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# Basis for developing biotechnology for plant protection means in Georgia

**Chkhubianishvili, Tsisia [*], Manana Kakhadze, Iatamze Malania, Mariam Chubinishvili, Rusudan Skhirtladze, Irine Rizhamadze**

Kanchaveli Institute of Plant Protection, Agricultural University of Georgia
David Aghmashenebeli Alley, 240 Tbilisi, 0159 Georgia

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Pest organisms cause the great damage to agriculture in Georgia. It becomes necessary to carry out the control measures against them. The plant protection from pest organisms requires the development of integrated pest management (IPM) system, where the biological agents will take the important place for human and environment.The greenhouse whitefly (GWF), *Trialeurodes vaporariorum* polyphagous insect is the most significant pest of agricultural crops in open and closed holdings. The specialized parasitoid of GWF – *Encarsia*, *Encarsia formosa* develops inside the body of whitefly larva. The local population of parasitoid has revealed (Tbilisi, Botanical gardens) and has used in experiments. The *Encarsia* biological effectiveness (BE) on vegetable crops (tomato, cucumber) and ornamental plantings at winter-spring period is 85-90% and 52-60% to the fall period. The results of study on the joint action of_entomopathogenic nematode (EPN) – *Steinernema feltiae* (introduced from Israel) and parasitoid *E. formosa* to the GWF have been established. The mass production of *Encarsia* in laboratory has elaborated. Pest insects – *Coccids* are widely distributed in Georgia. They damage the agricultural crops and ornamental plants. The relationship of EPN, *S. feltiae* to *Coccids* has studied and there is the prospect *S.feltia* using to control the *Coccids* in closed and open ground farms. *S.feltia* was tested also to main pest of vine the grape berry moth, *Lobesia botrana.* There is the preliminary data on biotechnology of nematode formulation on the base of local EPN, *S.feltia* "Georgian strain", tentatively called "*Geo-nema*". The accumulation of biomass by growing of EPN *in vivo* on laboratory cultures of *Galleria mellanolla* and *Tenebrio molitor* has elaborated. Bioformulation will be economically accessible to local manufacturers of environmentally friendly product. At present the fall webworm (FWW), *Hyphantria cunea* is a very dangerous quarantine pest insect, widely spread in urban holdings, where the use of chemical pesticides are prohibited. The formulations on base of entomopathogenic organisms (fungi, bacteria, viruses, EPNs) have examined. The possibility of using sex pheromones (Russia) for complex control to FWW at first time for Georgian conditions has established. The possibility of using entomopathogenic fungi to the very important pest insect for vegetable and technical cultures - the Colorado potato beetle, *Leptinotarsa decemlineata* has elaborated. The action of strain, *Beauveria bassiana* $LRC_{107}$ (introduced from Canada), enriched $KNO_3$ (Joint project, Durmishidze Institute of

[*] Corresponding author: e-mail: cisia@mymail.ge

Biochemistry and Biotechnology) to the different instars pest larvae and imagoes in natural conditions has studied. The average BE was achieved to 70-80%. The sunflower broomrape, *Orobanche cumana*, root-parasitic weed plant is the major pest of sunflower crops in Georgia. At present the investigations are carrying out on search the local pathogenic fungi at the *O. Cumana* populations. The high virulence isolate from genus *Fusarium* strain will serve as the base for mycopesticides production. The above mentioned biological agents are considered as basis for development the formulations, which offer a desired integrated pest management (IPM) - compatible alternative to broad-spectrum unselective chemical insecticides. The non-toxic and environmentally safe means gives possibility obtaining the ecologically pure production, which is a very important social problem.

**Key words:** biotechnology, insect, biocontrol

## Introduction

Georgia, a relatively small mountainous country in the Caucasus, has a variety of landscape zones and is rich by flora and fauna. It has a well-developed forestry, horticulture, viticulture, vegetable gardening, citrus, tea growing industry and subtropical crops. The great damage to economy of Georgia cause the pest organisms (insects, rodents, weeds, fungi, bacteria, viruses etc.) distributed on the different plants. The large-scale application of various chemical pesticides resulted in their accumulation in soils, water, plants and eventually in food. The needs for environment protection on the global level initiated the development of natural, biological means for pest control. The plant protection from pest organisms requires the development of integrated pest management (IPM) system, where the biological agents will take the important place for men and environment. The basic elaboration biotechnology of environmentally safe means plants protection is the presence of biological agents number regulation pest organisms.

The initial efforts in this area were directed with searching the local "natural resources" - entomophages and entomopathogens (fungi, virus, nematodes) in pest populations, testing the local, introduced strains and commercial formulations.

Currently, entomopathogenic nematodes (EPNs) are considered as the most promising means among the biological control agents for pest organisms number regulation and therefore predominate data prevail regard to them.

Materials and methods

The investigations were conducted in different agrocenosis – vegetable cultures (closed and open grounds), vineyards, potato sowings, ornamental plants, sunflower crops. The collected pathological materials were treated in laboratory.

The study of nematode pathology was started mainly on introduced (Israel, Germany) pathogenic nematodes, *Steinernema feltia*e (under the International projects).

The natural habitat of EPNs is a soil and the soil samples were collected in several agrocenosis (vineyards, orchards, forest, vegetable gardens, pasture fields etc.) of different regions in Georgia.

The analyzing of different pathological material was conducted by using of light, invert and stereoscopic microscopes.

The new methods of EPNs investigations have been developed at ARO, Israel under the Project CDR #TA- MOU-02-CA02-007 with supervision of Prof. I. Glazer) and the University of California Davis, USA (under the Project CRDF/GRDF/GNSF # NSS-05-07 with guidance of Dr. E Lewis). The bioassays were started with drowning up a list of investigated plots and sampling methods have done by appropriated methods in insect nematology (Kaya, Stock, 1997; Stock, Goodrich-Blair, 2012). The samples were taken from 7 different regions of Georgia: Kakheti, Qartli, Imereti, Guria, Samegrelo, Svaneti, Racha-Lechkhumi. Two main ways of nematode isolation were used: a) insect baiting method and b) nematode direct migration in water.

The mortality of tested individuals was corrected by control mortality index using Abbott's formula (Abbott, 1925).

The soil samples have been studied at laboratory by using of sieving methods for nematode direct migration in water and baiting methods for hatching the infective juveniles (IJs) with last instars larvae of wax moth, *Galleria mellonella* and the meal worm, *Tenebrio molitor.*

Determination of invasive ability of isolated nematodes has been carried out according to generally accepted methods. The infectivity of isolates on the laboratory insects' culture - the greater wax moth, *G.mellonella* and the meal worm, *T. molitor* has been approved. Infected material was divided by the pathological patterns and mass produced *in vivo*. The local EPNs bank has been refreshed and renewed by *in vivo* production on the laboratory test insects.

*Beauveria bassiana strain* $LRC_{107}$ was introduced from Canada (under the Project GNSF-STCU # 5011).

*Ketomium* mycofungicide formulation was submitted by Prof. Kasem Soytong (Thailand).

The phytopathogenic fungus, *Fusarium oxysporum* var.*orthoceras* was introduced from Israel (under the Project CDR TA-MOU-o2-CA22-016).

## Results

The greenhouse whitefly (GWF), *Trialeurodes vaporariorum* polyphagous insect is the most significant pest of agricultural crops in open and closed holdings. The specialized parasitoid of GWF – *Encarsia, Encarsia formosa* develops inside the body of whitefly larvae. The local population of parasitoid has revealed (Tbilisi, Botanical gardens) therefore has used in experiments. The *Encarsia* biological effectiveness (BE) on vegetable crops (tomato, cucumber) and ornamental plantings at winter-spring period is 85-90% and 52-60% to the fall period. The results of study on the joint action of entomopathogenic nematode (EPN) – *Steinernema feltiae* (introduced from Israel) and parasitoid *E. formosa* to the GWF have been established. The mass production of *Encarsia* in laboratory has elaborated (Skhirtladze, Rijamadze, 2014).

Pest insects – Coccids (*Hemiptera: Coccidae*) are widely distributed in Georgia. They damage the agricultural crops and ornamental plants. The relationship between EPN, *S. feltiae* (introduced from Israel) and Coccids: the soft scale - *Coccus hesperidum*, the vine scale - *Neophulvinaria innumerabilis* and the fern scale - *Pinnaspis aspidistrae* has been studied. The invasive coccids mortality after 12 hr was: *C. hesperidum* - 10%, *N.innumerabilis* - 40%, *P. aspidistrae* - 5%. The pathological materials microscopic investigations show the presence of IJs inside the cadaver of each coccids species. As the results of experiments there is the perspective for biological agent *S.feltia* using to control the coccids in closed and open ground farms (Mikaia, Skhirtladze, Rijamadze, 2012; Chubinishvili, Skhirtladze, Rijamadze, 2013).

The grape berry moth, *Lobesia botrana* Den. et Schiff *(Lepidoptera: Tortricidae)* is a very important pest of viticulture regions in Eastern Georgia. The pest damages the grape especially during the ripe phase when the larvae of third generation appear in mass. No chemical pesticides are recommended for vineyard treatment at this phase. At that time it is advisable the usage of biological means such as EPN. Generally *S.feltiae* is considered as the safe for men and environment biological agent for pest control. The typical situation of invasion causing by parasitic nematode has been recovered in of the grape berry moth organism. The invasive ability of new nematode generation, isolated from insect has been established in laboratory. The means on possibility of nematode suspensions insertion in the integrated pest management (IPM) of vineyards from pest organisms have been marked (Kakhadze at all., 2012).

The possibility of using entomopathogenic fungi to the very important pest insect for vegetable and technical cultures - the Colorado potato beetle, *Leptinotarsa decemlineata* has elaborated. The action of strain,

*Beauveria bassiana* $LRC_{107}$ (introduced from Canada), enriched $KNO_3$ (Joint project, Durmishidze Institute of Biochemistry and Biotechnology) to the different instars pest larvae and imagoes in natural conditions has studied. The average BE was achieved to 70-80% (Chkhubianisvili at all., 2010; 2011).

At present the fall webworm (FWW), *Hyphantria cunea* is a very dangerous quarantine pest insect, widely spread in urban holdings, where the use of chemical pesticides are prohibited. The formulations on base of entomopathogenic organisms (fungi, bacteria, viruses, EPNs) have examined. The possibility of using sex pheromones (Russia) for complex control to FWW at first time for Georgian conditions has established (Kakhadze, Malania, Leonidze, 2010; Chkhubianishvili at all., 2010; 2013; Chkhubianishvili, Malania, Kakhadze, 2013).

The susceptibility of EPN, *S.feltia* to the mulberry moth, *Glyphodes pyloalis* Walker) (*Lepidoptera: Pyralidae*) has established (Mikaia at all, 2010; Chkhubianishvili at all., 2011). *G. pyloalis* is considered as an urban pest damaging the mulberry plantings leaves and therefore the application of environmentally safe means is advisable.

The data on possibility of reproduction of EPN, *S feltia* on cotton aphid larvae, *Aphis gossypii* have presented (Mikaia, 2011).

As the EPNs are considered as potential biological control agents to various pest insects the investigations were carried out for searching and isolation of local EPN strains.

As the results of multiple researches the new model of nematode direct migration has been elaborated, which gives possibility to obtain more infective juveniles (IJs) from soil during short period. The experiments were continued on establishment of new isolates invasive ability. 100 IJs of strain-I, 100-150 IJs of strain-II, and 100-120 IJs of strain-III were used for contamination of 10 *G. mellonella* average size larvae. The last instars larvae of 10 *T. molitor* were infected by 150 IJs of all experimental strains. A typical pattern of nematode pathology has been obtained and IJs were used to the test insects in next trials of bioassays. The comparative virulence has been determined between strain-I, strain-II and strain-III. The preliminary results show perceptivity of new approach isolation nematodes for searching of EPN local strains, which are produced *in vivo* for identification.

The new strain of *Steinernema feltiae* was conventionally called - "Georgian strain". The several bioassays show, when the commercial strain invasive index reached to 84% - at that case Georgian strain's invasive ability significantly increased to 95%.

There is the preliminary data on biotechnology of nematode formulation on the base of local EPN, *S.feltia* "Georgian strain". The accumulation of

biomass by growing of EPN *in vivo* on laboratory cultures of *G. mellanolla* and *T. molitor* has elaborated. Bioformulation will be economically accessible to local manufacturers of environmentally friendly product.

The new methods of nematode direct extraction from soil, we used at laboratory, were successful that has enabled us to reduce labor work and simultaneously study multiple samples in a relatively short time interval, obtain more IJs, than it was possible by previously existing methods. The new model was tested successfully at laboratory bioassays and it is planned to be used for future investigations.

The experiments were continued on establishment of new isolates invasive ability. 1000 IJs of strain-I, 1000-1500 IJs of strain-II, and 1000-1200 IJs of strain-III were used for contamination of 10 *G. mellonella* average size larvae. The last instars larvae of 10 *T. molitor* were infected by 1500 IJs of all experimental strains. A typical pattern of nematode pathology has been obtained and IJs were used to the test insects in next trials of bioassays. The comparative virulence has been determined between strain-I, strain-II and strain-III. The preliminary results show perceptivity of new approach isolation nematodes for searching of EPN local strains, which are produced *in vivo* for identification (Chubinishvil at all., 2013; 2014).

The results of first attempt of *Ketomium* mycofungicide tests to control the causative agents fungi diseases of agriculture crops – tomato, eggplant, pepper, table beet, sunflower have been obtained (Kuprasvili at all., 2007).

The opportunity to use of introduced (Israel) phytopathogenic fungus, *Fusarium oxysporum* var.*orthoceras* for biological control with the sunflower broomrape, *Orobanche cumana* parasiting on sunflower has been studied. It was found out that action of fungus has not caused negative influence on sunflower, but there was a full dying of plants broomrape on a surface of ground (Malania at all., 2008).

## Conclusion

Biological means are not produced in Georgia and the import from foreign countries is very expensive. Therefore it is necessary to carry out self-production of biological means, as the potential possibilities have been already created in Georgia.

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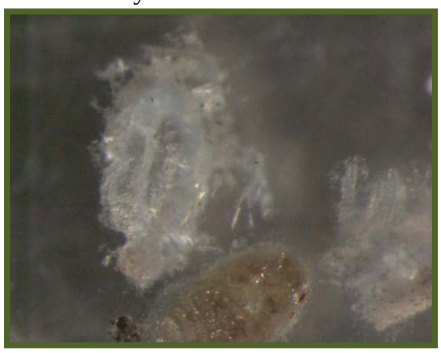
Rearing of *Encarsia* on tobacco plant of pot culture in laboratory

Development of *Encarsia* in greenhouse whitefly on tomato leaves



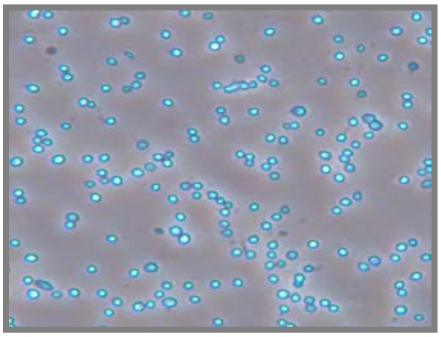
Action of *S. feltiae* on fern scale larvae

*S.feltiae* isolated from the body of scale

The invasive larvae of *L.botrana*

The isolated *S. feltiae* from *L.botrana*

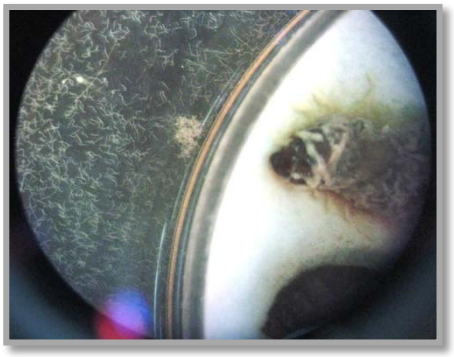
The Colorado potato beetle imagos with characteristic fungi pathology symptom



Spores of *Beauveria bassiana*

The fallen webworm infected with nuclear polyhedrosis virus

Natural invasion of the tested insects with nematodes



Migration of nematodes from tested insect

The action of *Fuzarium oxysporum* subsp. *Orthoceras* on the haustories of parasite plant, the sunflower broomrape, *Orobnche cumana*