International Conference on Research in Education & Science

ICRES2016

May 19-22, 2016 Bodrum/TURKEY

PROCEEDING BOOK

Editors

Wenxia WU
Mustafa Tevfik HEBEBCI
O. Tayfur OZTURK

ISRES Publishing

http://www.icres.net

USE OF ENVIRONMENTAL FRIENDLY ENTOMOPATHOGENIC NEMATODES FOR BIOLOGICAL CONTROL OF PEST INSECTS

Manana LORTKIPANIDZE,
lordkipanidze@dsl.geo

Oleg GORGADZE,
gorgadze@gmail.com

Nino GABROSHVILI,
gabroshvilin@yahoo.com

Madona KUCHAVA

ABSTRACT:Bioprotection is a holistic concept of direct relevance to the sustainability of agriculture, food safety, and the protection of the environment, including biodiversity. Bioprotection has become a major economic issue of concern to governments, agricultural industries and environmental organizations worldwide. It is known that harmful insects and agricultural plant diseases cause the fall of the crop capacity to ¼ on average. Phytophagous insects occupy a significant position among the living organisms that cause much damage to cultivated plants and forest species. There is an urgent need to accelerate the development and implementation of cost-effective, environmentally safe alternatives to chemical pesticides for insect control.

The study of insect diseases has enabled the scientists to state that, besides entomophages, there are other disease – causing organisms, such as bacteria, fungi and entomopathogenic nematodes EPNs, which have drastic effect on harmful insects. EPNs parasitize in plenty of organisms, insects among them. Insect-pathogenic nematodes of the family *Heterorhabditidae* and *Steinernematidae* have been known for decades as effective biological agents of insect pests. These nematodes can actively locate, infect and kill a wide range of insect species. Only the third-stage infective juvenile (IJs) can survive outside the insect host and move from one insects to another. Insect mortality, due to nematode infection, is caused by a symbiotic bacterium.Heterorhabditid nematodes have a symbiotic association with *Photorabdus* bacteria whereas Steinernematids are associated with.*Xenorhabdus*. After gaining access to the host haemocoel, the bacteria multiply, killing the host within 24-48 h, and convert the insect into a suitable environment for development and reproduction of the nematodes' parasitic stages.

Mass produced of EPNs can be carried out via their cultivation in specially processed and generally accepted feeding media. We suggest more effective and economic *in vivo* media for nematode cultivation.

Key words:bioprotection, entomopathogenic nematodes,bacteria, biological agents

INTRODUCTION

Insects have many types of natural enemies. As with other organisms, insects can become infected with disease-causing organisms, called pathogens. Soil serves as a natural home and reservoir for many kinds of insect pathogens, including viruses, bacteria, protozoa, fungi, and nematodes. We can take advantage of these natural enemies of insects to help manage insect pests. The use of natural enemies to manage pests is called biological control[1].

Biological control is the beneficial action of parasites, pathogens, and predators in managing pests and their damage. Biocontrol provided by these living organisms, collectively called "natural enemies" is especially important for reducing the numbers of pest insects [2].

In numerous studies, nematode communities showed possibilities to be good indicators of different kind of disturbances in ecosystems. Some groups of nematodes can survive under disturbed environmental conditions such as global climate change which also in the last decades influenced the water regimes of soil, which is crucial for a nematode survival [3,4].We suggest effective and economic *in vivo* mediasuchas -*Galleria mellonella, Tenebriomolitor*and *Bombyxmori*for cultivation ofnematodes.

METHODS

In the *in vivo* process, an insect serves as a bioreactor. *Galleria mellonella* larvae are most commonly used to rear nematode because of their commercial availability[5].Using *in vivo* process, yields between 0.5 x 10 and 4x102 IJ /larva depending on the nematode species, have been obtained. Currently in laboratory of Institute of Zoology we are develop technology mass-production ofentomopathogenic nematodes on local inexpensive substrate-*Bombyxmori* larvae and pupae. The number of nematodes in each*B. mori*larva and pupa reaches 400-450specimens[6]. When large quantities of nematodes are required, we will be cultured on an artificial medium (*in –vitro*) according to the method described by Woodring& Kaya[7]. In this method, nematodes are cultured on a crumbed polyether polyurethane sponge impregnated with emulsified beef fat and pig's kidneys, along with symbiotic bacteria. Using this method, approx 6 x105- 10x105 IJ / g of medium were achieved [8].

Mass-produced of EPNs by usage Tenebriomolitor larva. The larvae of T. molitor were placed on the Petri dish, on which there was the moisture filter paper. The Petri dishes were placed into a cuvette (80□80 cm), which was half filled with distilled water. In 10-12 days the Infective juvenile (IJ) appeared on the Petri dish and then they were found on the cuvette from which the nematode larvae with water were placed in the flasks. Then they were kept in the refrigerator at temperature 50C. Every day the suspension with nematodes was poured out of the cuvette and some distilled water was added to it. This procedure was repeated until the nematode emergence was completed. The appearance of nematodes from the larval cadaver continued during 10-12 days.
The same method was applied during nematode reproduction onthe *G.mellonella.*

Mass-produced ofEPNsby usage*B.mori*larvaeand pupae.Materials dealing with different stages of development of *B.mori* were obtained at the Institute of Breeding Silkworm at the end of May.

Version I. A middle-sized cuvette (80×80 cm) is covered with a filtered paper, on which the nematode suspension is spread in such a way that the filtered paper becomes completely saturated with it. Then live *B. mori*(L-3) previouslykept without food for two days are placed tightly side-by-side on the filtered paper. Then they are sprayed again with nematode suspension. The concentration of the suspension was equal to 250-300 IJs/ml water and is covered with another cuvette. In this position all larvae of *B. mori*died during in 24 h.

Version II. The only distinction between the first and the second version is that when the worms are placed on the saturated filtered paper, they are covered with fresh mulberry leaves and sprayed once more. Such invasion can be called 'double invasion', as in contrast to the first, leaves become invaded not only through anus and cuticle but also through mouth. In this case 100% of larvaedie in 12-14 hours.

Further work with invaded larvae goes on in accordance with the unified method: dead worms are situated on a Petri dish which is turned upside down and is laid by filtered paper. The worms are set carefully side-by-side so that they shouldn't cover one another. About 14 Petri dishes are placed on each cuvette. 1-1.15 liter of distilled water is poured into the bottom of the cuvette. The cuvette is covered with a transparent sheet of plastic film. Starting from the 11th day samples are taken from the bottom of the cuvette where the nematodes get from the Petri dish. Owing to accumulation of decayed substances in dead worm bodies, nematodes, apparently, leave their hosts (negative chemotaxis) and get into the water at the bottom of the cuvette. In this experiment 12 cuvettes were used and numbered. Every day, starting from the 11th day for two weeks, accumulation of nematode suspension was examined and checked up under the microscope. At the end of the experiment there was a mass of nematodes observed. They were moving actively in the liquid. Then Petri dishes were removed from the cuvette. The suspension was poured into the flasks and was kept in the refrigerator at a temperature $+4^0$ - $+5^0$C. The same method was applied during nematode cultivation in the *B. mori* pupae that were separated from their cocoons. The version with mulberry leaves was naturally excluded. All experiments were carried out at room temperature 22^0-24^0C.

RESULTS AND FINDINGS

After the cultivation of nematodes on the *T. molitor* larvae, have obtained the unequal number of IJs from each larva. The largest number of nematodes was for *S.carpocapsae* 120. 000 IJs, while for *H. bacteriophira* and *S. feltiae* average number was 90.000IJs. Approximately 150.000 IJs of *S.carpocapsae* and *H. bacteriophira* were accepted from pupae of *T. molitor* – whereas IJs of *S. feltiae* were lower- average 100.000(Fig. 1).

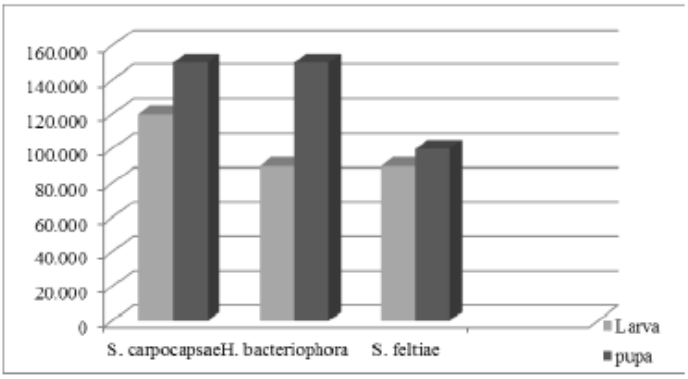

Fig.1. Number Of Infectivejuveniles After Cultivation On Tenebriomolitor Larvaandpupa.

During cultivation of nematodes on the *G. mellonella*, have obtained approximately 200.000 - for *S.carpocapsae*, 150.000 for *H. bacteriophira* and 100-110.000 for *S. feltiae*. The nematode emergence increased (15%) in case have been added hemolymphs and fat of insects larvae (Fig.2).

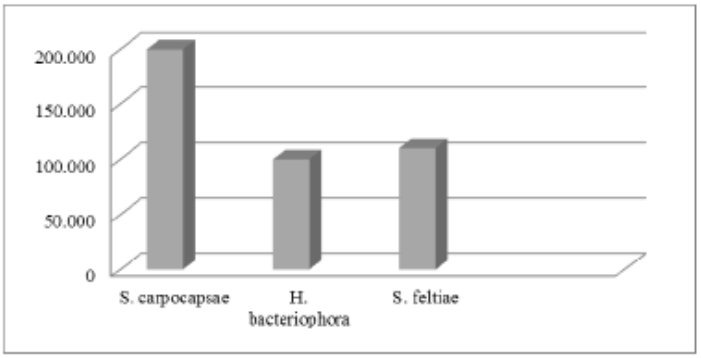

Fig.2. Number Of *Infective Juveniles*after Cultivation On *Galleria Mellonella* Larva

The number of nematodes in each *B. mori*larva and pupa reached 300-400per larva and pupa -the largest number of IJs were obtained for*S.carpocapsae* and *H.bacteriophira* - 400.000, but for *S. feltiae* averaged 340-350.000 IJs(Fig.3).

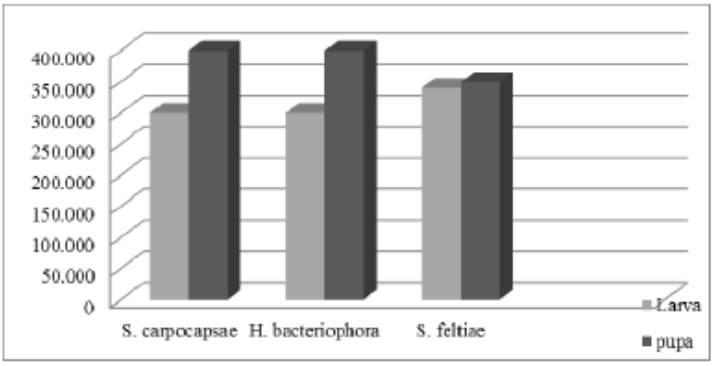

Fig.3. Number Of *Infective Juveniles*after Cultivation On *Bombyxmori* Larva And Pupa

CONCLUSION

The effect of the nematode suspension obtained in the laboratory has been approved in Georgia as means of pest control of 17 species of agricultural crops and forest plants: e.g. the *Leptinotarsadecemlineata, Pierisbrassicae, Agrioteslineatus, Hyphanthriacunea, Ocneriadispar,* etc. It has been estimated that insect death rate caused by spraying was 68-89%.

EPNs formulation development and implementation in agriculture will improve the quality of production of ecological pure products and will enhance income stability. According to preliminary data -based on performed experiments in the Laboratory, on small plots and in field conditions the loss of harvest will be decreased as a result of nematodes application.

A novelty, which needs further research, is the usage combination of EPN and other biological agents against various insect species and studding their effect.Such approaches will help in the development of an efficient biocontrol strategy and determination of their place in integrated pest management (IPM).

RECOMMENDATIONS

Microbial control agents can be effective and serve as alternatives to broad-spectrum chemical insecticides. However, their increased utilization will require (1) increased pathogen virulence and speed of kill; (2) improved pathogen performance under challenging environmental conditions (cool weather, dry conditions, etc.); (3) greater efficiency in their production; (4) improvements in formulation that enable ease of application, increased environmental persistence, and longer shelf life; (5) better understanding of how they will fit into integrated systems and their interaction with the environment and other integrated pest management (IPM) components; (6) greater appreciation of their environmental advantages; and (7) acceptance by growers and the general public. We envision a broader appreciation for the attributes of entomopathogens in the near to distant future and expect to see synergistic combinations of microbial control agents. Microbial control agents that have excellent potential for use in IPM programs.

REFERENCES

Barberchek M. (2005). Insect-Parasitic Nematodes For The Management of Soil Dwelling Insects, College of Agricultural Sciences Cooperative Extension Entomological Notes Department of Entomology. USA. http://ento.psu.edu/extension/factsheets/pdf/nematodes.pdf

Dreistadt, S. H., J. K. Clark, and M. L. Flint.(2004). *Pests of Landscape Trees and Shrubs: An Integrated Pest Management Guide, 2nd ed*. Oakland: Univ. Calif. Agric. Nat. Res. Publ. 3359.

Mikulec, V.-Stehlová, K.: (2006). Application of the climate change scenarios on selected meteorological chaacteristics for the purposes of water content course prognosis in time horizons 2010, 2030 and 2075 - Cereal Research Communications, Vol. 34 No. 45-49.

Vágó, K. - Dobó, E.- Kumar Singh, M.: (2006). Predicting the biogeochemical phenomenon of drought and climate variability. - Cereal Research communications, Vol. 34 No. 1. 93-97.

Dutky S.R., Thompson J.V. and Cantwell G.E. (1964).A technique for the mass propagation of the DD-136 nematode. J. Insect Pathol., v.6, p. 417-422.

Kakulia G., Lortkipanidze M., Chkhubianishvili C., Mikaia N. (2001). Mass rearing of entomopathogenic nematodes on *Bombyx mori*.8th European Meeting of the IOBC/WPRS Working Group "Insect Pathogens and Insect Parasitic Nematodes: Current Research and Perspectives in Pest Biocontrol", Abstracts, Athens, Greece, 40.

Woodring, J. L. and Kaya, H. K. (1998). Steinernematid and Heterorhabditid nematodes: A hand-book of techniques // South. Co-operative Serv. Bull.

Bedding R.A. (1981). Low cost in vitro mass production of *Neoaplectana* and *Heterorhabditis* species (Nenatode) for field control of insect pests. Nematology, 27, 109-114.