. by various photoprotectants. Leaves were treated with various B.t. complexes were exposed to solar irradiation for the time specified. Mortality of larvae feeding on B.t.-treated leaves was recorded 10 days later. Con. - control; Ber. - B.t.-berberine; AF - B.t.-acriflavin; Ch.Ba. - B t.-Chinese bark powder. A. *O. dispar*; B. *P. brassicae*; C. *Y. malinellus*.

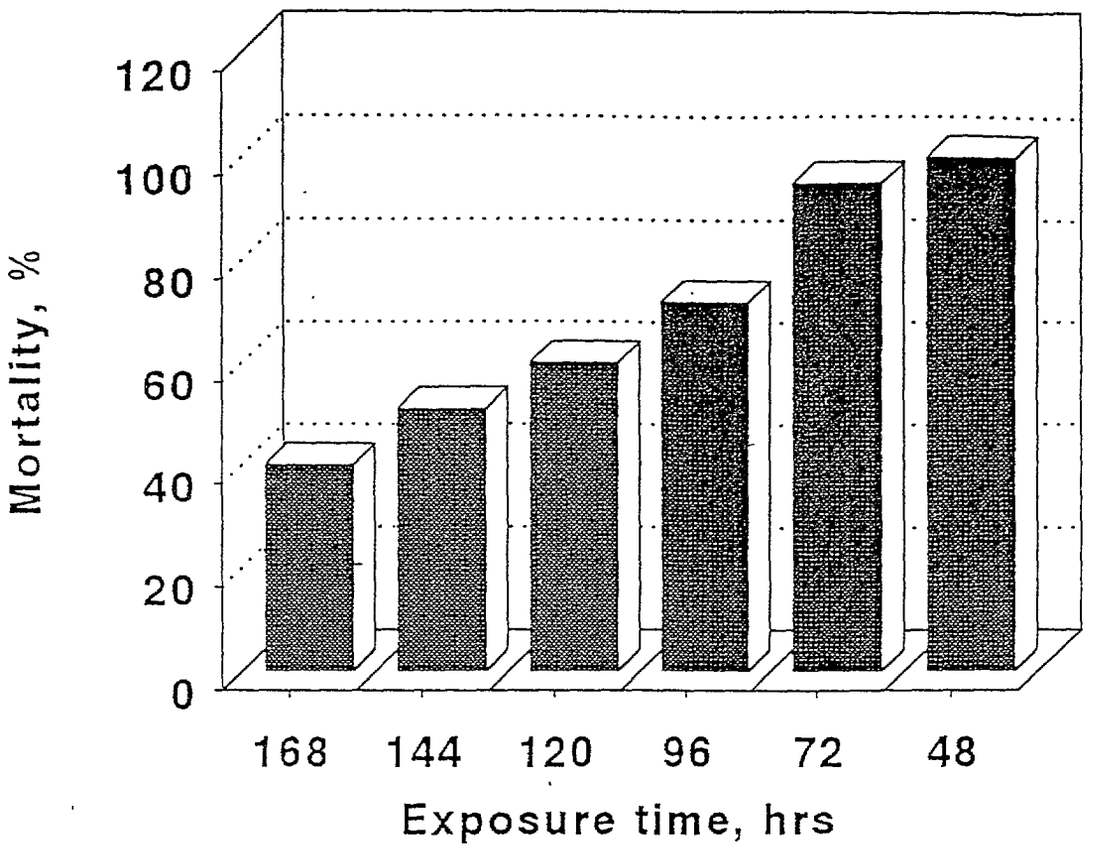


Figure 7. The effect of B.t.-acriflavin complex exposed to solar irradiation on mortality of *Hyphantria cunea*. Leaves were treated with the B.t. complex exposed to solar irradiation for the time specified. Mortality of larvae was recorded 10 days later.

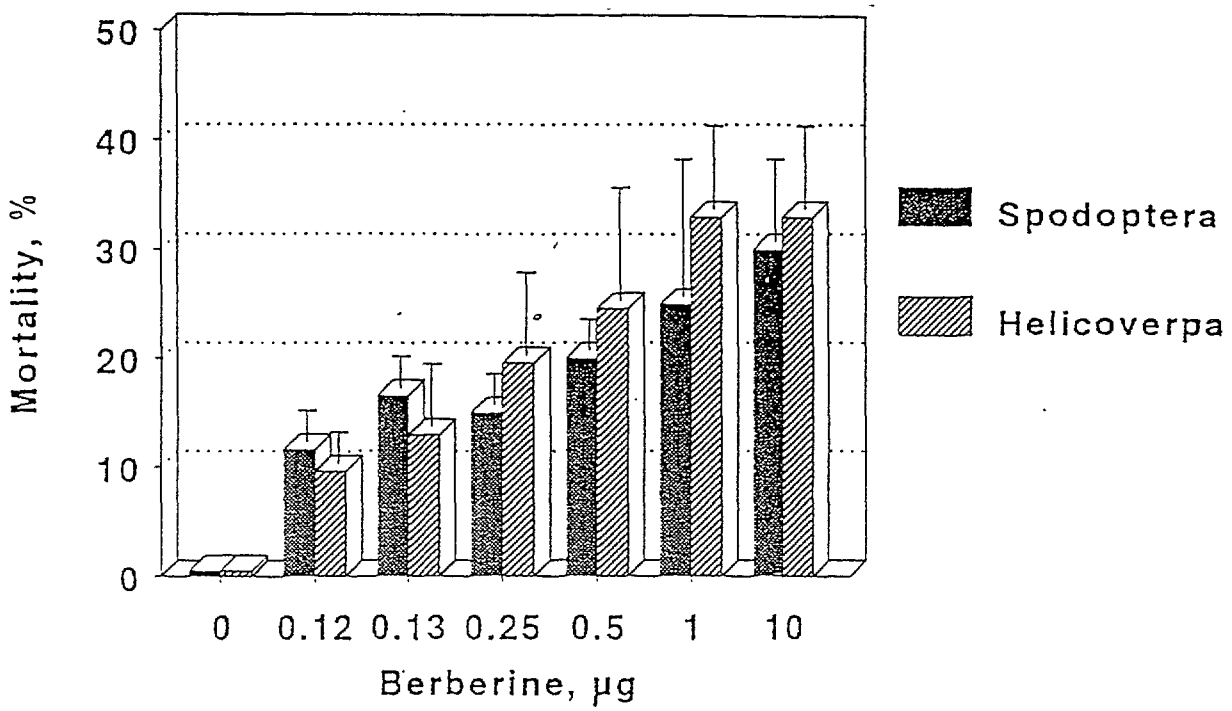


Figure 8. The effect of berberine on mortality of noctuid larvae. The specified amount of berberine in 20µl of distilled water was applied on 0.5 gr of artificial diet. Mortality of larvae (*Spodoptera littoralis* or *Helicoverpa armigera*) was recorded 7 days later.

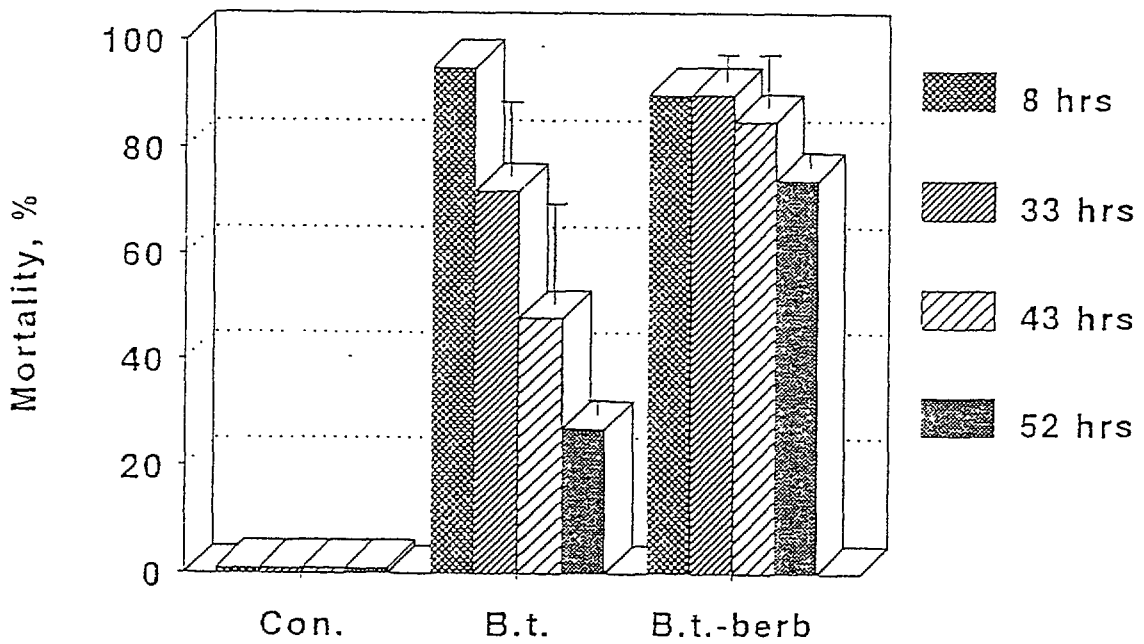
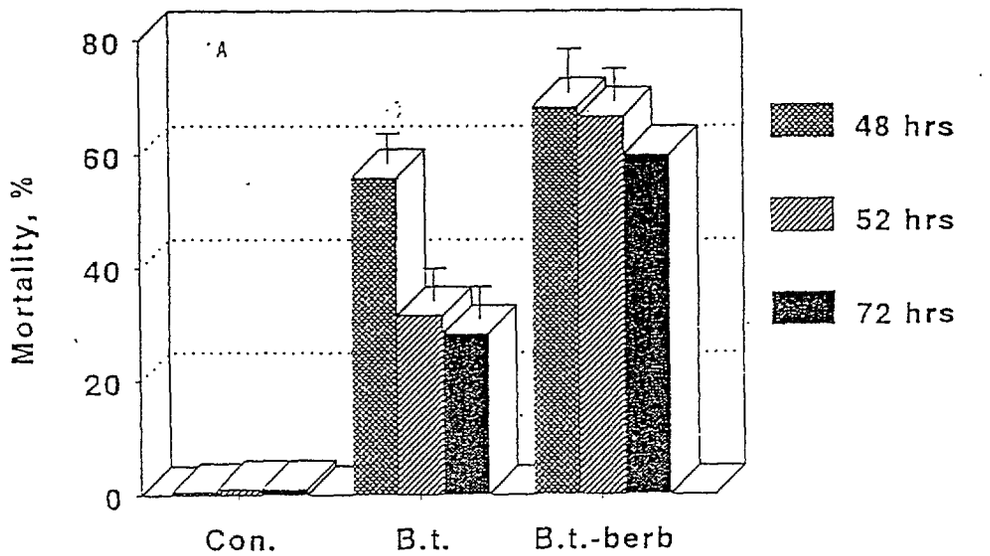


Figure 9. The photostabilizing effect of berberine mixed with B.t. B.t. or B.t. mixed with berberine were placed on slides and exposed to simulated UV irradiation for the time specified. 10 µg of B.t. suspended in distilled water was applied on 0.5 gr of artificial diet. The amount of mixed berberine was 1.9µg. Second instar *Spodoptera littoralis* larvae were bioassayed and mortality was recorded after 7 days. B.t. covered with aluminum foil and irradiated for the maximum UV exposure time caused 100% mortality. Con. - control larvae feeding on B.t.-free diet; B.t.-berb. - a mixture of B.t. and berberine.



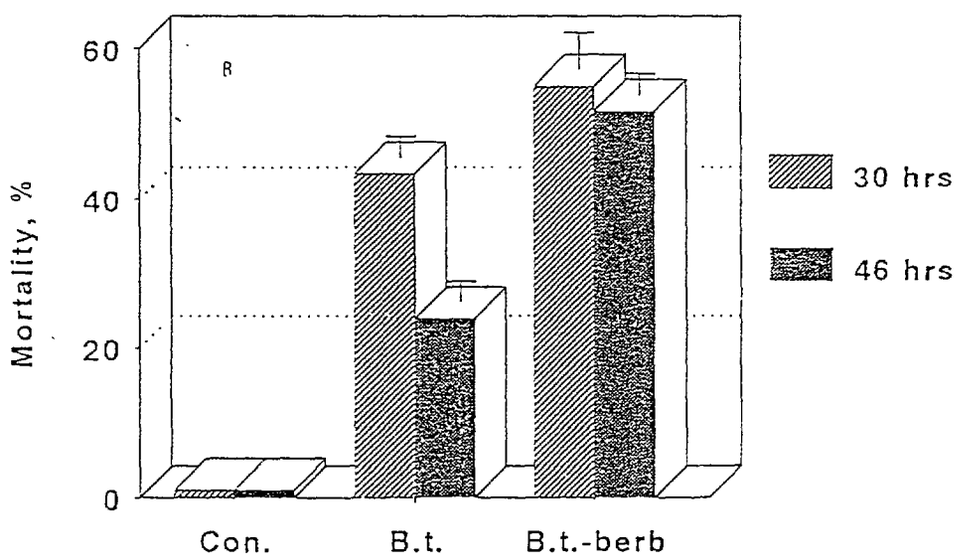
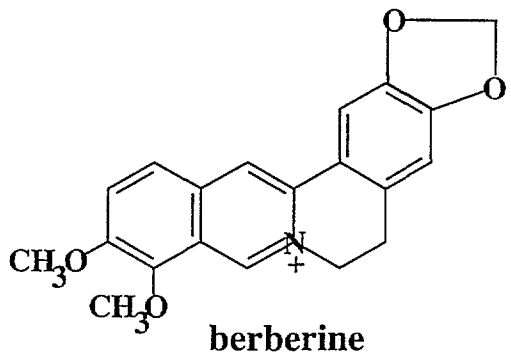
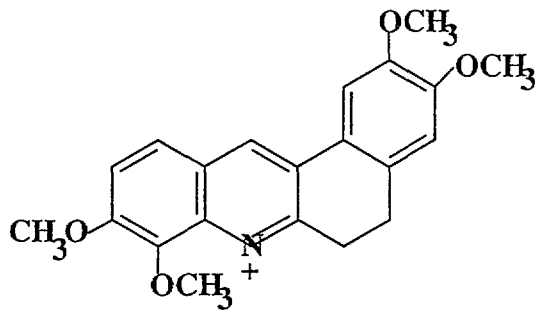


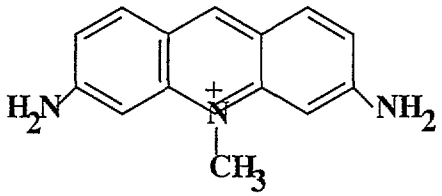
Figure 10. The photostabilizing effect of berberine- B.t. complex. B.t. or berberine-adsorbed B.t. were placed on slides and exposed to simulated UV irradiation for the time specified. 10μg of berberine-adsorbed B.t. suspended in 20μl distilled water was applied on 0.5gr of artificial diet. The amount of adsorbed berberine was 1.9μg per10μg B t. A. *Spodoptera littoralis* and B. *Helicoverpa armigera* second instar larvae were bioassayed, and mortality was recorded after 7 days. B.t. covered with aluminum foil and irradiated for the maximum UV exposure time caused 100% mortality. Con. - control larvae feeding on B t.-free diet; B.t.-berb. - a complex of berberine adsorbed on B.t.



berberine



palmatine



acriflavin

Figure 1. Chemical structures of chromophores mentioned in the report.

Experiments with photostabilized formulations of the entomopathogenic fungus *Beauveria bassiana* focused on effects related to spore viability.
medium was used for stock cultures and cultures prepared for harvesting conidia. The fungus was maintained for 14 days in the dark at 25°C. A selective medium composed of maltose, Bacto peptone, Bacto yeast extract, Bacto agar and the antimicrobial agents (Crystal Violet, chloramphenicol, benlate and dodine) was used following

various treatments. The conidia were harvested using soybean oil and filtered through 8 layers of gauze. The concentration of conidia per ml was determined using a hemocytometer. The oil was diluted to give about 10^3 conidia per 10 l. The oil-containing conidia were pipetted on cover glass, which following irradiation, were placed into 10ml of 0.05% Tween-20. After sonication and vortexing, 0.2 aliquots were applied on Petri dishes with the selective medium. Cover glasses wrapped with aluminum foil served as controls. The plates were kept for 7 days at 25°C, after which the *B. bassiana* colonies were counted.

Various combinations of clays (attapulgite, bentonite, kaolin) and chromophores (acriflavin, berberine, palmatine and Calcofluor White) were examined as to their capacity to photoprotect *B. bassiana* spores.

Irradiation experiments were carried out using a UV chamber for simulating solar irradiation. Inside this chamber a UV lamp (254 nm) and a lamp (120 cm) emitting light energy at 290-400 nm were placed. Part of the UV lamp was covered with aluminum foil exposing only 32 cm of its original length. Samples were placed at a 10 cm distance from the irradiation source.

Larval mortality was recorded during 10-15 days following treatment, corrected B.t. insecticidal activity was evaluated using probit-log dose analysis. To assess the effects of various chromophore-containing B.t. formulations, exposure to various irradiation regimens were followed by bioassays. In Georgia the leaves sprayed with the B.t. suspensions were exposed up to 168 hrs to sunlight; in Israel the B.t. formulations were irradiated inside a specifically-designed UV chamber in order to simulate solar irradiation conditions in the field.

Palmatine *per se*, palmatine mixed with B.t. and palmatine adsorbed to B.t. were bioassayed for their toxicity or photostabilizing capacity using 2nd instar larvae of *Spodoptera littoralis* and *Helicoverpa armigera*.

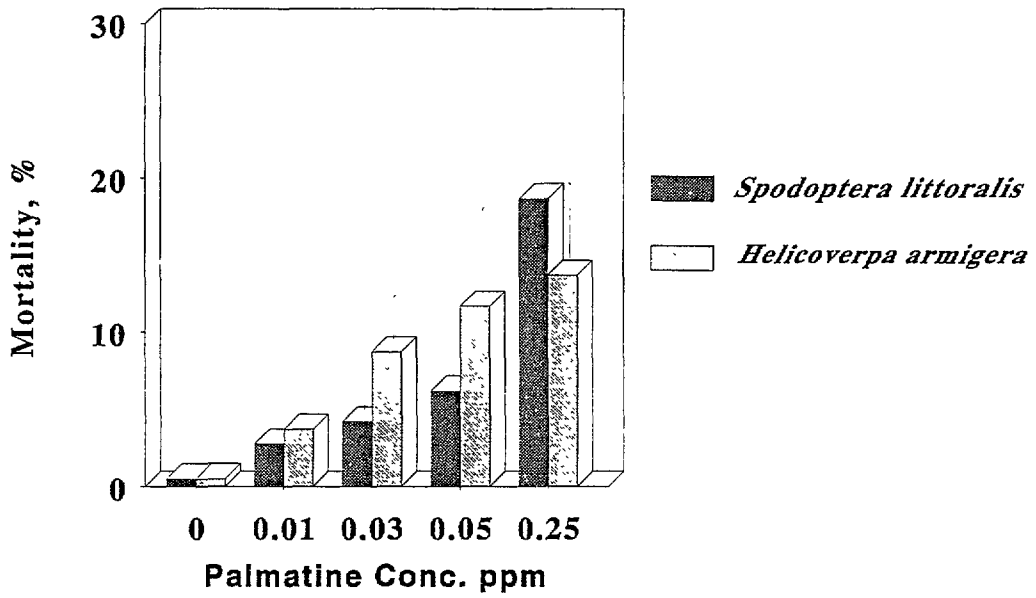


Figure 11. The effect of palmatine mixed in the diet on mortality of lepidopteran larvae. The specified amount of palmatine in 20 g of distilled water was applied on 0.5 gr of artificial diet. Mortality of *S. littoralis* and *H. armigera* larvae was recorded 7 days later.

To find whether palmatine *per se* has an effect on larvae of *S. littoralis* and *H. armigera*, it was mixed in the diet at the depicted concentrations. A dose response pattern is evident ranging from less than 3% mortality at 0.01 ppm up to 20% up to 20% mortality of *S. littoralis* larvae when the alkaloid level reached 0.25 ppm (Figure 11). Evidently, palmatine is toxic to the lepidopteran larvae, yet at the concentration adsorbed to B.t. its toxicity is negligible.

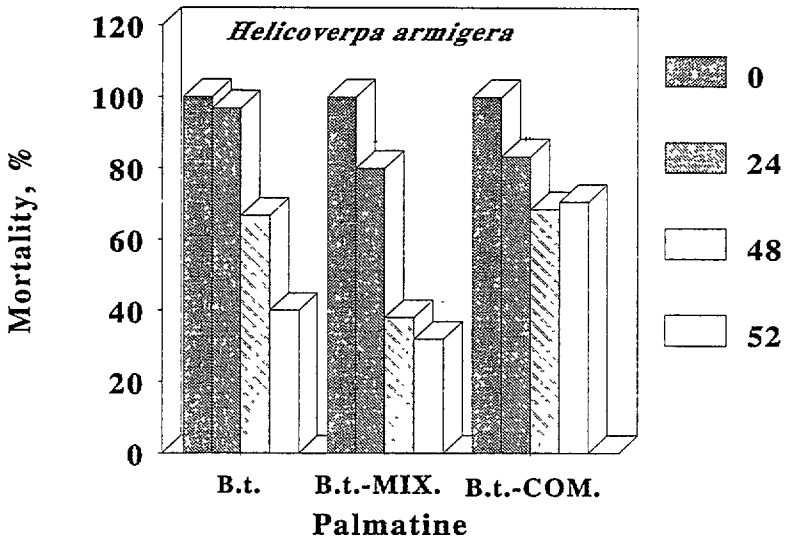
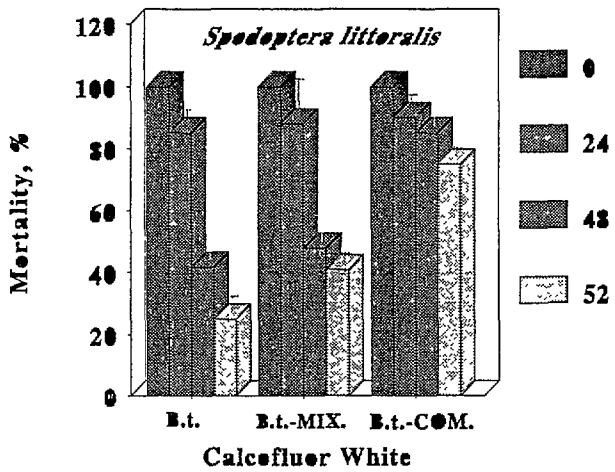
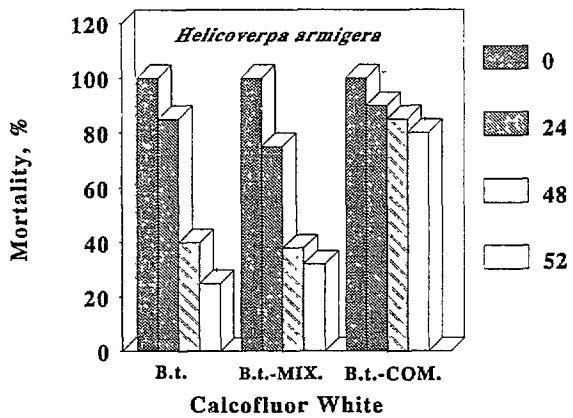


Figure 12. The photostabilizing effect of palmatine mixed and adsorbed to B.t. B.t., B.t. mixed with palmatine and B.t. adsorbed to palmatine were placed on microscope slides and exposed to simulated sunlight irradiation for the time specified up to 52 hrs. 10 g of B.t. suspended in 20 l distilled water was applied to 0.5 gr of artificial diet. The amount of mixed palmatine was 1.9 g per 1 gr; The level of adsorbed chromophore was 1.9 g per 10 g B.t. *H. armigera* second instar larvae were used for bioassay, and mortality was recorded after 7 days. B.t. covered with aluminum foil and irradiated for the maximum time exposure caused 100% mortality. B.t.-MIX. - palmatine mixed with B.t. in the diet; B.t.-COM. - palmatine adsorbed to B.t. and incorporated in the diet.

Depending on the length of irradiation B.t. lost its insecticidal activity to *Helicoverpa* larvae (Figure 12) . Significant reduction of activity occurred following 48 hrs of irradiation, and prolonged exposure (52 hrs) resulted in about 60% reduction in larval mortality. Mixing palmatine with the B.t.- containing insect diet did not protect the insecticidal toxin from inactivation. However, adsorption of the chromophore to the B.t. yielded a complex that photostabilized the toxic principle of B.t. Following 48 and 52 hrs irradiation of the complex, still 70% of larval mortality as compared to less than 40% mortality in the B.t. chromophore mixture, was evident. The stabilizing potential of palmatine is comparable to that of berberine and may be due to a close similarity in chemical structure.



(A)



(B)

Figure 13. The photostabilizing effect of Calcofluor White mixed and adsorbed to B.t. B.t., B.t. mixed with Calcofluor White and B.t. adsorbed to Calcofluor White were placed on microscope slides and exposed to simulated sunlight irradiation for the time specified up to 52 hrs. 10 g of B.t. suspended in 20 l distilled water were applied to 0.5 gr of artificial diet. Figure 13 (A) *S. littoralis* and Figure 13 (B) *H. armigera* 2nd instar larvae were used for bioassay, and mortality was recorded after 7 days. B.t. covered with aluminum foil and irradiated for the maximum time exposure caused 100% mortality. B.t.-MIX. - Calcofluor White mixed with B.t. in the diet; B.t.-COM. - Calcofluor White adsorbed to B.t. and incorporated in the diet.

Calcofluor White (Tinopal LPW) is a fluorescent brightener used to improve performace of entomopathogenic viruses. Part of its action has been attributed to photoprotection of photolabile components. In our experiments the brightener proved its potency as a powerful B.t. photostabilizer (Figure 13, A and B). Like as with palmatine, simple mixing of Tinopal LPW with B.t. did not protect against photoinactivation as loss of activity was similar to the B.t. *per se* following irradiation. Nevertheless, as a complex the brightener photoprotect B.t. from loss of insecticidal activity even after prolonged (52 H. hrs) exposure to irradiation. This effect was similar when bioassayed with either *H. armigera* or *S. littoralis* larvae. Close to 80%! mortality was observed following the maximum irradiation regimen.

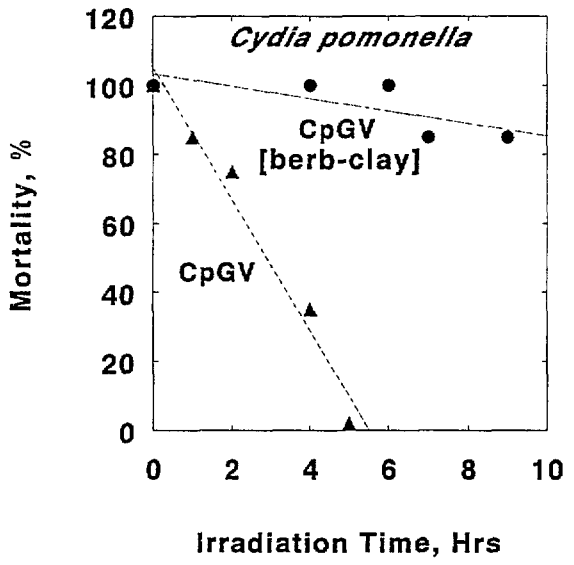


Figure 14. The photostabilizing effect of berberine on the codling moth *Cydia pomonella* Granulosis Virus (CpGV).

In Figures 14-16 the effects of chromophores and combinations of chromophores and clay are summarized. The *S. littoralis* NPV is highly sensitive to irradiation as more than 60% and 90% of infectivity was lost following irradiation of 4 and 6 hrs, respectively. Irradiation for 12 hrs completely destroyed the viral activity. It is noteworthy that addition of clay to the viral preparation is considerably effective as the complex lost only 40% of its original activity after 12 hrs of irradiation, while

after a prolonged exposure still 20% of the infectivity could be detected. Complexes of the virus with AF and berberine were equal with respect of bestowing

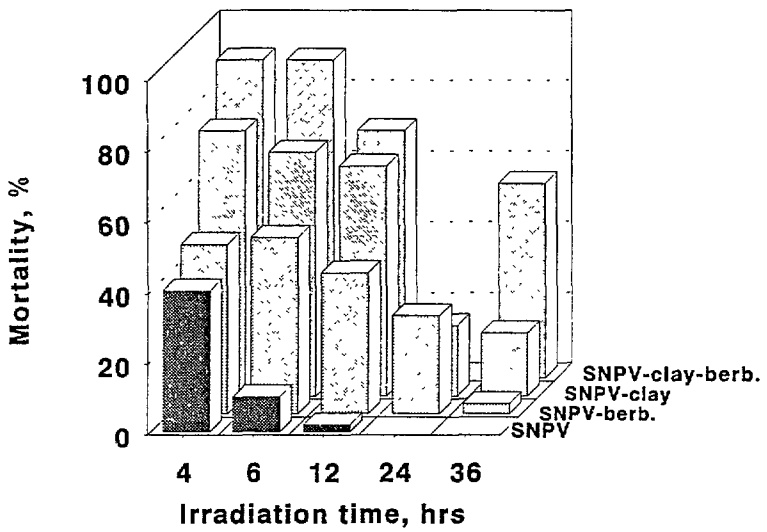


Figure 15. Photostabilization of *Spodoptera littoralis* NPV using combinations of clay and berberine. SNPV - *S. littoralis* nuclear polyhedrosis virus; berb. - berberine,

photoprotection. Both chromophores were capable of appreciable photoprotection reflected by 40% and 20% infectivity following irradiation periods of 12 and 24 hrs, respectively. Complexes consisting of three components (virus-clay-chromophore) were superior in photostabilizing the photosensitive virus. Using AF as a chromophore 70% of the original viral infectivity was still maintained after 12 hrs of irradiation. Even following a longer irradiation period (36 hrs) 60% of infectivity was retained. Similar results were obtained when the chromophore berberine is part of the complex.

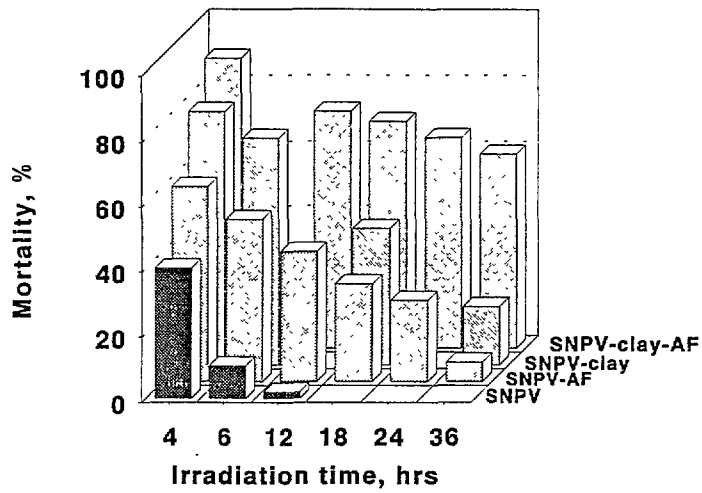


Figure 16. Photostabilization of *Spodoptera littoralis* NPV using combinations of clay and acriflavin. SNPV - *S. littoralis* nuclear acriflavin

In Figures 17-20 photostabilization of the entomopathogenic fungus *Beauveria bassiana* is illustrated. Irradiation of spores resulted in most cases in a drastic reduction of spore viability. More than 80% inhibition of germination was observed after 8-10 hrs of irradiation. Inclusion of various clays yielded different levels of photoprotection. Bentonite and kaolin are highly effective in keeping the viability of the fungus spores. Following 8-10 hrs of irradiation 60-80% of spore viability was retained. After 10 hrs of treatment 60% and 70% of the spores germinated when kaolin (Figure 17) and bentonite (Figure 19) were correspondingly included. Attapulgite was less effective as less than 40% of the spores germinated after 8 and 10 hrs irradiation (Figure 18). A complexe containing in addition to attapulgite also the chromophore berberine was extremely effective as almost 100% of spores germinated following 10 hrs of irradiation. A similar photoprotection was observed when a complex of spores-bentonite-berberine was examined following 10 hrs of irradiation (Figure 17). A complex, in which bentonite was replaced by kaolin, was less effective in protecting the fungal spores as germination was inhibited by 40% (Figure 19).

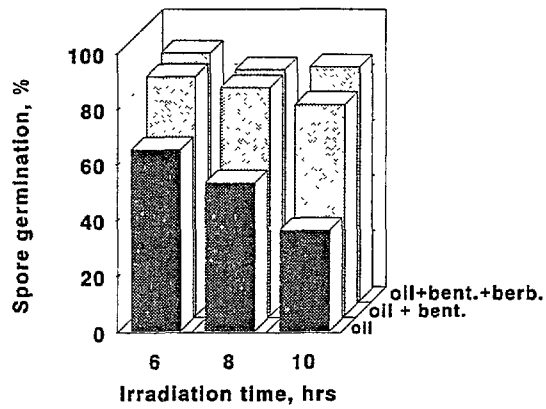


Figure 17. The photostabilizing effect of bentonite and a bentonite-berberine combination on the viability of *Beauveria bassiana* spores. The viability was assessed by the number of germinating spores. Bent. - bentonite; berb.- berberine.

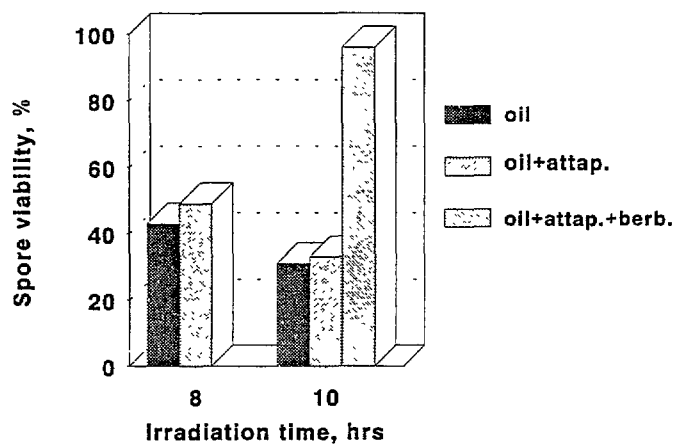


Figure 18. The photostabilizing effect of attapulgite and an attapulgite berberine combination on viability of *Beauveria bassiana* spores. Viability was assessed by the number of germinating spores. Attap. - attapulgite; berb. - berberine.

The potent chromophore acriflavin, which displayed considerable photostabilizing effects on microbials and entomopathogens, was effective in protecting spores of *B. bassiana* from photoinactivation (Figure 11). More than 80% of spores germinated following 8 hrs of irradiation. It may be concluded that AF and berberine are similar in their photostabilization potential.

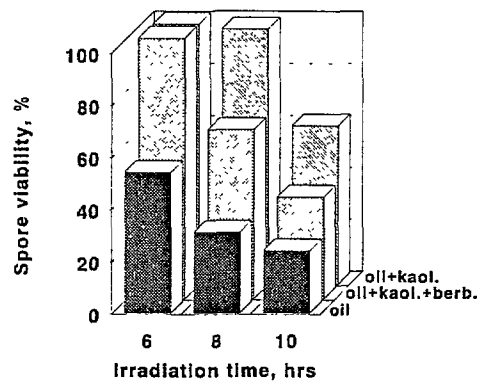


Figure 19. The photostabilizing effect of kaolin and a kaolin-berberine combination of the viability of *Beauveria bassiana* spores. The viability was assessed by the number of germinating spores. Kaol. - kaolin; berb.- berberine.

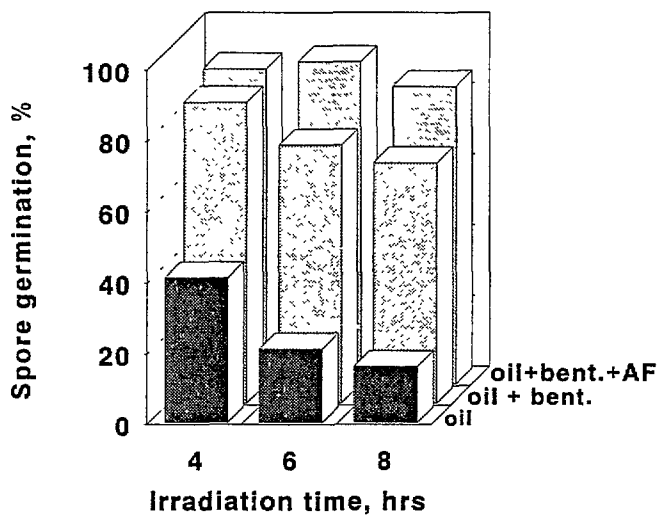


Figure 20. The photostabilizing effect of bentonite and a bentonite-acriflavin combination on the viability of *Beauveria bassiana* spores. Viability was assessed by the number of germinating spores. Bent.- bentonite; AF - acriflavin.

The B.t.-diatomite complex was prepared by mixing equal volumes of 1% B.t. suspension (227,000 units per gr) with 1% of diatomite suspension. The mixture was sonified and shaken for 24 hours. The suspension was centrifuged for 30 min and the precipitate was collected and lyophilized. The results demonstrate that B.t. lost its activity by 40% following 36 hours of solar irradiation (in Georgia). However, B.t.

complexed with diatomite was stabilized as under the same irradiation conditions only 20% of its initial activity were lost. It could be that like the photoprotection of B.t.-clay compexes shown before a light scattering effect might be involved.

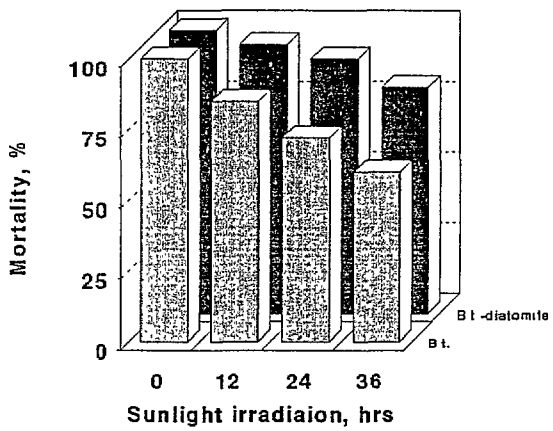


Figure 21. The photostabilization effect of diatomite on the flour moth *Ephestia kuehniella*. Ist instar larvae of the flour moth were used for the bioassay. The B.t. and B.t.-diatomite complex were exposed to sunlight for the indicated time periods and applied to the insect diet. Mortality of larvae was recorded 10 days later.

B.t.-diatomite and B.t.-zeolite complexes after being irradiated inside a UV-simulated chamber and were bioassayed using *H. armigera* larvae. Both complexes were very effective as mortality of larvae fed B.t-diatomite and B.t.-zeolite was 78% and 56% after 24 hrs of irradiation. Unprotected B.t. under the above conditions lost 83% of its initial activity.

The project has accomplished most of its proposed goals. In fact, more information as initially planned in the research proposal has been gained relative to use of clays, chromophores and biocontrol agents. Nevertheless, more trials should have been performed to evaluate the efficacy of new photostabilized formulations under field conditions. Such field trials are quite expensive, and unfortunately the overall money

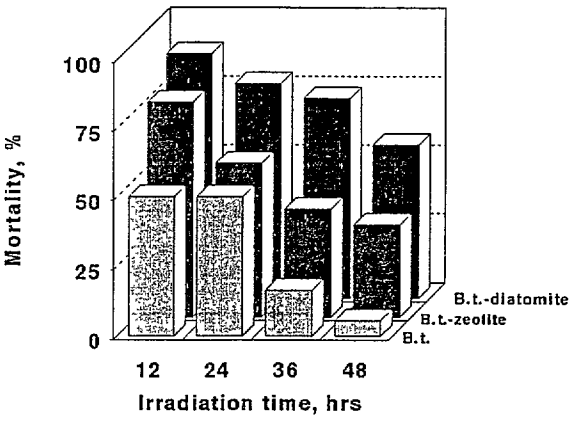


Figure 22. Photostabilization of B.t. by zeolite and diatomite. B.t. B.t.-zeolite and B.t.-diatomite complexes were irradiated in a solar UV simulator for the time periods specified and applied to the insect diet. Bioassays were carried out using 1st instar *Helicoverpa armigera* larvae. Mortality of larvae was recorded 10 days later.

allocated for this project did not meet the needs. The above summarized data and information are being incorporated into several publication that are in the process of preparation.

Impact Relevance and Technology Transfer

Georgia is largely an agricultural country and as such is plagued by pests causing severe damage to crops. Applying B.t. as an environmentally-benign control agent is advantageous in particular due to the possible production of this microbial insecticide in Georgia. In fact, the collaborating PI (Prof. Chkhubianishvili) has been looking seriously into such a potentiality. Sunlight irradiation has been recognized as the major environmental factor affecting efficacy and performance of microbial insecticides such as B.t. and a variety of viral and fungal entomopathogens. Stabilization of photosensitive biocontrol agents is a major objective world-wide in studies aimed at finding toxicologically-safe replacements to synthetic chemicals.

The training of scientists from the Kanchaveli Institute at Rehovot (see Project Activities section) greatly contributed to expertise in the various technological and conceptual aspects of the research project. The above includes the stabilization of photosensitive microbial insecticides; use of clays and chromophores in the preparation of photoprotected formulations as well as the design of suitable bioassays and analysis of data. The knowledge has been transferred to Georgia and implemented there as evidenced by the conducted experimental part. The knowledge and skill gained provide a solid basis for a sustainable capacity to independently perform same or similar research. Although rather elementary, the equipment purchased (see below) and left behind, should help in this regard. Of enormous importance are the visits, training periods and attending an international scientific conference on plant protection. Such activities disrupted the scientific isolation of the Georgian collaborators and exposed them to international scientific community, modern scientific approaches, new skills and improved technologies. Obviously, such exposure will have a profound impact on their labs, colleagues and Institution. An up-to-date sophisticated PC (Compaq, Prolinea Pentium 150 MH) and peripheral accessories as well as a Laser printer (HP Laser-jet 5L) were purchased in Israel to be used by the Georgian team. Software including Word, Excel and graphic programs were installed. The newly purchased PC was used for processing data and preparing an account on experiments conducted in Georgia which eventually served as a basis for the previous annual reports and the present final report. In addition, the Georgian scientists carried back a multi-purpose temperature-controlled shaking bath for growing microorganisms, small laboratory items such as a vortex, set of pipetors, tips, racks etc., as well as miscellaneous materials hard-to-purchase in Georgia.

Project Activities/Outputs

The money transferred to a Swiss bank account has been used by the Georgian partner for research according to the project plan, as well as for travel. All together The Israeli and Georgian principle investigators traveled twice to the corresponding countries. The overall collaboration proceeded according to schedule. An additional one year extension beyond the original expiring date of the project (January 1998) was requested and approved by Mr. William Crane the scientific attache at the US Embassy, Tel-Aviv. The Israeli principle investigators (E. Cohen) visited the

Kanchaveli Institute in Tbilisi twice. The first visit, which took place on March 1996, was aimed at coordinating and starting the collaborative research. The Israeli investigator brought along B.t., chromophores, B.t complexes and formulations for the speedy start of the project. The second visit to Tbilisi on July 1998 focused on summarizing the research, discussion the overall achievements of the joint project and for obtaining the last experimental data and associated information for the final report. The collaborating scientist (C. Chkhubianishvili) paid a reciprocal visit to Rehovot, Israel on July 1996. Essentially, the previously reported research accomplishments reflect the successful outcome of such discussions and coordination. Dr. Mamuka Kotetishvili and Dr. Iatamze Malania from the Kanchaveli Institute arrived at Rehovot on November the 24^{th,} (1996) for a three week training in photostabilization techniques and bioassay procedures. Also the guests performed experiments with anew chromophore and visited the B.t.-producing plant in Kibbutz Dalia. The training period was planned in an efficient way so that during a short time the collaborating scientists became familiar with the relevant methodologies and approaches. The techniques and procedures were transferred to the Kanchaveli Institute and successfully implemented in the studies performed there. Dr. Kotetishvili spent an additional period of two months (October and November, 1998) at the laboratory in Rehovot. He conducted experiments aimed at photostabilizing B.t. by diatomite and zeolite. He also presented a poster (see abstract and poster in the appendix) at the International Plant Protection Congress (IPPC) in Jerusalem (July 25-30, 1999) summarizing his results on photoprotection of B.t. using a diatomit-containing formulations. Moreover, his poster along with three other posters was selected for presentation in the relevant poster discussion session. Dr. Kotetishvili presented orally his experimental results before an international audience of scientists. Prof. Chkhubianishvili also attended the same international conference and presented a poster related to photostabilized Bt formulations.

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PLANT PROTECTION CONGRESS (ICPP)

Diatomite as a possible UV protectant for the photostabilization of *Bacillus thuringiensis*

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Objectives: The efficiency and field performance of *Bacillus thuringiensis* (Bt) formulations largely depend on their stability under field conditions. Sunlight irradiation is regarded as one of the major environmental factors in decreasing the biological activity of Bt. The purpose of the study was to find out whether diatomite added to Bt formulations may be considered as a possible UV photoprotectant. **Methods:** A Bt-diatomite (Bt-d) complex (0.15% in solution) was prepared using Bt *Kurstaki* HD1 strain. The efficiency for photostabilization was assayed using 1st instar larvae of the European cabbage worm, *Pieris brassicae* L. on cabbage plants in the field. **Results:** Unprotected Bt samples underwent rapid inactivation reflected by insecticidal activity that did not exceed 63.4%. In contrast, Bt-d complexes yielded considerable photostabilization, as on the average the insecticidal activity reached 88%. **Conclusions:** It appears that inclusion of diatomite as an additive in Bt formulations will provide phtostabilization to the biocontrol agent exposed to sunlight irradiation.

DIATOMITE AS A POSSIBLE UV PROTECTANT FOR THE PHOTOSTABILIZATION OF *BACILLUS THURINGIENSIS*

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Introduction

The commercial microbial insecticides, which are most widely used in integrated pest management (IPM) programs are derived from the entomogenous bacterium *Bacillus thuringiensis* (Bt). The highly virulent strains of the bioagent, producing parasporal inclusions of protein toxins, are characterized by a broad spectrum of insecticidal activity against various insect pests belonging to the different insect orders: Coleoptera, Lepidoptera, Diptera. The formulations on the basis of this group of insecticides are biodegradable, non-accumulative and their long-term application is environmentally friendly

However, it must be indicated that the full scale-application of Bt in IPM programs is still limited. Under field conditions the microbial insecticide is unstable primarily due to solar irradiation. Thus, significant importance is attached to develop photostable Bt formulations

Many attempts to acheve Bt photostabilization such as encapsulation, addition of various screeners or isolation of mutants with increased UV resistance were largely inadequate. The problem of photostabilization of Bt and various entomopathogenic insecticides has not been solved yet. The purpose of our study was to test diatomite, as a possible UV protectant for Bt.

Materials and Methods

Bt *Kurstaki*, (strain-HD1) containing δ-endotoxin crystals and lysed bacterial cells was obtained from Biodalia-Becker, Israel. The source of diatomite powder (about 10^2 intact shells of diatoms/cm^3) was the Khusaubi deposit (Georgia).
A Bt-diatomite complex (Bt-d complex) was prepared as follows: Aqueous suspension (1%) of either Bt and diatomite were prepared separately and mixed at a 1:1 volume ratio. The resulting mixture was shaken overnight and the precipitate, obtained by centrifugation, was washed with distilled water and subsequently lyophilized.
Preliminary experiments were carried out with field-collected 1st instar larvae of the European cabbage worm (ECW) (*Pieris brassicae* L.) to determine insecticidal activity of Bt. Photostabilization of the Bt-d complex was evaluated by exposure to UV irradiation inside of UV chamber that simulate solar irradiation conditions. Small-scale field experiments were conducted using the Bt-d complex. Cabbage plants naturally infected with 1st and 2nd instar larvae of ECW were sprayed with the stabilized complex. Unprotected Bt was applied as controls. The insecticidal activity was recorded up to 15 days post treatment

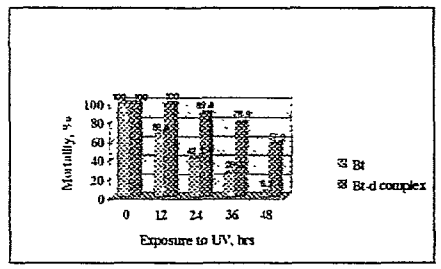


Figure 3. Photostabilizing effect of diatomite

Bt and the Bt-diatomite complex were exposed to UV irradiation inside the UV chamber for the specified time periods. Cabbage leaves treated with the samples were fed to 1st instar *P. brassicae* larvae. Mortality of insects were recorded 10 days later

Bt is highly sensitive to UV irradiation as after exposure of 24 hrs about 60% of the treated larvae survived. The effect is already manifested after a 12 hrs exposure period with a reduction of mortality by 32%. Following a prolonged exposure of 48 hrs only 5% of the insects died. In contrast, the Bt-diatomite complex is effective in protecting Bt from UV inactivation as after 24 hrs of irradiation close to 90% of the larvae died. Even following exposure to 48 hrs resulted in about 60% mortality

Figure 1. Scanning electron micrograph of diatomite

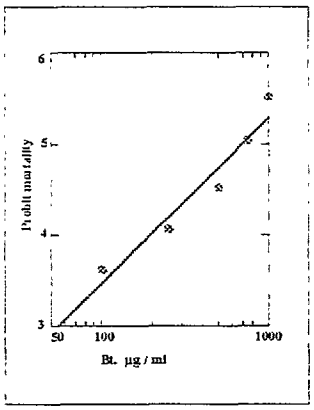
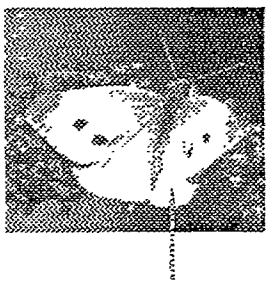


Figure 2. Dose mortality response of Bt in *Pieris brassicae* larvae

Cabbage leaves treated with various concentrations of Bt were fed to 1st instar larvae of *P. brassicae*. Mortality was recorded 10 days later and insecticidal activity of the microbial agent was estimated by Probit analysis. The LC_{50} value of the Bt under the condition of the experiment is 800µg per mL



Pieris brassicae L.

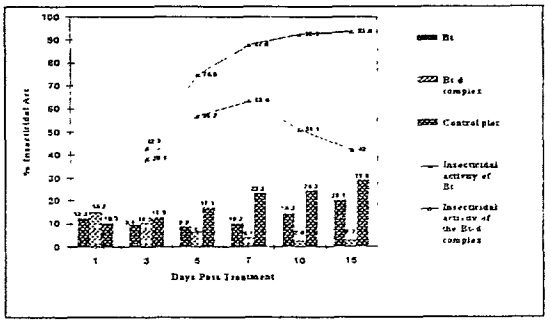


Figure 4. Photostability of the Bt-d complex under field conditions

Cabbage plants naturally infected by ECW larvae were treated with suspensions of Bt (300 gr per hectare) and Bt-d complex (at a rate of 400 gr per hectare). The insect population density was recorded for 15 days and the insecticidal activity was calculated by Franz's formula that takes into account survival rate of the insects in treated vs. control plots. The numbers above bars represent average number of larvae per plant

Field observations showed that due to photoinactivation of Bt as a result of solar irradiation, more larvae of the pest survived on cabbage plants. In the experimental plots treated with Bt the decline of the population density was not significant on the 3rd, 5th and 7th day post application. On the 10th day even an increase in the insect population density was observed. This phenomenon is probably related to larvae that hatched later from eggs. Overall, the insecticidal activity of Bt under field condition was low. In contrast, the Bt-d complex was much more stable resulting in an extended insecticidal activity. This trend can be observed already after 5 days where a difference of 20% is evident. The gap is increased dramatically (by more than 50%) at day 15.

Discussion and Conclusions

The effectiveness of the application of biorational insecticides developed on the basis of different strains of Bt, depends largely on their photostability under field conditions. Sunlight irradiation is evidently the major environmental factor causing loss of biological activity of this group of bioinsecticides. Many attempts to develop photostable Bt formulations based on UV screeners as additives, are problematic due to environmental risks related to water and soil pollution.
By using diatomite as a photostabilizing additive we took into consideration its toxicological and environmental properties. Diatomite is characterized by a unique microscopic structure and adsorption properties, which might be useful in application as a UV protectant of Bt.

Our studies have shown that the presence of diatomite in the complex, contributed photostability of the active component of Bt. We consider that this effect was a result of sunlight deflection by the additive that resulted in protecting the δ-endotoxin from UV-induced photoinactivation. Based on our experiments we may conclude that application of diatomite as a UV protectant, can be one of the possible ways of photostabilization of Bt formulations

Acknowledgement

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