ISSN No:-2456-2165

Examination of Phytopathogenic Nematode *Aphelenchoides sp.* towards Spruce Bark Beetle (*Ips Typographus*) in Georgia

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Abstract:- The aim of our research was to study the entomopathogenic properties of phytopathogenic nematodes and to use these properties for biocontrol of *Ips typographus*. Bark beetle (*Ips typographus*) is from the family of beetles. It is just as damaging to forests as grasshopper for farmers. The damage caused to the pine and spruce population by pest *Ips typographus* in Georgia, Samtskhe-Javakheti region, reached a large scale. Experiments on phytopathogenic nematodes, their cultivation and obtaining nematode suspension were performed under laboratory conditions.The results showed that high entomopathogenic properties of phytonematodes (Aphelenchoides-sp.) were observed on beetle-typographs, 59.5%. Whose mortality depended on the species of nematode, on the temperature, and the concentration of nematodes. Finally, we can conclude that the effectiveness of phytopathogenic nematodes against beetle (Ips typographus) in laboratory conditions is really justified and it is possible to biocontrol this insect.

In the future, the study will be conducted in forest conditions, with nematode suspension treatment of specific coniferous trees.

Keywords:- *Entomopathogenic nematodes, Aphelenchoides sp. Ips typographus, Spruce bark beetle.*

I. INTRODUCTION

The damage caused to the pine and spruce population by pest *Ips typographus* in Georgia, Samtskhe-Javakheti region, reached a large scale. Significant damage to planted forest and changes in its ecosystem are possible in the near future. The aim of our research was to study the entomopathogenic properties of phytopathogenic nematodes and to use these properties for biocontrol of *Ips typographus*. Bark beetle (*Ips typographus)* is from the family of beetles. It is just as damaging to forests as grasshopper for farmers. Bark beetle is distinguished from other wood pests by its great reproductive ability. Due to their vital activity, beetles cause great damage to tree plantations and forest massifs. Exactly, the bark beetle represents the main target of tree plantations and forest massifs. While forming pairs male beetle makes a small tunnel in the wood, drawing the female's attention with the help of special substances - pheromones. The fertilized female lays her eggs.

It is important to remember, that the beetles themselves do not significantly damage the tree. The main danger is caused by the worm, which creates the number of labyrinths of tunnels in the wood, after which it becomes unusable [1].

II. THE BARK BEETLE (Ips typographus) DEVELOPMENT PROCESS AND CONDITIONS

Ips typographus undergoes complete and long metamorphosis. The worm stage can last for several years, during which time, damage to the tree can give irremediable results. Therefore, timely detection and destruction of bark beetle or its worms is quite an important task. It takes about two years to reach to adult imago from the moment the eggs turn into worms. And during this time the pest reveals a very high level of activity. Herewith, foresters note that the bark beetle has always lived in forests and performed useful sanitary functions. It has only recently become the most harmful enemy of the coniferous trees. The number of *Ips typographus* and the harmfulness of its worms are increasing day by day, because neither humans nor other insects living in the forest can control its population.*An sharp increase in the number of Ips typographus can occur for a variety of reasons.* For example, in the Samtskhe-Javakheti region, in the Borjomi-Tsaghveri-Bakuriani forests, recent conditions (frequent fires, which was caused by various reasons) have weakened coniferous trees and this circumstance has led to a sharp increase in beetle populations. As a result of the weakening of the trees, the forest massifs became the basis for the feeding and reproduction of the Ips typographus. Up to a thousand beetles from one family of *Ips typographus* can appear per year. The problem is that the *Ips typographus* can fly. This property of the beetle accelerates the process of infecting trees. It is therefore necessary to take immediate measures to combat against it. All known methods are used against pest such as timely cleaning of diseased trees. This process shall be carried out before the *Ips typographus* come out from the anabiosis and reproduce on them. It will also facilitate the selection of newly infected trees by the *Ips typographus* and the removal of bark before the beetles come out. Sanitary cutting is also often applied, although these methods show temporary effectiveness and only reduce and not completely eliminate the spread of the pest [1,2,3].

Fig. 1

Fig. 2

Fig. (1,2): Bark beetle (*Ips typographus*) spread of imago and worms at pine bark outlets

Fig. 3

Fig. 4

Fig.(3,4): Bark beetle (*Ips typographus*) distribution of imago and worms at the outlets of spruce bark

The experiments conducted in laboratory conditions showed entomopathogenic efficacy of phytopathogenic nematodes and this makes it possible, that through them (by inserting nematode suspension under the bark of the plant) it is possible to fight against the above-mentioned pests (bio-control) with great efficiency.

Phytopathogenic nematodes *(Aphelenchoides sp.)* are in symbiosis with bacteria (microbacterium). The joint action of a bacterium and a nematode causes the death of an insect, which plays an important role in the regulation of pests populations. Nematodes of this species are distinguished by their effectiveness, and they can be used as biological agents for biological control of the pest [4].

Phytopathogenic nematodes (*Aphelenchoides sp.*) are plant parasites. Their sizes are not large (length not more than 5 mm). Fertility is relatively low. Rare are the species whose females produce several hundred or slightly more than 1000 eggs in their individual lives. The eggs are usually relatively large. In all phytoparasitic nematodes the oral cavity is altered into the so-called stiletto, its tip resembles the needle of a medical syringe, an inclined, thin canal passes inside the stiletto. The back (proximal) end of the stiletto mostly bears special basal heads. Three packages of myofibrils extend from the heads to the partitions of head capsule. These are the protractor muscles, that push the stiletto out. Phytoparasitic nematode (phytohelminths) pierces with its stiletto the walls of plant cells. In parallel with the impact of stiletto, special esophageal enzymes are released into plant tissue, which are produced from the esophagus gland, especially from the dorsal gland. These enzymes destroy plant proteins. The change of hosts does not occur in phytoparasitic nematodes. Many of them reproduce themselves repeatedly in the body of the plant, accumulate in it in huge quantities and lead the plant to death [6].

Fig. 5: Phytonematode Aphelenchoides sp. under a light microscope

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III. MATERIALS AND METHODS

Experiments on phytopathogenic nematodes in order to determine entomopathogenic properties and as well as, their cultivation on the feed area (agar-agar) were performed in the laboratory conditions at 23-25^{0}C. The suspension obtained as a result of cultivation was stored in a refrigerator at a temperature of 4-6^{0}C. At this temperature the nematodes moved to anabiosis, and at 24-25^{0}C, after 6-10 hours they were reactivated again and it was possible to use nematode biomass for further experiments.

Our experiments were focused on studying entomopathogenic properties of phytopathogenic nematodes *(Aphelenchoides-sp.)* in laboratory conditions against coniferous pest *Ips typographus.* Experiments on phytopathogenic nematodes, their cultivation and obtaining nematode suspension were performed under laboratory conditions. The feed area agar-agar was used for cultivation and the temperature regime was protected in the range of 22^0-25^{0}C. The population of nematodes was obtained in 14 days in the form of suspension, which was stored in a refrigerator at a temperature of 4-6^{0}C. By which it was possible to transfer nematodes to anabiosis and then use them again in experiments, which established the identification and study of the entomopathogenic properties of phytopathogenic nematodes on the worms of *Ips typographus* and its adult form-imago. We put filter paper on two petri plates and placed separately on them beetle, second-age worms in the amount of 27 units and adult beetle imago in the amount of 14 units, and sprinkled an equal amount of 500 n/ml of nematode suspension on both. After 48 hours, the following results were obtained by examining petri plates: 25 out of 27 worms were died, and from 14 units of adult imago, all were died. After spraying the additional suspension, the 2 units that survived during 48 hours were also died. The obtained results showed, that the phytopathogenic nematodes *Aphelenchoides sp.* revealed the property of entomopathogenic nematodes. The experimental results also showed, that the mortality of the *Ips typographus* depended on time, species of phytonematodes, their number and concentration. This confirms the entomopathogenic properties of phytopathogenic nematodes by 59.9%. This experiment was performed under laboratory conditions and it is planned to determine the effectiveness of phyto-nematode suspension in field (forest) conditions in the future [5,7,8].

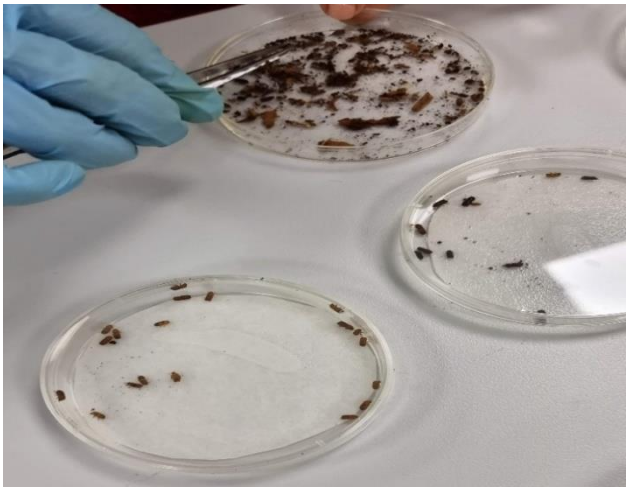

Fig. 6

Fig. 7

Fig. (6,7): Plant parasitic nematode *Aphelenchoides sp.* virulence in adult and worm of Ips typographus in laboratory conditions

IV. OBTAINED RESULTS

The results showed that high entomopathogenic properties of phytonematodes *(Aphelenchoides-sp.)* were observed on beetle-typographs, 59.5%, whose mortality depended on the species of nematode, on the temperature, and the concentration of nematodes.

Finally, we can conclude that the effectiveness of phytopathogenic nematodes against beetle (*Ips typographus*) in laboratory conditions is really justified and it is possible to biocontrol this insect.

In the future, the study will be conducted in forest conditions, with nematode suspension treatment of specific coniferous trees.

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