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Biological control of Entomopathogenic nematode *Steinernema carpocapsae* against Colorado potato beetle *Leptinotarsa decemlineata*

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Abstract:- This study aimed to determine the efficacy of biological control of the Colorado potato beetle *(Leptinotarsa decemlineata)* through Entomopathogenic nematode (EPNs) *Steinernema carpocapsae* in both laboratory and field conditions.

Before starting the experiment, Entomopathogenic nematodes (EPNs) were isolated from the soil, which were obtained at different locations of Samtskhe-Javakheti region, in particular, soil samples were taken for the experiment from borough Bakuriani, village Timotesubani, borough Dviri, borough Tsaghveri (from the agricultural plots of land, various parts of Arjevanidze forest). The experiments conducted in the laboratory conditions determined, that all insects participating in the experiment died, including Imago (95%), while the mortality of larvae reached 99,5%, which undoubtedly indicates the efficacy of entomopathogenic nematodes. This experiment showed, high efficacy of *Steinernema carpocapsae* towards imagos and larvae of Colorado potato beetle.

Accordingly, the determination of the efficacy of Entomopathogenic nematode (EPNs) in the field conditions became necessary, particularly in the plot of land of the potato. This experiment also showed, the efficacy of *Steinernema carpocapsae*. Almost all Metamorphic forms of adult Colorado potato beetle and final stage larva died, as a result of suspension treatment of entomopathogenic namatodes of potato bush. Of which, adult beetle mortality reached (75 %), while the mortality of final stage larvas amounted to 88,5%. As a result of filtrate inspection and microscopic examination, it was determined, that nematode suspension showed good results even in field conditions. It is also noteworthy, that the target insect for entomopathogenic nematode is only an insect-pest and at the same time it does not influence the growth and development of the plant and its fruit (tuber), which is a quite effective method in terms of ecology, because it does not require the use of pesticides and other poisonous chemical products and therefore it is an environmentally safe natural process.

Keywords:- *Entomopathogenic nematode, Steinernema carpocapsae, Leptinotarsa decemlineata*

I. INTRODUCTION

Colorado potato beetle causes great damage both in the Samtskhe-Javakheti region and in various agrarian zones of Georgia. Mainly, it damages cultivated plants of a Nightshade family *Solanaceae* (Potato, pepper, tomato, eggplant). Colorado potato beetle (*Leptinotarsa decemlineata*) is a dangerous quarantine pest of the leaf beetle family (Galerucella). It gives 1-2 generations per year. The adult beetle spends winter in the soil. The beetle and worm eat leaves. They can damage green plants and significantly reduce crop yield. *Leptinotarsa decemlineata* lays up to 3000 eggs on the lower side of a potato leaf. This study aimed to determine the efficacy of entomopathogenic nematodes *Steinernema carpocapsae* towards Colorado potato beetle (*Leptinotarsa decemlineata)* and larvae of its metamorphic form.

Fig 1, 2. Colorado potato beetle (Adult Imago, chrysalis, larva and eggs).

Based on our study, insect biological control is ecologically clean, effective and safe for human health, through entomopathogenic nematodes *Steinernema carpocapsae*, because entomopathogenic nematodes do not adversely influence on the organisms of humans and animals, as well as useful insects, soil worms and plants, which allowed the World

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Health Organization (WHO) to recommend entomopathogenic nematodes in agricultural practice.

As it is known, entomopathogenic nematodes (EPNs) belong to Steinernema carpocapsae genus and is associated with the bacteria *Xenorhabdus*. The combined symbiotic action of bacteria and nematodes leads to the death of the insect, which plays an important role in the regulation of the number of pests. Nematodes are characterized by the following form of development: within 24 hours after puberty, females begin laying eggs, and on the fifth day after becoming infected, larvae of the first generation appear in the body of the insect. The second-generation larvas are covered with cuticles. These stages of development, unlike first-generation larvas and adult nematodes, are resistant to drying. In case of the death of the host and lack of food, they can go out into the environment and migrate in search of new victims. If there is no insect nearby, after 3-4 days the nematode stops moving and can be in an anabiotic state for several months. As well as, it spends winter in this form. The rate of insect mortality and the generation of nematodes developed in its organism depend on the number of invasive larvas initially introduced. In this case, the bodies of dead insects receive a characteristic reddish-brown color. Invasive larvas (of stage 3rd) find and infect the host in the soil. Nematodes penetrate into the insects, both passively (by feeding) and actively (through the mouth, spicula, and anus). Nematodes develop in an insect's body, until the entire content of its body is not destroyed. Up to 1.5 million infectious larvas are reproduced in one gram of insect mass. The nematode is active at a temperature of 12-30°C and influences all stages of insect development. The pathogenic efficacy of nematode depends on soil moisture and temperature. The range of invasiveness was the biggest at the high temperature of the soil and in the case of soil humidity.

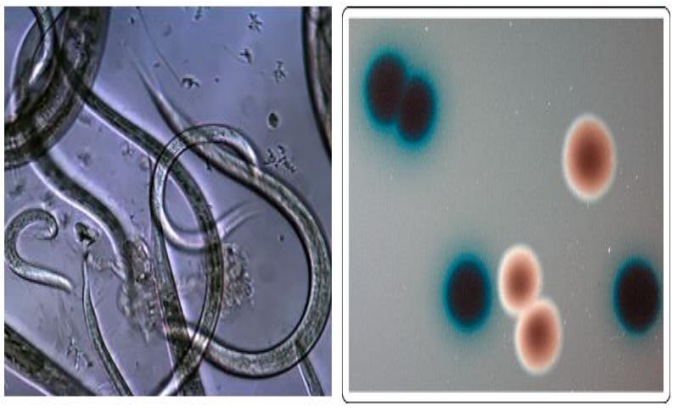

Fig 3,4. Entomopathogenic nematodes *Steinernema carpocapsae* and its bacteria symbiont *Xenorhabdus*

II. MATERIALS AND METHODS

Before participating in the experiments, we isolated entomopathogenic nematodes from the soil (period 14 days) and then carried out their cultivation initially, in the food area agar-agar (Period-12 days), then in the bodies of the host organism, the Colorado potato beetle (Imago) and its larvas at a temperature of 23-25^{0}C. The process was carried out in Petri dishes in which drying paper of appropriate diameter was spread out. One dish was used for imago of Colorado potato beetle and the other one for larvas. The insects placed on a Petri dish (in the amount of 50 units) were treated with nematodes suspension. The invasiveness process of the insects lasted for 7 days. At the end of the past period, during the examination of the insides of the Petri dishes, it was found that all the insects participating in the experiment died, which undoubtedly indicates the efficacy of the entomopathogenic nematode (99,5%). The cultivation of Nematodes, according to its life cycle, lasted in the body of the insect and larvae, and on the 8th day the dead beetles and their larvas were transited to the "White trap" (White trap, White G.1927). The isolation of Nematodes from the body of an infected dead insect and their transition to the aquatic environment are carried out through this method (White G.1927), from the bodies of the Colorado potato beetle and its larvas. We also used nematode suspension collected in this way in the field conditions to determine the efficacy of the experiment. In particular, we treated 2 potato bushes with suspension, on which lots of Colorado potato beetles and their larval stage were presented. After treatment, we wrapped the bushes with polyethylene bags hermetically, so that no other obstructive factor entered the experiment area. By this action, we maintained the "purity" of the experiment. The outdoor temperature at the experiment site was 27^{0}C and the duration of the experiment was 4 days. After passing this period, we carefully removed the polyethylene bag so as not to spill the liquid existing inside with dead insects. As a result of filtrate inspection and microscopic examination, it was determined, that nematode suspension showed good results even in field conditions (the mortality of insects reached 90,2%)

Fig 5. Soil samples from different locations obtained for the experiment.

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Fig. 6. The process of weighing the soil sample.

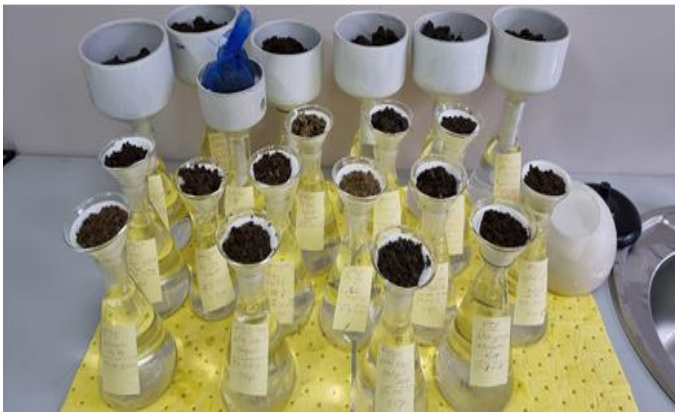

Fig.7. Isolation of Nematodes from the soil in the aquatic area.

III. RESULTS

As a result of the experiments conducted in the laboratory conditions, it was determined, that all insects participating in the experiment died, including Imago (95%), while the mortality of larvas reached 99,5%, which undoubtedly indicates the efficacy of entomopathogenic nematodes. This experiment showed, the high efficacy of *Steinernema carpocapsae* in the laboratory conditions towards imagos and larvas of Colorado potato beetle.

Accordingly, the determination of the efficacy of entomopathogenic nematode (EPNs) became necessary in the field conditions, particularly in the plot of land of the potato. This experiment also showed, the efficacy of *Steinernema carpocapsae*. Almost all metamorphic forms of adult Colorado potato beetle and final stage larva died, as a result of suspension treatment of entomopathogenic namatodes of potato bush. Of which, adult beetle mortality reached (75 %), while the mortality of final stage larvas amounted to 88,5%. As a result of filtrate inspection and microscopic examination, it was determined, that nematode suspension showed good results even in field conditions. It is also noteworthy, that the target insect for entomopathogenic nematode is only an insect-pest and at the same time it does not influence the growth and development of the plant and its fruit (tuber), which is a quite effective method in terms of ecology, because it does not require the use of pesticides and other poisonous chemical products and therefore it is an environmentally safe natural process.

Fig. 8, 9. White trap method: Coming out of nematodes from the bodies of dead infected insects into aquatic environment

Fig. 10. Approbation of the efficacy of nematode suspension in field conditions (potato bush).

Fig. 11. Nematodes isolated through White Trap-method under the microscope.

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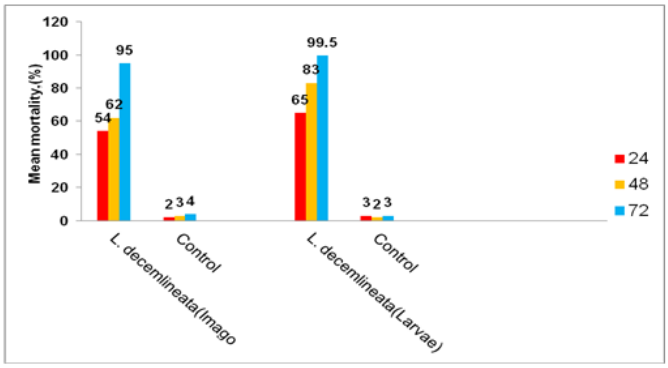

Fig. 12. Virulence of *S. carpocapsae* on imagos and larvas of Colorado potato beetle in the laboratory conditions

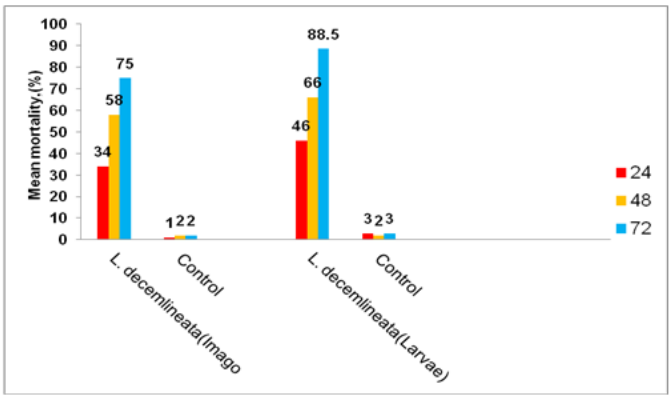

Fig. 13. Virulence *of S. carpocapsae* on imagos and larvas of Colorado potato beetle in the field conditions

IV. CONCLUSION

It was determined that, efficacy of the Colorado potato beetle (Leptinotarsa decemlineata) can be controlled by Entomopathogenic nematode *Steinernema carpocapsae* and further studies should be continiued, as laboratory, also in field conditions.

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