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Title - Biocontrol of Insect Pests Using *Aschersonia* Species

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Executive Summary

The purpose of the project is to investigate to possible use of entomopathogenic fungi to control important agricultural pests such as diaspidids and whiteflies. Entomopathogens belonging to the genus *Aschersonia* was isolated from diseased insects, bioassayed in the laboratory and mass-cultured on autoclaved millet. The sensitivity of spores to solar-simulated radiation was investigated and photostabilization experiments were conducted using clays and clay-adsorbed photoprotectants. The range of insect pests sensitive to the entomopathogen was studied primarily in Georgia in order to identify the ones towards which control measures will be directed in the future.

It has been shown that the entomopathogenic fungus *Aschersonia* F. Georgia is effective against species of armored scales (Diaspididae) like *Cryosomphalus dictyospermi* and the soft scale (Coccidae) *Coccus hesperidum*. The entomopathogen was also very effective against 1st and 2nd instar larvae of the cottony scale *Neopulvinaria innumerabilis*. The fungus was not effective against softscales (Coccidae) like *Ceroplastes japonicus* and *C. sinensis* (all stages) and against coccids of the family Pseudococcidae (Mealybugs). The favorable developmental stages for infection of the sensitive coccids were the 1st and 2nd instar larvae.

We examined 5 different potent photostabilizers for their effects on the viability of *Aschersonia* f. Georgia. It was found that the positively charged photoprotectants such as acriflavin, berberine and palmatine are toxic to the fungal spores. The three compounds caused severe reduction in spore viability at extremely low concentration. Two anionic dyes (Naphthol Yellow (NY) and Fast green (FG)) did not affect spore viability at very high concentrations. Consequently, the research proceeded using the two safe anionic photostabilizers adsorbed to surface of clays (attapulgitite, montmorillonite and bentonite) converted positive by the aminosugar biopolymer chitosan. The composite matrices were highly effective as they provided excellent photoprotection of spores from UV irradiation.

Section I

Note of Publications

Abstracts

V. Yasnosh, L. Chkhaidze, E. Tabatadze. 1999. Biocontrol and IPM strategy in citrus orchards in Georgia. XIV International Plant Protection Congress. Israel, Jerusalem.

E. Tabatadze. 1999. New pathogen of armoured scale in Georgia. Biocontrol and IPM strategy in citrus orchards in Georgia. XIV International Plant Protection Congress. Israel, Jerusalem.

E. Tabatadze, V. Yasnosh. 1998. Population dynamic and biocontrol of the Japanese scale, *Lopholeucaspis japonica* Cocherell in Georgia. VIII International Symposium on Scale Insects Studies. UK, P.36

L. Chkhaidze. 1999. Biocontrol of *Chrysomphalus dictyospermi* in citrus orchards of Georgia. XIV International Plant Protection Congress. Israel, Jerusalem.

Publications

E. Tabatadze. 2000. New host insects of *Aschersonia* in Georgia. Advanced Study on Plant Pest Biological Control. Heilongjian.

E. Tabatadze, V. Yasnosh. 2000. New entomopathogen of coccids - *Aschersonia*. Journal of Plant Protection and Quarantine. No 10, P.39.

A) Research Objectives

The main objectives of the project were to find the range of insect pests sensitive to the entomopathogen as well as to provide measures of protection to photosensitive spores. As the fungus was isolated from homopteran species, insect pests taxonomically related to this group were selected for bioassay. To overcome environmental constraints like UV radiation, the photolabile spores were protected using a composite matrix composed of clay, a positively charged biopolymer like the asminosugar chitosan and toxicologically safe anionic photostabilizers like Fast Green and Naphthol Yellow.

B) Research Accomplishments

The research carried out in Georgia has identified the range of insect species sensitive to the entomopathogenic fungus, *Aschersonia* f. Georgia (Table 1). The fungus is effective against species of armored scales (Diaspididae) like *Crysomphalus dictyospermi* (58-75% mortality) raised on two different plants (Magnolia and Quercus) and the soft scale (Coccidae) *Coccus hesperidum* (85% mortality). The entomopathogen was also very effective against the larval stages of the cottony scale *Neopulvinaria innumerabilis* on vine. The 1st instar larvae were most sensitive to the entomopathogen as mortality reached values of 77-83%. The 2nd larval stage is less sensitive yielding mortality rates of only 35%. The fungus was not effective against softscales (Coccidae) like *Ceroplastes japonicus* and *C. sinensis* (all stages) and against coccids of the family Pseudococcidae (Mealybugs). The favorable developmental stages for infection of the sensitive coccids were the 1st and 2nd instar larvae.

Five different photostabilizers were examined as to their effect on spore viability of *Aschersonia*. Viability of spores exposed to very high concentrations of the toxicologically-safe anionic dyes such as Naphthol Yellow and Fast Green were not affected (not shown). The positively charged photoprotectants such as acriflavin, berberine and palmatine drastically reduced spore germination (Figures 1 and 2).

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Concentration of spores	Insect species	Plant	Mortality, % Larvae	Mortality, % Females
1.5x10 ⁶ /ml (46-80)*	<i>Chrysomphalus dictyospermi</i>	Magnolia	6.3±4.8	0.98±0.94
2.4x10 ⁹ /ml (44-81)*	<i>Chrysomphalus dictyospermi</i>	Magnolia	57.5±11.1	14.0±6.5
1.5x10 ⁶ /ml (197-502)*	<i>Chrysomphalus dictyospermi</i>	Quercus	9.6±4	0.9±1.2
2.4x10 ⁹ /ml (67-405)*	<i>Chrysomphalus dictyospermi</i>	Quercus	74.5±9.4	
6.0x10 ⁶ /ml (23-82)*	<i>Neopulvinaria innumerabilis</i>	Vine	77.9±7.6 (stage I)	
7.2x10 ⁷ /ml (43-110)*	<i>Neopulvinaria innumerabilis</i>	Vine	77.0±20.0 (stage I and II)	
3.5x10 ⁷ /ml (30-137)*	<i>Neopulvinaria innumerabilis</i>	Vine	82.7±13.5 (I) 34.6±6.9 (II)	31.7±8.9
6.0x10 ⁶ /ml (19-102)*	<i>Coccus hesperidum</i>	Ornamental plants	84.9±5.9	

Table 1. Mortality of insect pests following infection with the entomopathogen *Aschersonia* f. Georgia. Three insect pests raised on various plant species were selected, and mortality of either larvae or females was recorded.
*Range of insect numbers per replicate.

Acriflavin was highly inhibitory as a concentration of about $7\mu\text{M}$ caused 50% reduction in spore germination. Berberine and palmatine were less toxic as a rate of only about 10nM reduced spore viability by 50%.

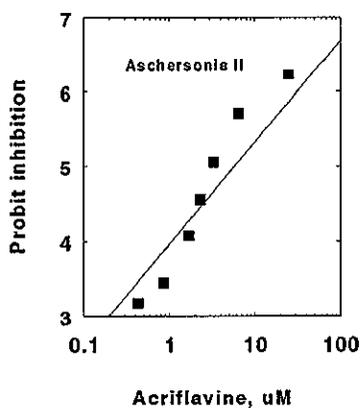


Figure 1. Effect of acriflavin on spore germination of *Aschersonia* f. Georgia. Percentage inhibition of germination was transformed to Probit units.

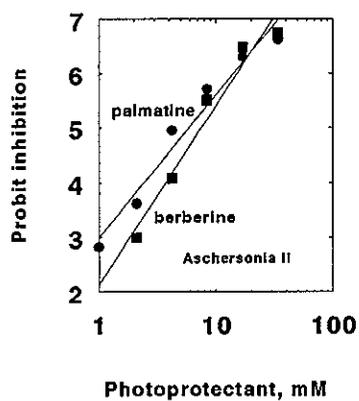


Figure 2. Effect of the alkaloids berberine and palmatine on spore germination of *Aschersonia* f. Georgia. The percentage of mortality was transformed into Probit units.

The spores of *Aschersonia* are highly sensitive to UV irradiation. Quantitative data as to the level of sensitivity, Figure 3 represents experiments conducted with spores exposed to near UV irradiation in a UV chamber that simulate solar radiation.

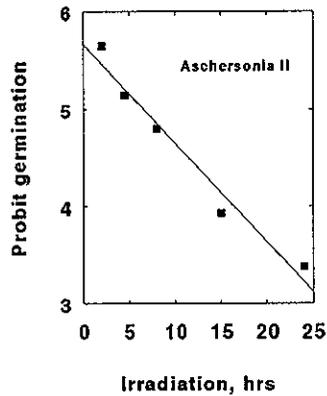
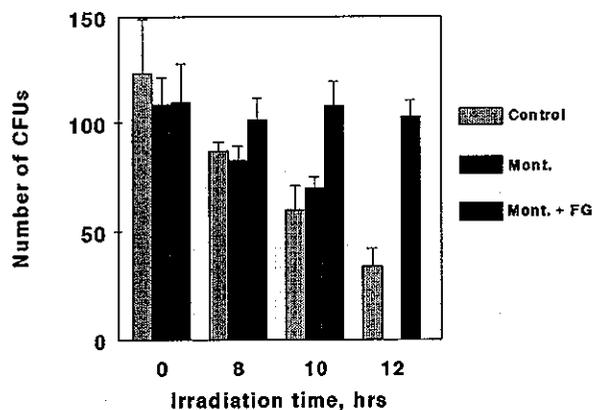


Figure 3. Reduction in germination rate of *Aschersonia* spores exposed to UV irradiation. Percentage germination was transformed to Probit units.

It is quite evident that irradiation is detrimental to spore viability. About 5 hours of irradiation caused a 50% reduction in spore germination.

Aschersonia spores are sensitive to UV irradiation as germination rate was drastically reduced after 12 hours of irradiation. A significant reduction was observed already after 8 hours of exposure (Figures 4-8). Montmorillonite *per se* did not protect spores from photoinactivation. The montmorillonite-chitosan-Fast Green (FG) complex was highly efficient in photostabilizing the spores as germination rate Figure



4. Photostabilization of *Aschersonia* spores by montmorillonite and montmorillonite - Fast Green complex.
 Mont.- montmorillonite; Mont.-FG - montmorillonite -Fast Green complex.
 Spores were exposed to simulated solar irradiation for the time period specified. CFU - colony forming units.

following 12 hours of irradiation was close to the non-irradiated control (Figure 4). As clay and clay complexes obscure spores, number of colony forming units (CFUs) were used as a measure of germination.

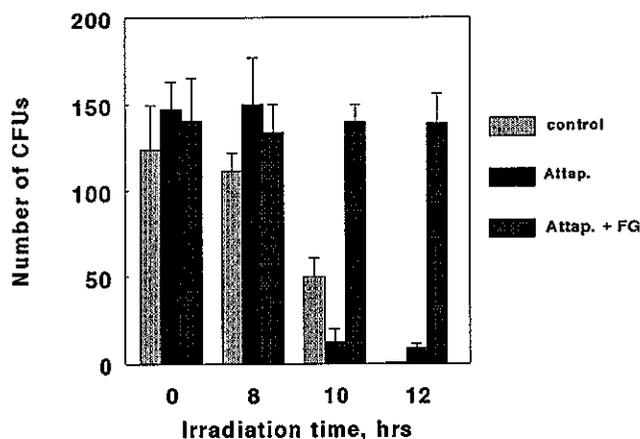


Figure 5. Photostabilization of *Aschersonia* spores by attapulгите and attapulгите-Fast Green Complex.

Attap. - attapulгите; attap.+FG - attapulгите plus Fast Green complex. Spores were exposed to simulated solar irradiation for the time period specified. CFU - colony forming units.

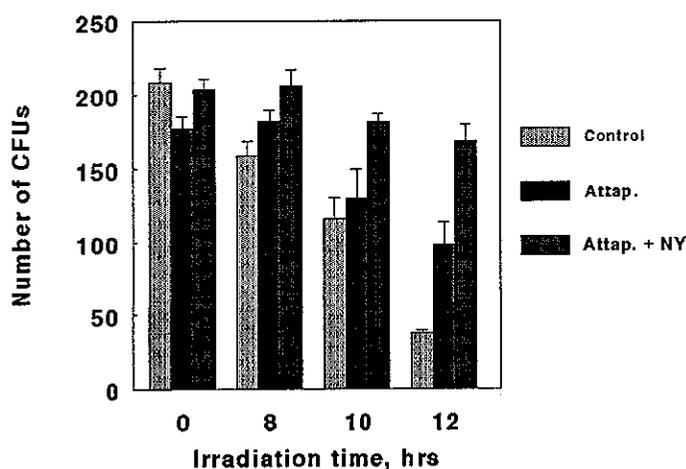


Figure 6. Photostabilization of *Aschersonia* spores by attapulгите and attapulгите-Naphthol Yellow Complex.

Attap. - attapulгите; attap.+NY - attapulгите plus Naphthol Yellow complex. Spores were exposed to simulated solar irradiation for the time period specified. CFU - colony forming units.

Similar to montmorillonite, the complex of attapulgite-chitosan-Fast Green is a potent matrix which bestowed photoprotection to *Aschersonia* spores (Figure 5.) The germination rate of spores did not diminish after 12 hours exposure to irradiation. It is noteworthy that the attapulgite *per se* also contributed some measure of photoprotection.

As observed in Figure 5, attapulgite alone is able to provide some photoprotection to *Aschersonia* spores (Figure 6). After 12 hours of irradiation the unprotected spores yielded 12% CFUs as compared to 38% CFUs when spores were mixed with the clay. After this exposure period, the attapulgite-chitosan-Naphthol Yellow complex was very effective as the germination rate was about 80% of the control.

Bentonite is a third clay used for photostabilizing *Aschersonia* spores (Figure 7). Compared to the two other clays, bentonite alone bestowed a high degree of photoprotection as the germination rate was decreased by only 34% after 12 hours of exposure to irradiation. Photostabilization was slightly improved by the complex of clay-chitosan-dye.

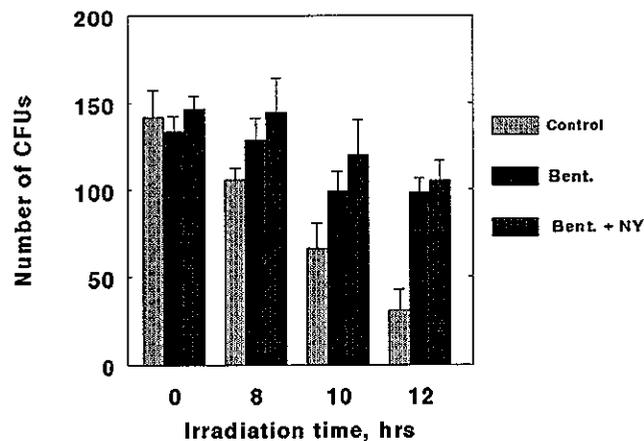


Figure 7. Photostabilization of *Aschersonia* spores by bentonite and bentonite-Naphthol Yellow Complex.
 Bent. - bentonite; Bent.+NY - bentonite plus Naphthol Yellow complex.
 Spores were exposed to simulated solar irradiation for the time period specified. CFU - colony forming units.

C) Scientific Impact of Collaboration

The collaboration with the team of scientists in Georgia proceeded in the reporting year as planned. We were able to show the range of insect pests sensitive to the entomopathogen and identified the target pests for their future biocontrol. The data gathered in Georgia during the reporting year were submitted on time and served well

for preparing the present report. Since one of the collaborating team, Dr. Tabatadze, spent 6 week in Israel, it gave us the opportunity to discuss the results as well as future experiments. On her way back to Georgia we provided her with several photostabilizing clay-dye complexes.

D) Description of Project Impact

The results from the project have not yet been used for controlling insect pests. More information should be generated prior to the practical application of the biocontrol agent in the field. We anticipate that by the end of the project a photostabilized formulation of *Aschersonia* will be available for thorough testing in the field.

E) Strengthening of Developing Country Institutions

The efforts invested during the first year of the project (extensive training, the purchase of equipment and the computer, necessary supplies) served the project well. It has been demonstrated in the quality of laboratory and field experiments, as well as in the reporting. The above has obviously provided a solid basis for a sustainable capacity to independently perform similar research. It is noteworthy that Prof. Yasnosh (principal PI collaborator) and Dr. Tabatadze attended the International Congress of Plant Protection (IPPC) which took place in Jerusalem (July, 1999) and also presented there three abstracts. I believe that this participation have had a profound scientific impact on labs and colleagues at the Kanchaveli Institute of Plant Protection.

F) Future Work

The project has proceeded as planned and is on schedule. I have asked that the project will be extended for one more year. If such request is granted, field experiments using photostabilized formulations could be conducted in the coming Summer in citrus orchards in the Batumi region (near the Black Sea). Such experiments will be performed against pests like coccids demonstrated to be sensitive to the biocontrol agent.

Section II

A. Managerial Issues

During the second year of the project we have encountered the same problem as experienced in the first year i.e. the proper way of transferring money to Georgia was, to put it mildly, inadequate. I don't have any idea where is the weak link in such transfer, but the end result was that my collaborator, Prof. Yasnosh, did not receive the allocated budget money which consequently caused problems in carrying out the experiments in Georgia. Throughout this year the Authority of Research and Development at the Hebrew University received many complaints related to the above issue which still remains unresolved.

B. Budget

No changes were made in budget.

C. Special Concerns

No changes relative to the "special concerns".

D. Collaboration, Travel, Training and Publications

The previous year training of the team from Tbilisi at the Rehovot Laboratory has been implemented in the research carried out later in Georgia. The experimental results presented in Section I is a demonstration of the impact of this training. All travels planned occurred during the first year of the project and none is anticipated in the next months until completion of the project. A number of publications by Prof. Yasnosh and colleagues concerning the project (see section I) is an additional proof for the scientific progress achieved by the collaborating Georgian team.

E. Requests for A.I.D. or BOSTID Actions

The exchange of data and managerial issues via fax and electronic mail has greatly improved. It is noteworthy to mention that the staff at the U.S. Embassy in Tel-Aviv handling the A.I.D. matters has been very helpful.