FINAL REPORT

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TITLE OF PROJECT: **Entomopathogenic Nematodes as Pest Control Agents in Georgia: Application and Genetic improvement of Beneficial Traits for Enhancement of Effectiveness**

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Section I: Technical Progress

A) Research Objectives:

Our overall aim is to implement the use of entomopathogenic nematodes (EPNs) as biological control agents of important pests in Georgia, thus to provide a viable alternatives to chemical insecticides. This goal was achieved through completion of the following specific objectives:

a. Test the efficacy of nematodes under laboratory, environmental chamber and greenhouse conditions, in combination with effective antidesiccant, against key pests of vegetables.

b. Isolate indigenous EPNs in Georgia. Characterize their infectivity against important pests in Georgia as well as their tolerance to environmental extremes.

c. Enhance EPNs efficacy under the climatic conditions. Enhancement of nematode desiccation tolerance either by selective breeding or hybridization to increase their potential as biological control agents.

B) Research Accomplishment

Objective 1: Test the efficacy of nematodes against key pests of vegetables:

In Georgia the nematodes were tested against the following following insect:

At the beginning of the project it was realized (see first report) that the most important pests in Georian greenhouse are: The green peach aphid (*Myzodes persicae*), the greenhouse whitefly (*Trialeurodes vaporarium*) and the web mite (*Tetranichus urticae*). Another devastating and dangerous pest is the Colorado potato beetle, *Leptinotarsa decemlineata*. It infects vegetable cultures damages the tomato seedlings in hotbeds at early spring (Gardabani region).

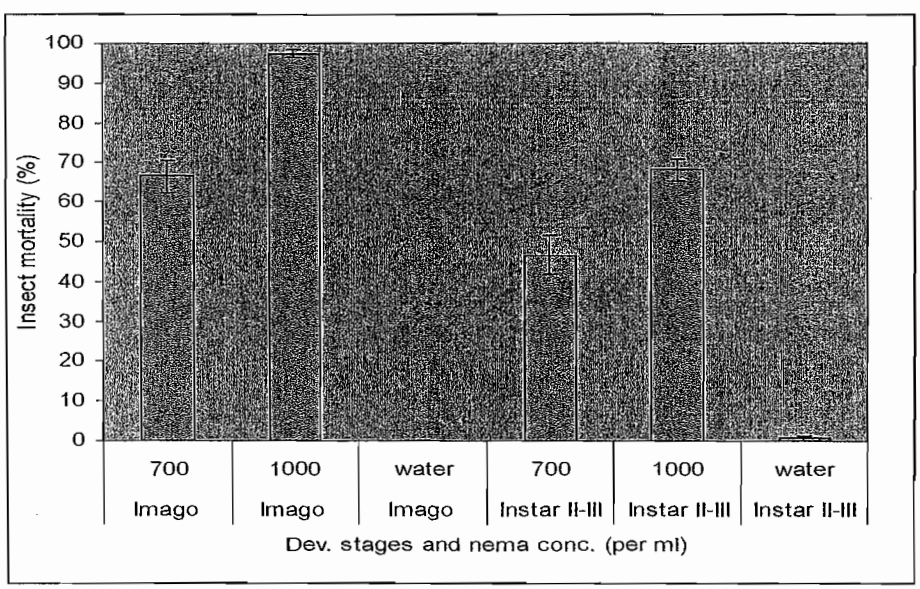
Georgian local entomopathogenic nematodes from collection of the Laboratory of Entomo-nematology (Institute of Zoology) - *Steinernema thesami, S. disparica, S. carpocapsae, Steinernema sp.* and introduced *Heterorbabditis bacteriophora* (from USA) were cultivated on the larvae of *Galleria mellonella* (reared on the beeswax), *Bombyx mori* (obtained from Institute of sericulture of Georgian Agrarian State University), *Tenebrio molitor* (reared on flour bran) and *Melolontha hipocastani* (collected in the soils of several agrocenosis, Mtskheta region).

Nematode species	Insect species	Life stage of Insect	Mortality (%)
S. thesami	*M. persicae*	I-IV instars, Imago	70-75
S. carpocapsae		Imago	100
H. bacteriophora			
S. thesami	*T. waporarium*	Eggs, Larvae, Imago	20-51
S. carpocapsae			23
H. bacteriophora			5
S. thesami	*T. urticae*	Imago	12
S. carpocapsae			
H. bacteriophora			
S. thesami	*Aphis pomi*	Larvae II-III	83,5
S. disparica			31,4
Steinernema sp.			42,6
S. carpocapsae			47,3
S. thesami	*Leptinotarsa decemlineata*	Larvae III-IV	58,8
S. disparica			67,4
Steinernema sp.			67,4
S. carpocapsae			65,9
Control	*M. persicae*	Eggs, Larvae, Imago	0
	T. waporarium	Eggs, Larvae, Imago	0
	T. urticae	Eggs, Larvae, Imago	0

Insects tested under greenhouse conditions:

The experiments were carried out for establishment of *Steinernema thesami* effectiveness to the Colorado potato beetles both laboratory and field conditions. The results are presented in table 1.

Table 1: Infectivity of *S. thesami* to Colorado potato beetle



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Field experiment with 2000±150 IJs/ml resulted in 62.3% mortality of Larvae II-III instars

The **greenhouse whitefly (GHW), Trialeurodes *vaporarium (Homoptera).*** The action of EPNs to the GHW already established (Report, 4; Chkhubianishvili C., Mikaia N., Kakhadze M. 2006).

The goal our research was to establish the efficacy of joint action of entomoparasite nematode *Steinernema feltiae* and emulsifiable suspension – mycoinsecticide *BotaniGard ES* to GHW. Mycoinsecticide contains active ingredient Beauveria *bassiana* Strain GHA - 11.3%, inert ingredient 88, 7%. B

The experiments were conducted by using 1% preparation and 1000 IJs/ml concentration, the mixture of nematode+mycoinsecticide (1: 1) on I-II instars GHA larvae was spraying in laboratory and closed environment. The results are given in Fig. 1.

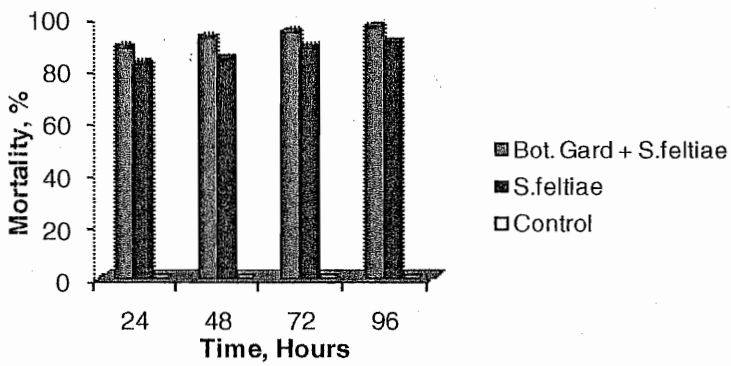


Fig.1. Results of *BotaniGard +S.feltiae* and S.feltiae action in laboratory

The high mortality (88%) of the GHW by contamination of *BotaniGard+S.feltiae* after 24 hr was detected, later on 96 achieves 96%; the action of *S.feltiae* to pest after 24 hr is 82%, while 96 after it is achieves 90%.

Results of treatment by the combined suspension of tomato plants in greenhouse are given in fig. 2.

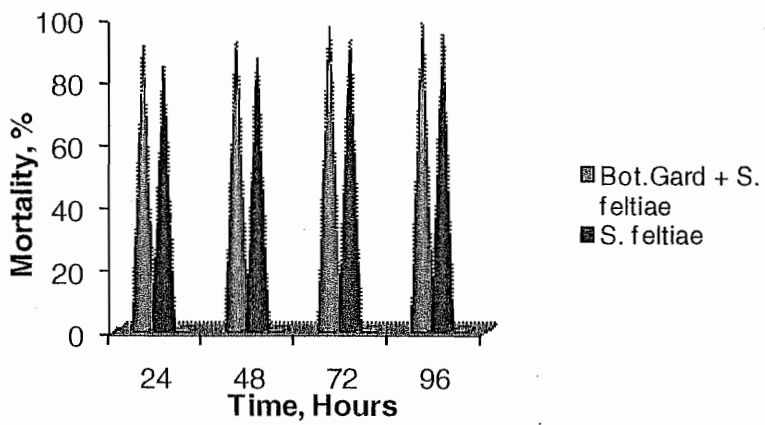
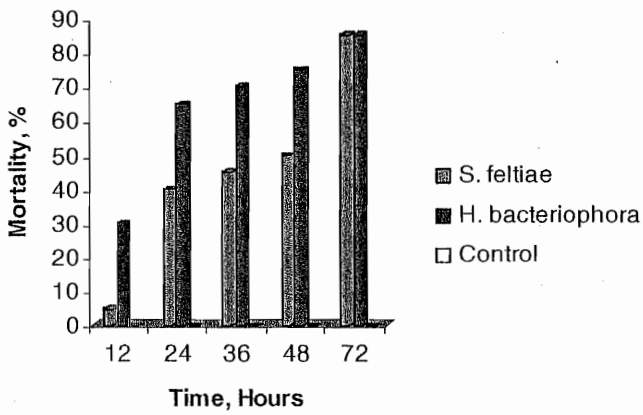


Fig. 2. Results of *BotaniGard +S.feltiae* and S.*feltiae* action in greenhouse

It was be expected The high mortality (90%) of the GHW by contamination of *BotaniGard+S.feltiae* after 24 hr was detected, later on 96 achieves 98%; the action of *S.feltiae* to pest after 24 hr is 84%, while 96 after it is achieves 94%.

The investigations are conducted on efficacy of *Steinernema feltiae* (SFG) and *Heterorhabditis bacteriophora* (HB) (Israel' strains) to some species of coccids *(Homoptera),* as a serious pests of agriculture crops in the Black Sea regions of Georgia - **the soft brown scale - *Coccus hesperidum*** L., **the cottony maple (or vine) scale- *Neopulvinaria innumerabilis*** **(Rathvon) and the grapevine mealy bug - *Planococus ficus*** **Signoret *(Homoptera: Coccidae).*** The results are given at fig. 3, 4, 5.

Coccus hesperidum



Neopulvinaria innumerabilis

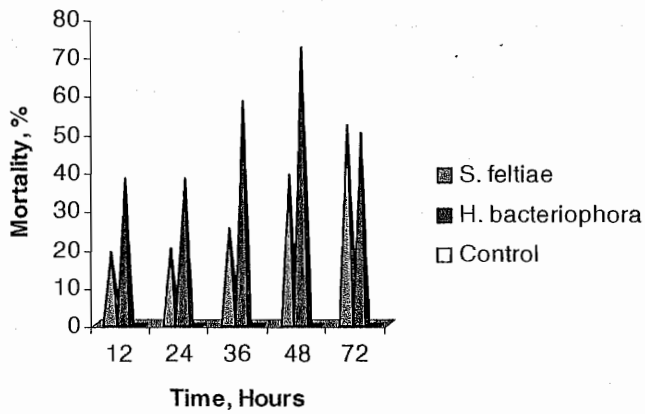


Fig. 3,4. Resuts of action SFG and HB to *C. hesperidum* and *N. innumerabilis*

Planococus ficus

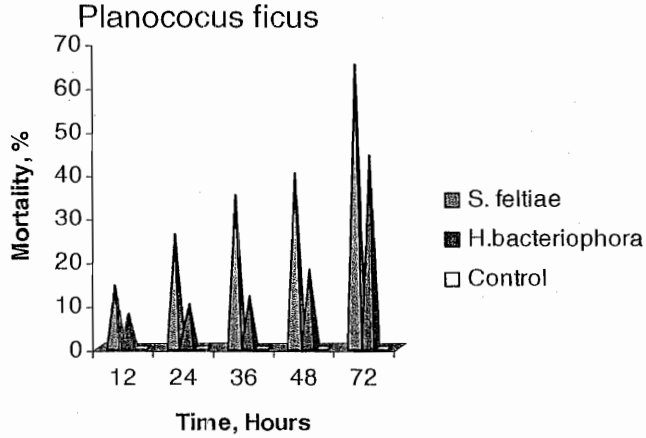


Fig. 5. Resuts of action SFG and HB to P. ficus

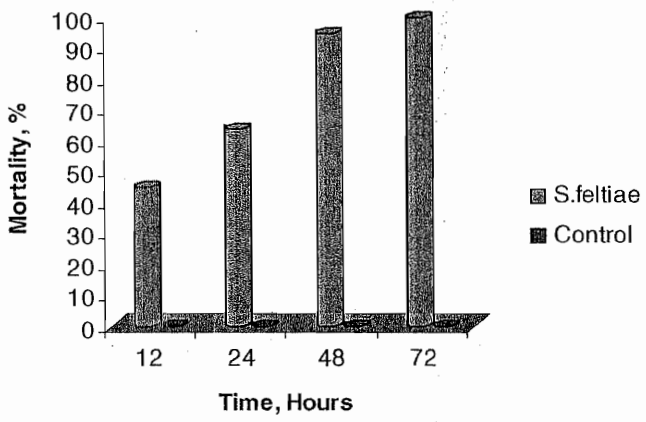
The fall webworm, *Hyphantria cunea Drury (Lepidoptera: Arctiidae)*, that is quarantine pest insect damaging more than 600 species of plants: forest and bush plants, orchard and berry plants, field and vegetable cultures, ornamental trees, herbs, etc.

The main goal of investigations was the study of interrelationship between the fall webworm and entomopathogenic nematodes from genus *Steinernematidae* and *Heterorhabditidae*. No date on presence of entomopathogenic nematodes in the populations of fall webworm in literature.

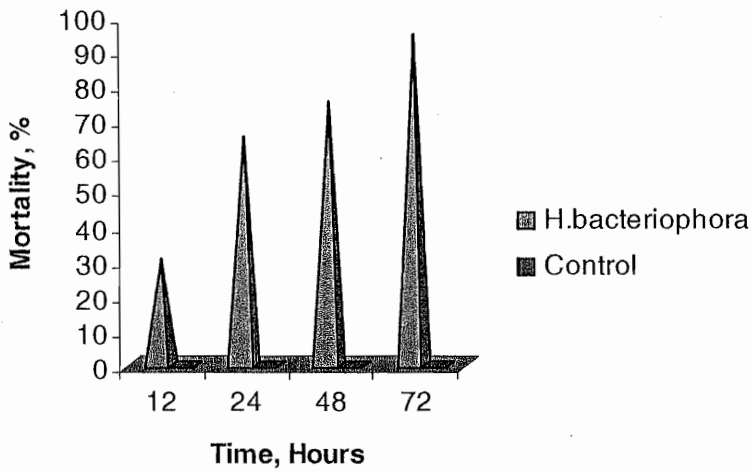
To invasion larvae of fall webworm in laboratory conducted by – *Steinernema feltiae* (SFG) and *Heterorahabditis bacteriophora* (HP), which cultivated of infective juveniles on the wax moth, *Galleria mellonella* larvae reared on artificial diet at 25°C and 75% humidity to according method.

The SFG suspension - 500 nematodes/ml was used in tests and in the case of HP -1000 nematode/ml. Experiments were carried out in three replicated trials. As a control the distilled water was used. The accounting of invasion larvae was carried out in every 12 hours, during 72 hours. Mortality of larvae was determined by Abbot's formula. Investigations the nematode pathology of fall webworm was conducted by the generally accepted methods in insect's nematology.

SFG (Fig. 1)



HP (Fig. 2)



As results of investigations the efficacy of SFG and HP to the fall webworm has established. These biological agents are considered as the potential, environmentally safe means to plant protection.

S.feltiae was tested to the fall webworm combined with bacterial formulation XenTari DF (*Bacillus thuringiensis subsp.aizawai) and* mycoinsecticide *BotaniGard ES (Beauveria bassiana)*

It was established that II-III instars of the fall webworm larvae at action of simultaneously infection the mortality in third day was achieved 100% at laboratory conditions. The results are presented in Fig. 3.

This experiments show the possibility of compatibility of *S.feltiae* with another biological agents, in this case with the bacteria - B.thuringiensis and fungous – *Beauveria bassiana.* The tests are continued.

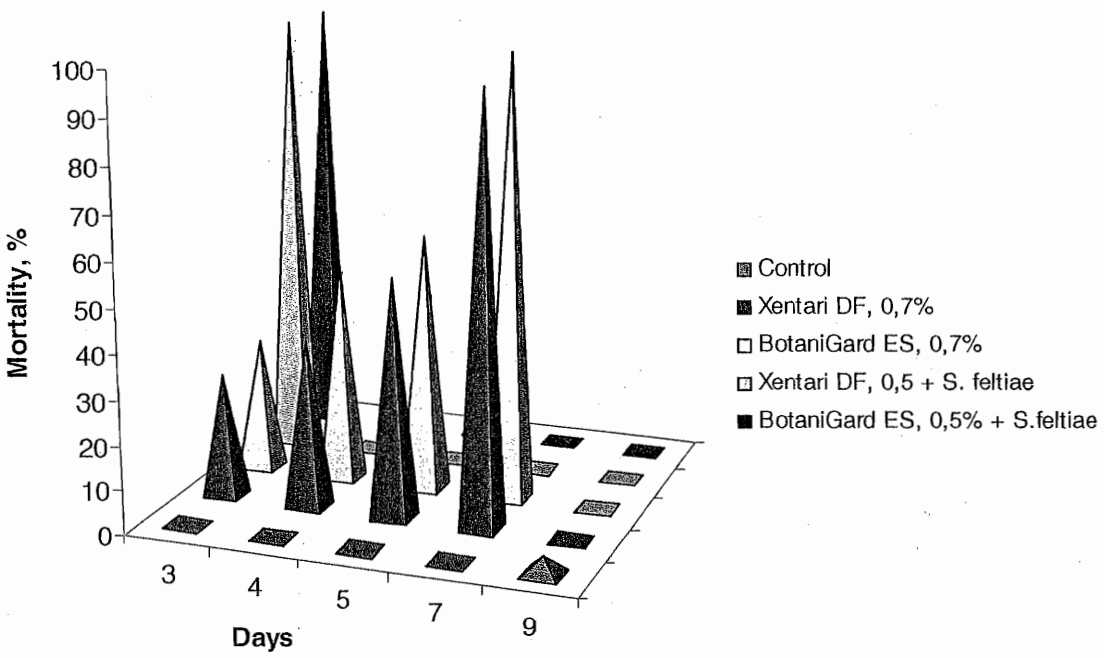


Fig. 3. Results of invasion of the fall webworm larvae by S.feltiae with bioformulations

Objective 2: Isolate indigenous EPNs in Georgia- A search for entomopathogenic nematodes in nature (Tbilisi surroundings) was conducted. The research works were conducted by using of the generally accepted methods in insect nematodes pathology, mainly (Kaya H., Stock P. 1997. Techniques in Insect Nematology. In: *Manual of Techniques in Insect Pathology,* Chapter VI, Ed. L.Lacay, Academic Press Limited, pp. 281-324.) As results the *Steinernematidae* were revealed. The nematodes isolated were used to evaluate their efficacy against pests as described above.

In Israel

According to the research plan the Israeli team was suppose to:

a. Training of one Georgian Student/Technician in Israel for survey and rearing nematodes techniques (7-8 months)
b. Survey and collection of EPNs in Israel and Georgia.
c. Characterization of the new isolates in desiccation tolerance and laboratory assays against key pests.

At the beginning surveys for entomopathogenic nematodes were conducted throughout the country: We obtain samples from 78 locations from the south (arid and semi-arid), the center (subtropical) and the north part (semi temperate). Five random soil samples were taken from each site. The samples were taken by auger to a 10-15-cm depth and a volume of 1 liter per soil sample. In this process we isolated 11 new populations of Heterorhabditids and Steinernematids. The population are currently subjected to laboratory bioassays in order to determine their beneficial characteristics:

a. Infectivity to *G. mellonella*
b. Survival under suboptimal conditions- desiccation and heat tolerance assays.
c. Reproduction potential

The following table provide preliminary information on some of our recent findings:

Population Code	Location of Isolation	Species	Infectivity assays		Survival bioassays		
			Penetration assay[a]	LD_{50}[b]	Desiccation tolerance[c]	Heat tolerance[d]	Repruduction[e]
IS_{6}	Gvulot	*S. feltiae*	28.0 ± 3.5	22.4±5.7	41% ± 3	55% ± 3	32,700
IS_{35}	Zewelim	*S. feltiae*	42.1 ± 7.2	21.3±4.6	67% ± 5	78% ± 7	57,400
IS_{36}	Gruphit	*S. feltiae*	14.4 ± 5.0	14.5±3.7	81% ± 4	81% ± 5	102,300
IS_{37}	Holit	*S. feltiae*	33.6 ± 5.1	7.5 ± 1.1	27.5% ± 3	49% ± 4	67,900
HP88	US (Reference pop.)	H. bact.	4.3 ± 1.2	12.6±2.7	14.7% ± 3	38% ± 3	112,000
IS_{38}	Shadmot Dvora	H. bact.	9.4 ± 3.1	4.1±2.0	29.6% ± 2	59% ± 5	143,000
IS_{39}	Nahal Zin	H. bact.	2.6 ± 0.8	8.7±3.5	33% ± 4	63% ± 4	160,000
IS_{40}	Besor	H. bact.	5.0 ± 1.1	15.2±3.1	41% ± 6	44% ± 6	99,000

[a] Number of infective juveniles that invaded *G. mellonella* larvae following 8 h exposure to 200 nematodes in Petri dish padded with moist filter paper at 25°C .

[b] LD_{50} – Lethal dose needed to kill 50% of *G. mellonella* larvae following exposure to 5, 25, 50, 100 and 200 infective juveniles for 48 h in Petri dish padded with moist filter paper at 25°C.

[c] Survival of infective juveniles (%) after 72 h exposure to 97% RH followed by 48 h exposure to 85% RH at 25°C.

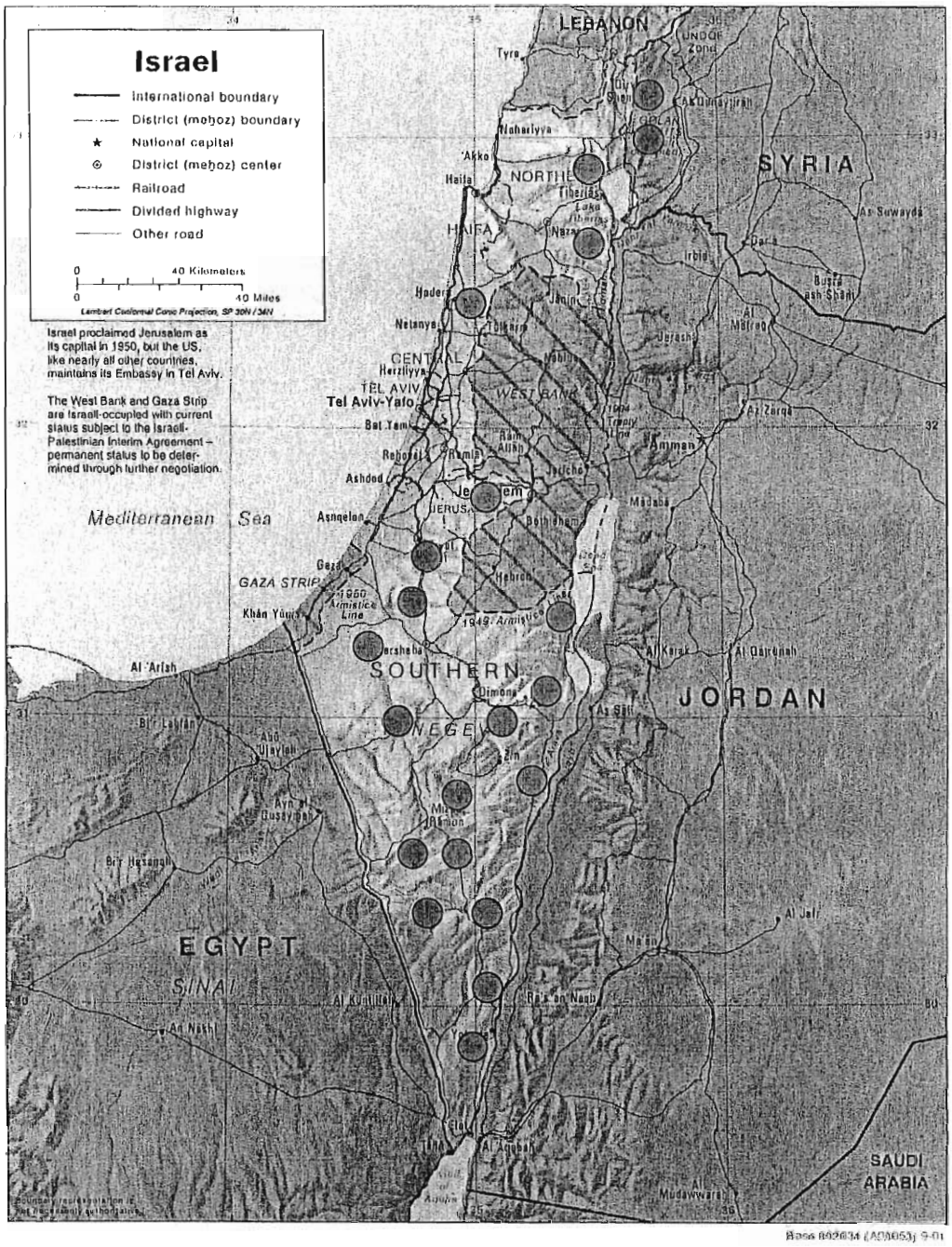
[d] Survival of infective juveniles after 6 h exposure to 38°C.

[e] Number of infective juveniles obtained per 1gr insect wait of *G. mellonella* reproduced on larvae of the wax moth after 14 days at 25°C.

The Georgian first trainee, Nona Mekaia, arrived on September 2004 and immediately joined our team in conducting whole range of experiments. She was trained in the following techniques:

a. Conducting surveys, soil sampling and extraction of EPNs from different soils (Baiting and physical extraction techniques).
b. Rearing nematodes on susceptible host (the wax month *Galleria mellonella*) as well as on artificial medium. She also acquired the rearing technique of *G. mellonella*).
c. Taxonomical identification of EPNs- was done in collaboration with Dr. Yevgeni Kozodoi from the Plant Protection Services.
d. Characterization of beneficial traits among the new EPN populations. That included:
    1. Infectivity assays- Does response, penetration assay, infectivity to different insects/arthropods (*G. mellonella*, *Tenebrio molitor*, the tick *Boophilus annulatus*)
    2. Reproduction potential in susceptible insect- *G. mellonella*.
    3. Tolerance to environmental stresses- Heat and desiccation tolerance assays
    4. Movement in soil profile- sand column assay.

Together with Nona we continued our survey for nematodes and obtained samples from 114 locations from the south (arid and semi-arid), the center (subtropical) and the north part (semi temperate). See map bellow:



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We isolated in this process we isolated 15 new populations of Heterorhabditids and Steinernematids. See table 2 for details:

Name/place	Place Characterization	Date Found	Nematode Species
Holit	**Lemon orchard**	**11.6.04**	***Steinernema feltiae***
Besor	**Avocado orchard**	**11.6.04**	***S. feltiae***
Susia	**Pasture**	**5.3.04**	***S. feltiae***
Nir Itzhak	**Medow**	**18.304**	***S. feltiae***
Zeelim	**Mango**	**18.304**	***S. feltiae***
Zeelim	**Citrus orchard**	**9.6.04**	***S. feltiae***
Besor Hb	**Avocado orchard**	**11.2.04**	***Heterorhabditis* sp.**
Devora	**Olive trees**	**5.7.04**	***Heterorhabditis* sp.**
Grofit	**Palm trees**	**20.7.04**	***Heterorhabditis* sp.**
Pardes Hana	**Citrus orchard**	**25.8.04**	***Heterorhabditis* sp.**
Mishmeret	**Citrus orchard**	**25.8.04**	***Heterorhabditis* sp.**
Kfar Hess	**Citrus orchard**	**25.8.04**	***Heterorhabditis* sp.**
Nahal. Zin	Bushes	3.6.04	Un unidentified
Nahal Lavan	Bushes	24.7.04	Un unidentified
Nahal Akrab	Bushes	24.7.04	Un unidentified

Intermediate conclusions:

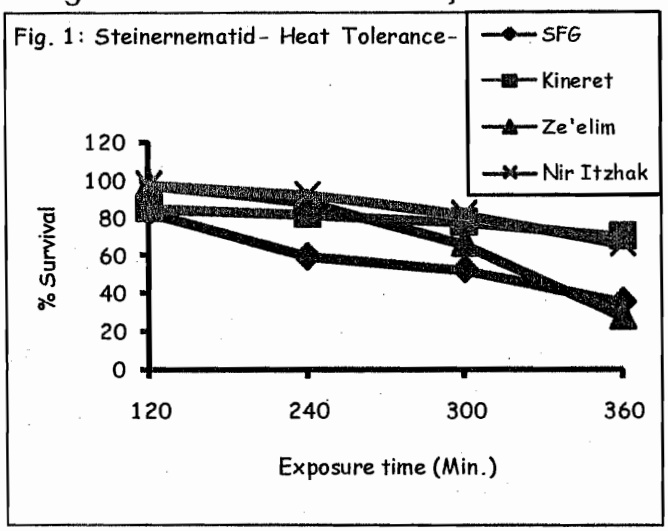
1. The proportion of our success in finding natural populations of EPNs (ca. 13%)from the total sample taken is higher than commonly reported in previous publications (7-10% success).
2. Throughout our survey, we could never recover steinernematid and heterorhabditid populations from non irrigated habitats in arid region. This phenomenon was also confirmed with other colleagues from US (Arizona), China and Spain.

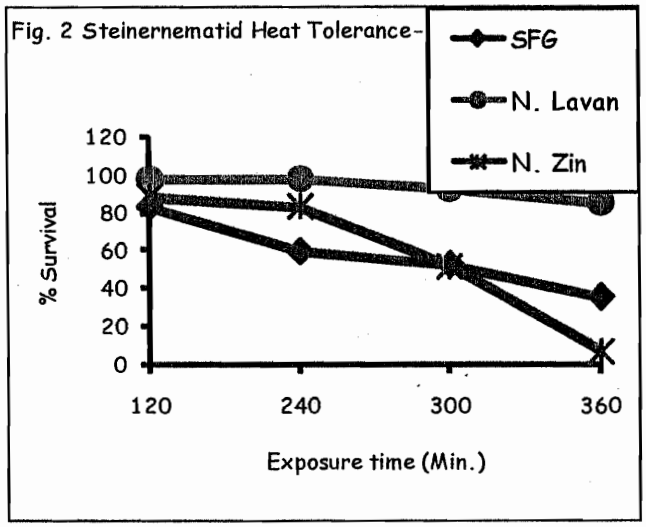
The population were subjected to laboratory bioassays in order to determine their beneficial characteristics:

a. Survival under suboptimal conditions- desiccation and heat tolerance assays.
b. Reproduction potential

Bellow are the results of our more completed experiments:

Fig. 1-3: Survival of infective juveniles after exposure to 37°C and 40°C.





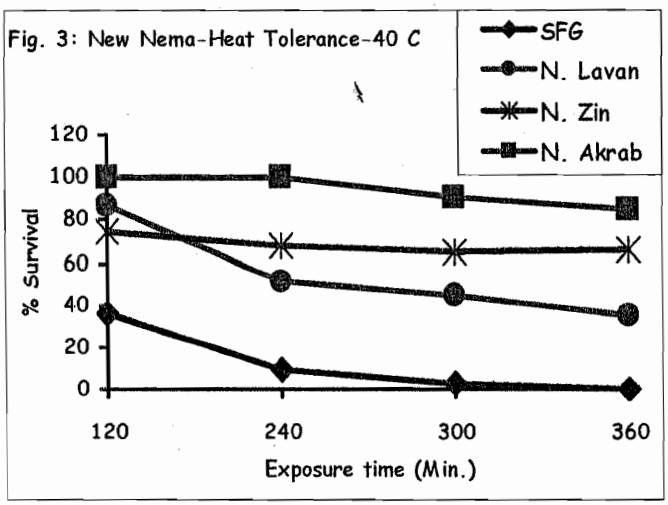
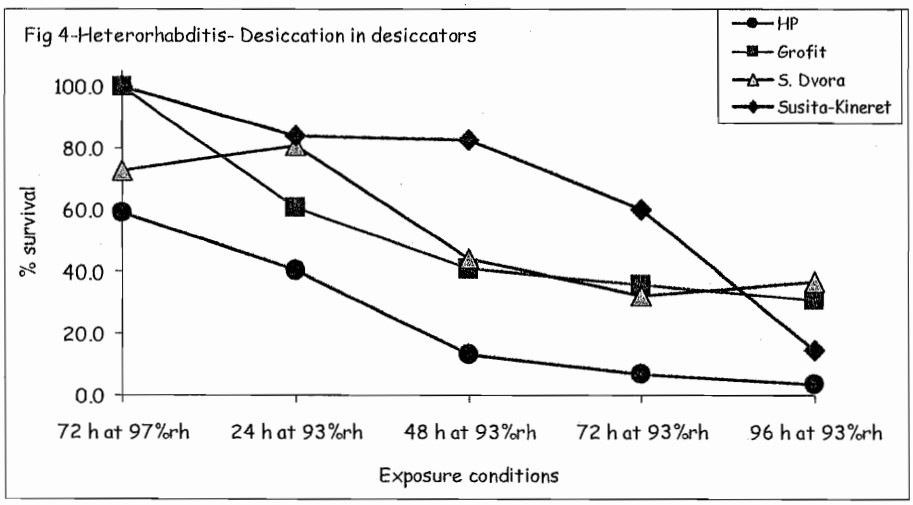
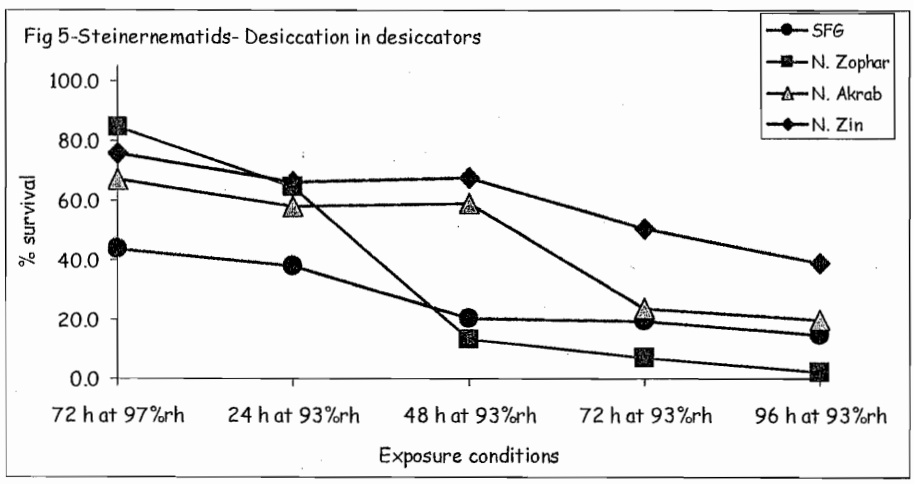


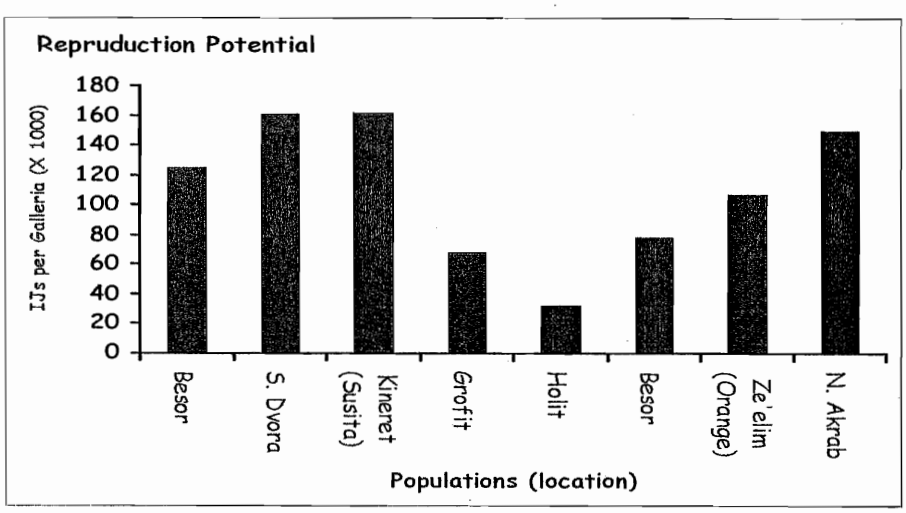
Fig. 4-5: Survival of infective juveniles (%) after 72 h exposure to 97% RH followed by 96 h exposure to 93% RH at 25°C.





Reproduction potential- Number of infective juveniles obtained per 1gr insect wait of *G. mellonella* reproduced on larvae of the wax moth after 14 days at 25°C.

The assays show that there are several populations with enhanced desiccation and heat tolerance. One population has greater reproduction potential than our common lines in the lab.



Invasion rate- Since instars of *G. mellonella* are highly susceptible to nematode infection we used the procedure described by Ricci *et al.* (1996- *Biocont. Sci. and Tech.* 6: 235-245). In short, 6 instars of *G. mellonella* are exposed to 500 IJ in 5 cm diam. Petri dish padded with moist filter paper. The plates are kept at 25°C in the dark. After 4, 8 and 24 h of exposure the insects are removed from the plates, washed in tap water (to remove IJs from their surface) and placed in nematode-free Petri dish padded with moist filter paper for additional 48 h incubation under the same conditions. At the end of the incubation period insect mortality is recorded. The insect cadavers are dissected under stereo-microscope and the number of invading larvae in each insect is recorded. Each treatment consisted of 4 replicates (24 insects) and was repeated twice. The results are presented in figures 1 and 2 bellow:

Fig 1: *Invasion rate of Infective juveniles from different steinernematid populations into last instars of* G. mellonella *following 4, 8 and 24 h exposure in Petri dishes.*

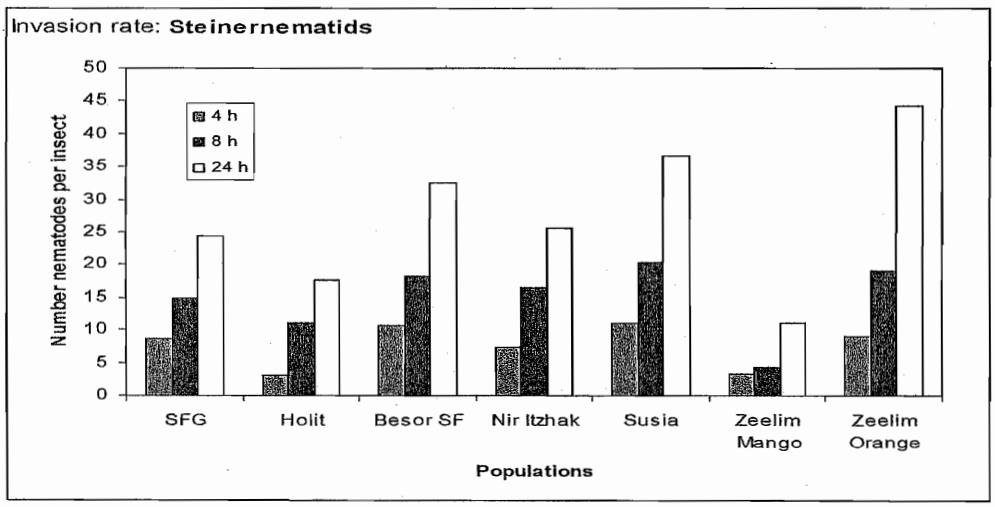
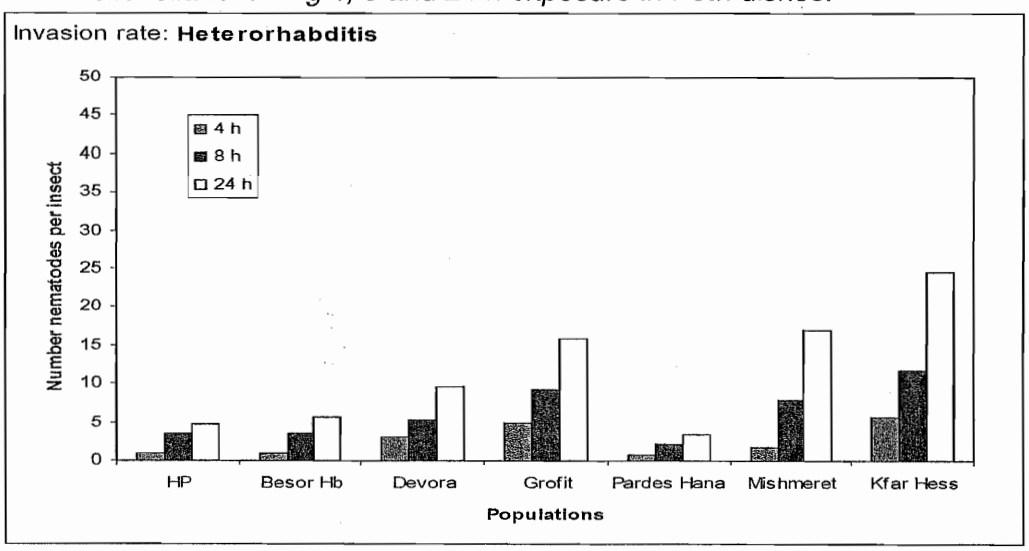


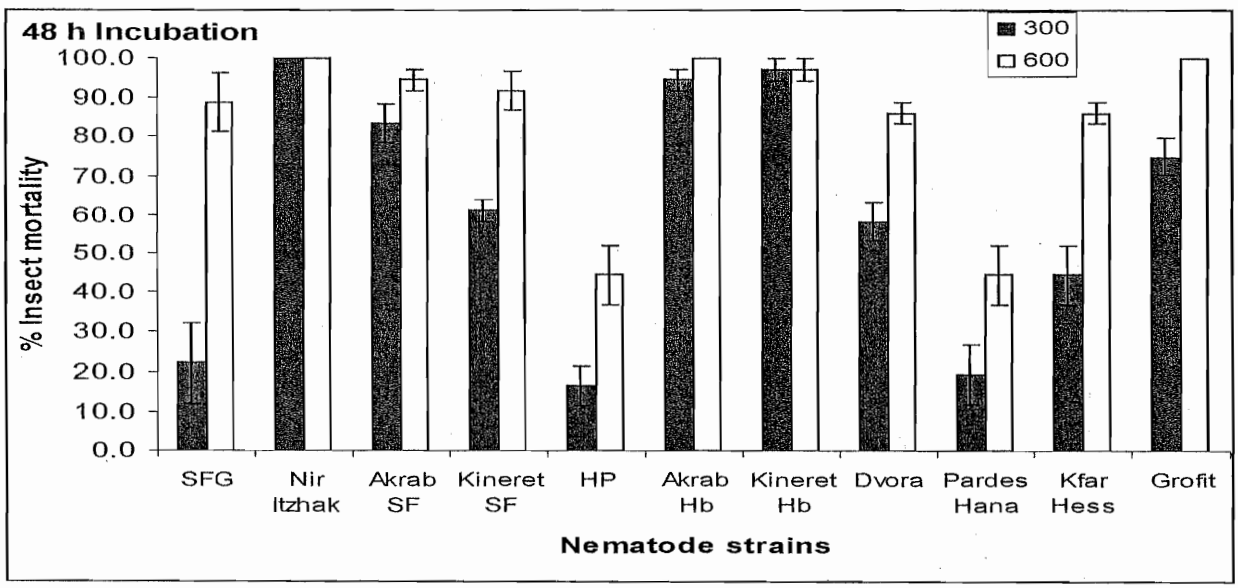
Fig 2: *Invasion rate of Infective juveniles from different heterorhabditid populations into last instars of* G. mellonella *following 4, 8 and 24 h exposure in Petri dishes.*



Substantial differences in invasion rate were found between the various population. Similarly to previous studies the over number of Steinernematid IJs invading the insect host was greater than that of heterorhabditids. In both genera several new isolates showed a significant higher invasion capability into an insect host than the laboratory references (*S. feltiae* G for steinernematids and *Heterorhabditis bacteriophora* HP for heterorhabditids).

Infectivity to the mealworm *Tenebrio molitor*- Last instar of the mealworm *T. molitor* was exposed to 300 or 600 IJs in 5 cm diam. Petri dish padded with moist filter paper. The dishes were incubated at 25°C in the dark. Insect mortality was recorded after 48 h and 72 h incubation. In Fig. 3 the level of insect mortality is given after 48 h exposure (most insect died in all treatments after 72 h).

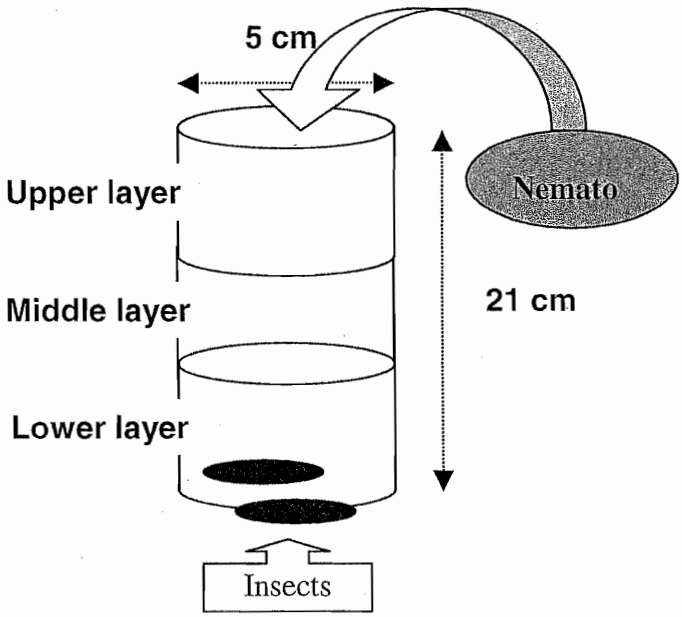
Fig 3: *Mortality of* Tenibrio molitor *instar following exposure to 2 concentrations of different EPNs strains.*



Substantial differences were observed between the effect of the different populations. Several isolates caused >90% mortality within 48 h even at the lower nematode concentration (Nir Itzhak, Akrab Hb, Kinerent Hb) while others displayed poor infectivity (< 50% at the higher concentration. Within 72 h of incubation most (90-100%) insect died in all treatments (not presented).

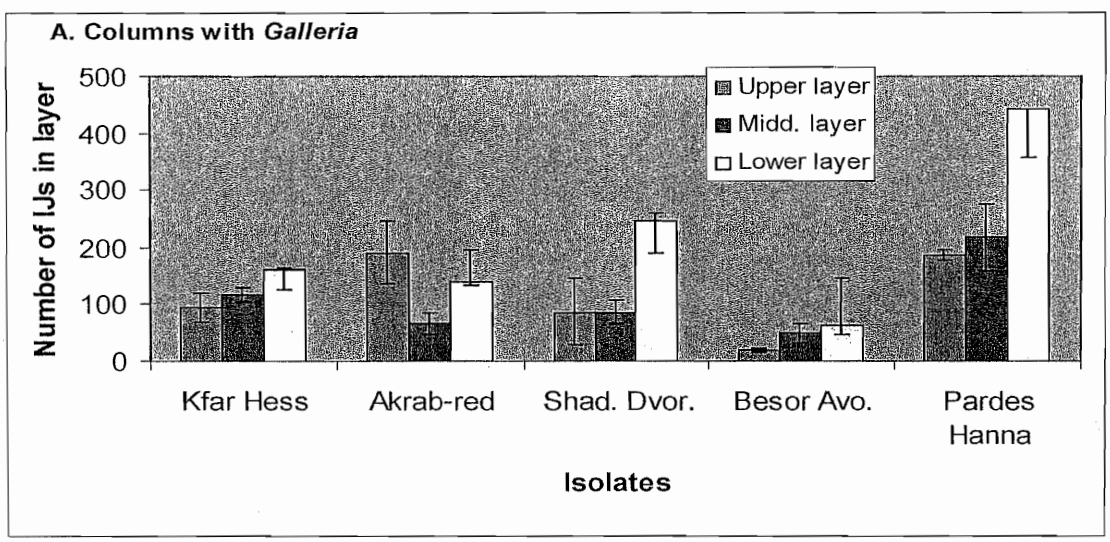
Motility in soil- The ability to move through the soil and locate a target host is an important feature for EPNs as biological control agent. We evaluated their capability to do so in a "sand column assay" (see scheme 1)-

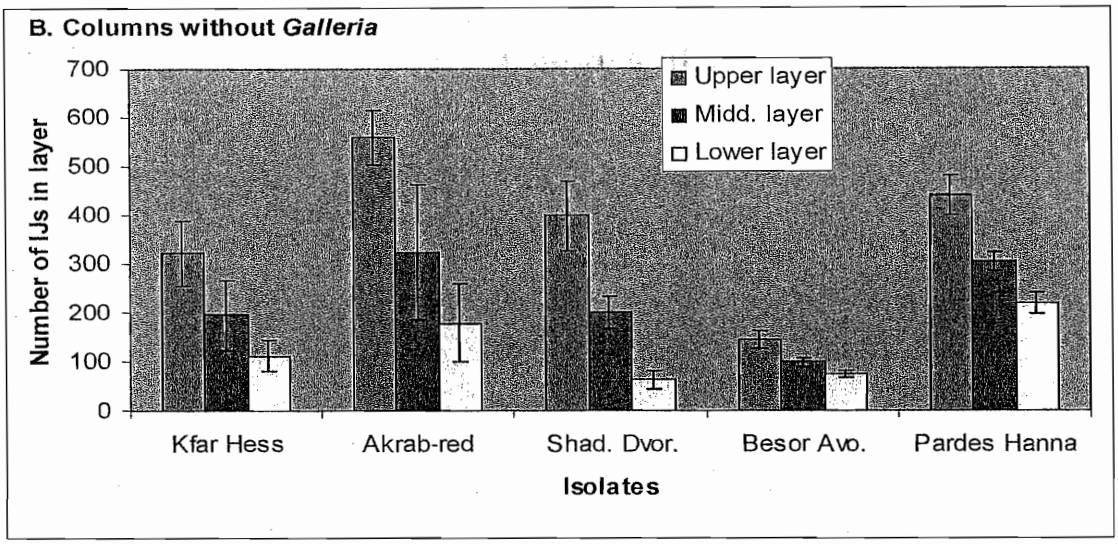
Scheme 1: Sand column



Plastic pipe (5 cm diam and 21 cm long) was filled with pre-oven dried sandy soil (8% moisture w/w). Infective juveniles from applied on top of the column. In half of the column 4 last instars of *G. mellonella* were placed at the bottom of the column. The columns were incubated at 25°C in the dark for 72 h. After the incubation period the soil was pushed out from the column and divided to 3 sections- upper, middle and lower section (see scheme 1). The number of nematodes per section was determine by placing the soil samples on "Bermann funnel" overnight. The results are given the following fig.:

Fig: Distribution of different nematode isolates in sand column following 72 h incubation in the presence (A) of insect (*G. mellonella*) or without an insect (B).





While the presence of insect at the bottom of the columns attracted the nematode, when insect was not available larger proportion remained at the top layer.

During this part of the project vast amount of information was accumulated in regard to the different strains isolated in Israel. In order to compare between the strains we scored each of the different traits that was tested on a 1-10 scale. The evaluation is given in the following table:

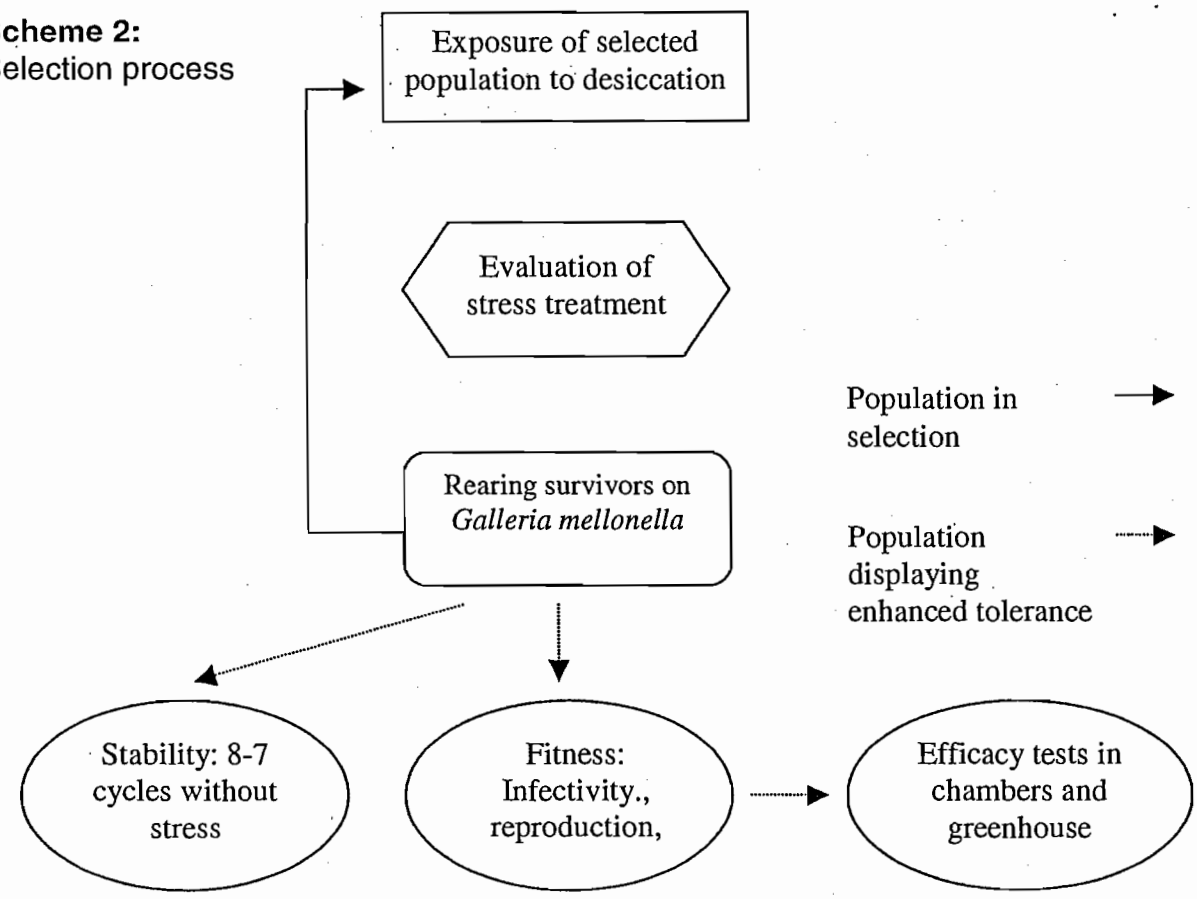
Genera	Strains/ Location	Location Characteristics	Date Found	Desic. Room	Desiccator	Heat tolerance	Motility in Column	Invasion rate	Infec. Tenebrio	Reprod. Potential	Average
Steinernematids	SF G*	Lab reference	--	6	4	4	4	5	6	5	4.3
	Holit	Lemon orchard	11.6.04	4	5	6	4	4	7	2	4.0
	Besor	Avocado orchard	11.6.04	3	6	4	3	6	7	4	4.1
	Susia	Pasture	5.3.04	4	6	5	4	6	9	10	5.5
	Nir Itzhak	Medow	18.3.04	4	5	6	6	5	10	6	5.3
	Zeelim	Mango	18.3.04	4	5	5	5	3	7	5	4.3
	Zeelim	Citrus orchard	9.6.04	3	5	5	4	8	7	5	4.6
	Nahal Akrab	Bushes	24.7.04	6	8	9	5	5	9	7	6.1
Heterorhabditids	HP*	Lab reference	--	2	2	4	4	2	3	5	2.8
	Besor Hb	Avocado orchard	11.2.04	4	4	8	3	4	7	6	4.5
	Devora	Olive trees	5.7.04	7	6	8	5	4	8	7	5.6
	Grofit	Palm trees	20.7.04	3	5	9	5	5	9	4	5.0
	Pardes Hana	Citrus orchard	25.8.04	3	4	6	8	4	4	6	4.4
	Mishmeret	Citrus orchard	25.8.04	3	4	6	6	5	6	6	4.5
	Kfar Hess	Citrus orchard	25.8.04	3	4	6	5	7	7	6	4.8
	Nahal Akrab	Bushes	24.7.04	3	4	9	4	5	10	7	5.3

We used this analysis as basis for selection of population for genetic improvement as the next stage of the research program.

At the beginning of February 2006, Ms. Mariam Chubinishvili., a trainee from Kanchaveli L. Institute of Plant Protection arrived to our laboratory. Her main research program was focused on the genetic selection of beneficial traits in the entomopathogenic nematodes strains isolated previously (during the stay of the first trainee, Dr. Mikaia).

At first we mixed the populations and cultured them together on *Galleria mellonella* larvae. This was done to enhance the genetic variability of the population. In parallel, Miriam defined the selection regime for the following traits rapid desiccation (RD), gradual desiccation (GD) and movement in soil (MS). The conditions at each selection assay are described bellow. Theses assays were used for selection cycles of the nematodes as described in research program (See scheme 2).

Scheme 2:
Selection process



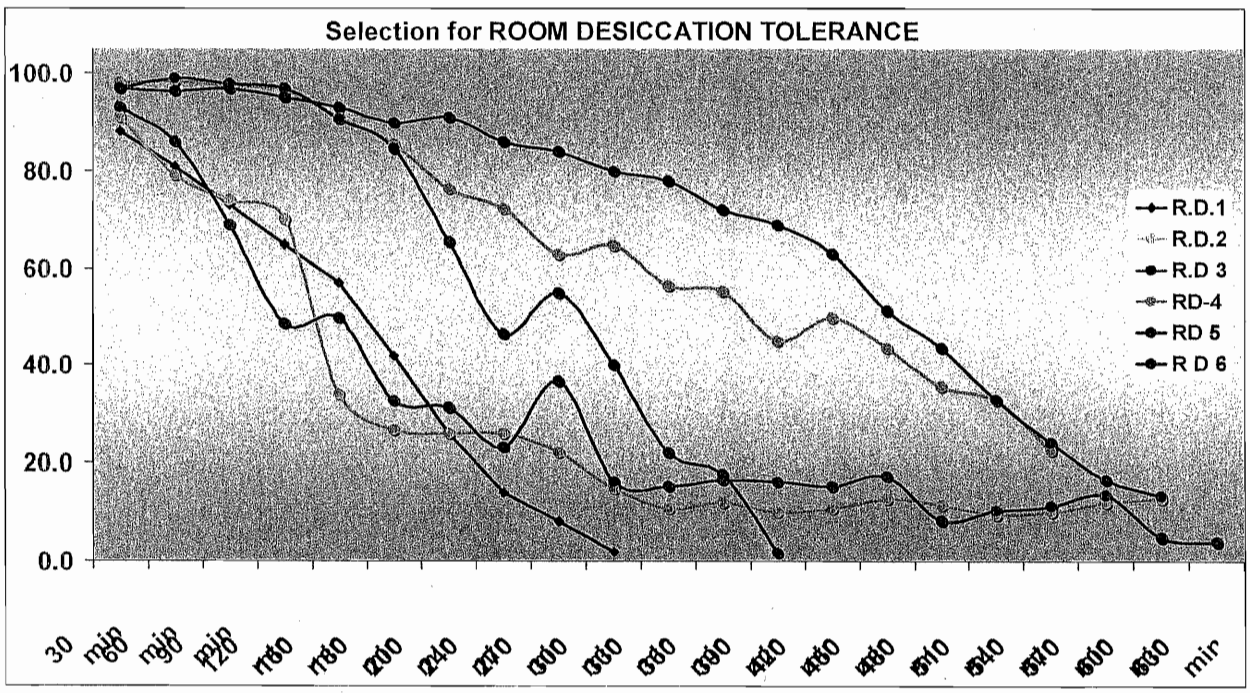
We started the actual selection in mid April 2006 and it took until December 2006 to finalize the experiments (upon the return of Ms. Chubinishvili back to her home land.

The following are the results:

Rapid desiccation- Exposure of the nematodes to room conditions (25°C, 50-70% RH). The LT_{20} (exposure time required to cause 80% mortality, 20% survival) was evaluated. The surviving nematodes were re-cultured on *G. mellonella* larvae. The selection regime simulated conditions of rapid desiccation which occur on plant foliage. The selection of population with high tolerance to rapid desiccation will allow the users (in Georgia) to use the nematodes against foliage pests.

i. The survival was recorded by taking samples at 20 min intervals for a period up to 640 min. The results are given in Fig. 1.

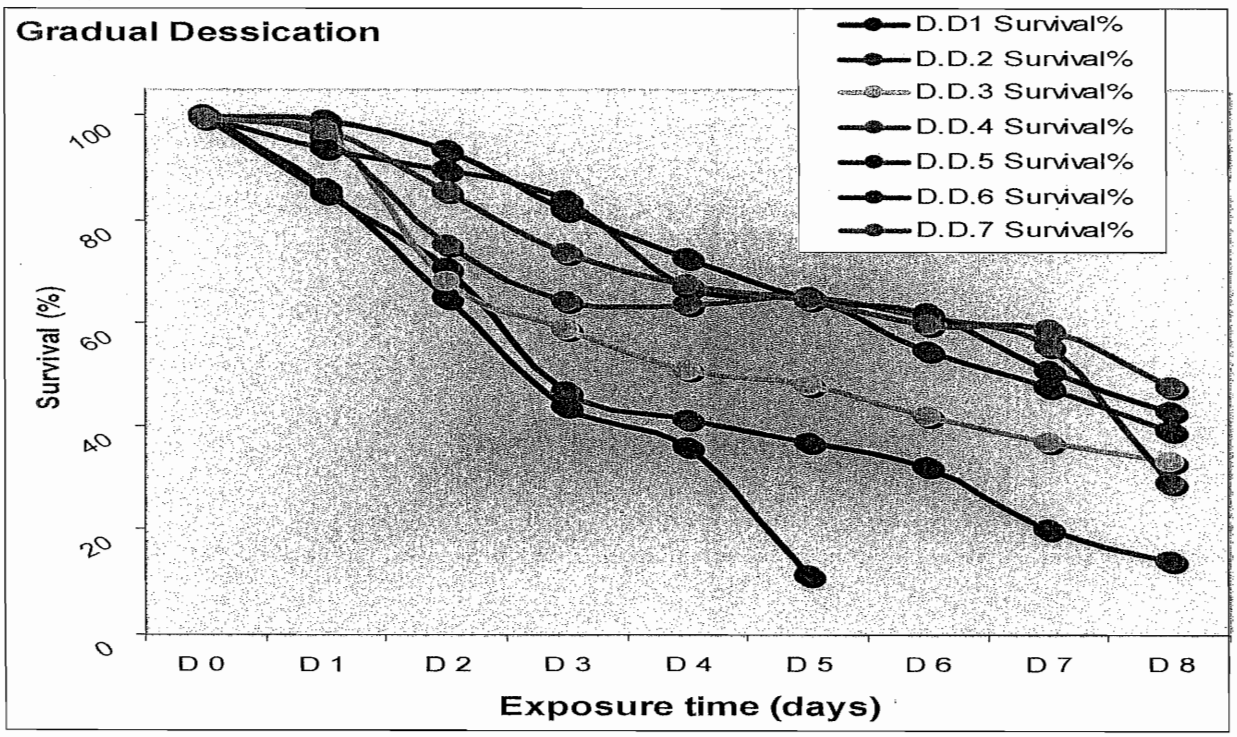
Fig. 1: Survival of selected population during exposure to room condition (50-70% RH).



Over time survival of nematodes increased. Under room conditions the RH is not consisted and varied considerably between one selection round to another. Measurements indicated that the RH varied between 50% to 70%. This variation explains the fact that more advanced genetation survived somewhat less than the previous population (see the difference between R.D.4 and R.D. 5. However an overall increase in survivability at room conditions is apparent.

Gradual desiccation- The nematodes were pre-exposed to 97% RH for 72h (this process induces dormancy state on the nematode, term 'Anhydrobiosis'). Than they were exposed to 85% RH. The LT_{20} value at 85% RH was evaluated. The surviving nematodes were re-cultured on *G. mellonella* larvae. These gradual desiccation conditions simulate the apparent condition in the soil. Increase in gradual desiccation tolerance will increase nematode persistence in the soil.

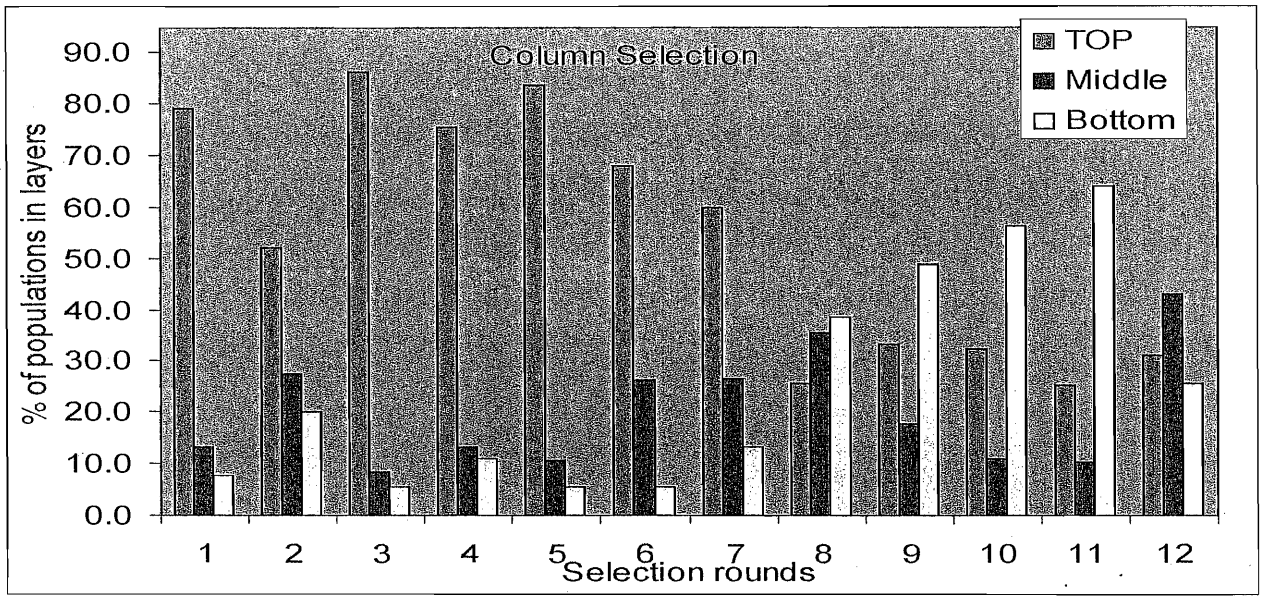
Fig. 2: Survival of selected population at 85% RH following pre-exposure to 97% RH for 72h.



Within 4 selection cycles survival of the IJs was increase substantially. Between the 5th and 7th cycles no significant increase was recorded. That is probably due to the fact that survival was high and no additional selection pressure was applied. To obtain further increase we will exposed the nematode to more severe conditions to cause 10-15% survival within 4-5 days.

Sand columns- According to the procedure described in the 3rd report Sand columns were set up (See scheme 1). Ca 5000 infective juveniles (IJs) were placed on top of each column and 10 *G. mellonella* larvae at the bottom. After 48 h incubation at 25°C the insects were removed from the columns and were incubated. Dead insects (infected by nematodes) were further incubated for nematode culture. After 4 selection cycles where no detection of change was recorded, the number of IJs placed on top of each column was reduced to 2500.

Fig. 2: Proportion (in %) of nematodes found in different layers of columns after 48 h incubation at 25°C.



After the 7th selection cycle substantial increase in the proportion of nematode found at the lower layer of the column suggesting that high proportion of the population was attracted and move faster towards the insects at the bottom.

Conclusions-

a. Genetic selection is applicable for improvement of beneficial traits among population of the entomopathogenic nematode *S. feltiae*.

b. The traits aimed for selection will provide the nematode with higher ability to withstand environmental conditions:

   1) Rapid desiccation tolerance- Will increase nematode ability to withstand the conditions on the plant surface or on the upper soil layers immediately after application.

   2) Gradual desiccation tolerance- Will increase nematode survival in the soil following gradual drying of the soil.

   3) Movement is soil towards target insect- will enhance the efficacy of the nematodes against the insect. It will also increase the survivability of the nematode in the insect cadaver and their reproducibility.

Section II: Project Management and Cooperation

In Georgia

Participation in meetings and publications:

- Dr. Nona Mikaia reported on this project at the XXV International Plant Protection Congress, Beijing, China (13, May, 04). (Mikaia N., Chkhubianishvili C. 2004. Occurrence of entomoparasitic nematode in *Scarabidae* beetles). The visit of Dr. Mikaia in China was reported in Georgian press - Periodical Organization of Tbilisi Javakhishvili I. State University "Sokhumis Sadroso", N 25 (138), and 17 June, 2004.
- Research team participated in the International Conference "Integrated Plant Protection in the beginning of XXI Century", Kiev, 1-5 November, 2004 (Institute of Plant Protection, Ukrainian Academy of Agricultural Sciences) by the publication: C. Chkhubianishvili, M. Kakhadze, I. Malania. 2004. Perspectives of biological plant protection from quarantine pest insects in Georgia. In: Materials of International Scientific-Practical Conference, Kiev, pp.566-570. Report was included in the plenary session of Conference (C. Chkhubianishvili).
- Prof. C. Chkhubainishvili participated at the annual conference of IOBC/WPRS Working Group "Insect Pathogens and Insect Parasite Nematodes", Locorotondo (Bari), Italy, 10-15 June 2005. Dr. Glazer also participated in the meeting. The progress and future pans of the current projects were discussed.
- The poster and abstract titled: "Isolation and characterization of new populations of entomopathogenic nematodes from Israel" – Mikaia N., Glazer I. were submitted to participate at Conference – Biological methods in Integrated Plant Protection and Production, Poznan, Poland, 15-19 May, 2006.
- The poster titled: "Isolation and characterization of new populations of entomopathogenic nematodes from Israel" – Mikaia N., Glazer I., Chkhubianishvili C. was submitted to participate at Summit Workshop COST ACTIONS 850 "Biocontrol Symbiosis", Salzau, Germany, 1-6 June, 2006. The presentation was made by Dr. I. Glazer.

Publications:

1. Chkhubianishvili C., Mikaia N., Kakhadze M. 2006. Towards relationship between the greenhouse whitefly and entomopathogenic nematode. "Science and Technologies", Monthly Scientific-Reviewed Magazine of Georgian Academy of Sciences, N 1-3, Tbilisi, pp. 127-129.

2. Chkhubianishvili C., Mikaia N., Kakhadze M. 2006. Effectivity of joint action of entomoparasite nematode and mycopesticide to the greenhouse whitefly. Proceeding of Georgian Academy of Agricultural Sciences, Tbilisi, and N 16, pp. 78-81.

3. Chkhubianishvili C., Kakhadze M., Mikaia N., Skhirtladze R. 2006. The complex protection of vegetable cultures in closed environment. International Scientific-Practical Conference "Technology of creation of the biological means of plant protection on the base entomophages, entomopathogens, microbs-antagonists and their use in outdoor and closed environments", Krasnodar, Russia.

4. Mikaia N. 2005. Towards the tolerance on some entomopathogenis nematodes. Proceedings, Proceedings Institute of Zoology, Georgian Academy of Sciences (in press).

5. Chkhubianishvili C., Kakhadze M., Mikaia N., Skhirtladze R. 2006. The complex protection of vegetable crops in closed environment. In: "Biological Plant Protection – base of agroecosystem' stabilization", Krasnodar, issue 4, pp. 301-302.

6. Chkhubianishvili C., Glazer I., Mikaia N., Kakhadze M. 2005. Perspectives insect parasite nematodes research development in Georgia. IOBC/WPRS Bulletin, Bulletin OILB/SROP, Italy (in press).

7. Tabatadze E., Mikaia N., Chkhubianishvili C. 2006. Effectiveness of Steinernema *feltiae* and *Heterorabtidis bacteriophora* to Coccidae. Proceedings of Academy of Agricultural Sciences of Georgia, Tbilisi, N 17, pp. 55-57.

8. Chkhubianishvili C., Mikaia N., Malania I., Kakhadze M. 2006 Susceptibility of entomopathogenic nematodes to the fall webworm *Hyphantria cunea* Drury (*Lepidoptera: Arctiidae).* Bull. Georgian Academy of Sciences, Tbilisi.

9. Chkhubianishvili C., Malania I., Kakhadze M., Mikaia N. Development of the joint action of Entomopathogenic nematodes with the microbial means to the fall webworm. "Science and Technologies", Monthly scientific-reviewed magazine of Georgian Academy of Sciences, Tbilisi.

10. Chubinishvili M., Salame L, Chkhubianishvili C., Glazer I. 2007. Genetic Improvement of beneficial traits mixed population of *Steinernema feltiae* for enhancement of persistence and efficacy. In: 11th European Meeting IOBC/WPRS working group "Insect pathogens and Insect parasitic Nematodes" Ales (Gard), France, June, p. 36.

11. Chkhubianishvili C., Malania I., Kakhadze M., Mikaia N. 2007. Study of joint action of microbiological means and entomopathogenic nematode to the fall webworm. "Metsniereba da Technologiebi" ("Science and Technologies") Monthly Scientific-Reviewed Magazine of Georgian Academy of Sciences, N 1-3, pp. 81-83.

12. Chkhubianishvili C., Mikaia N., Malania I., Kakhadze M. 2007. Susceptibility of entomopathogenic nematodes to the fall webworm *Hypanthia cuneal* Drury (*Lepidoptera: Arctiidae).* Georgian National Academy of Sciences, Bulletin, v. 175, N 2, pp. 112-114.

13. Mikaia N., Glazer I. 2007. Isolation and Characterization of New Populations of Entomopathogenic nematodes from Israel. In: IOBC EPS, Information Bulletin N 36, Biological Methods in Integrated Plant Growing and Plant Protection, Poznan-Pushkino, pp. 134-135

14. Mikaia N., Salame L., Chkhubianishvili C, Glazer I. 2007. Isolation and Characterization of New Populations of Entomopathogenic nematodes from Israel. IOBC, In: 11th European Meeting IOBC/WPRS working group "Insect pathogens and Insect parasitic Nematodes" Ales (Gard), France, June, p.90

15. Chkhubianishvili C., Glazer I., Mikaia N., Kakhadze M. 2005. Perspectives insect parasite nematodes research development in Georgia. IOBC/WPRS Bulletin, Bulletin OILB/SROP, Italy (in press).

16. Mikaia N., Kakhadze M. 2007. Results of study the action of entomopathogenic nematode to the Colorado potato beetle. Bulletin of Academy of Agriculture Sciences of Georgia. (in press).

17. Chkhubianishvili C., Chubinishvili M., Glazer I. 2007. Genetic improvement of beneficial traits for enhancement of entomopathogenic Nematodes effectiveness. Bulletin of Academy of Agricultural Sciences of Georgia (in press).

Final conclusions:

The present project was aimed to introduce a new approach for control of agricultural pests in Georgia. Further was indented to enhance the research and development capabilities of the Georgian scientists. The results presented here indicate that it fulfilled its purposes in full. The Georgian partners established an active laboratory (with a great support for purchases of equipment by CAR) dealing with entomopathogenic nematodes (EPN). In addition the trainees (Nona and Miriam) obtained in Israel substantial experience in relevant techniques and research approaches to develop the research program in Georgia. Furthermore, the results obtained here indicate that EPN has potential use against key pests in Georgia. Finally we strengthened the relations of the two laboratories (Georgia and Israel) and hope to collaborate in the future.