

Phytonomus (Phytonomus Variabilis Hbst) Infected With the Parasite Batiplektis and Pathogenic Bacteria

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Abstract: For the first time in the conditions of the Karshi desert, the bioecology of alfalfa weevil was studied in depth. Important features of insect bioecology were identified, its description and phenological scheme was developed. In the agrocoenosis of alfalfa weevil different microorganisms and epizootic microorganisms cultural-stamps were isolated. The hitherto unknown aspects of the bioecology and activity of the beneficial insect *Bathyplectes* which naturally reduce the amount of alfalfa weevil, have been identified. *Bathyplectes* is highly active in drought-tolerant years, infesting alfalfa weevil larvae in large numbers in 3-4-year-olds alfalfa field, while pathogenic microorganisms are more active in relatively moist seasons when alfalfa weevils are abundant. The composition of alfalfa weevil epizootics in the alfalfa agrocoenosis of the Karshi desert consists of 21 species of bacteria belonging to 4 families, including 1 species belonging to the family *Aerobacter*, 13 species belonging to the family *Vacillus*, 3 species belonging to the family *Micrococcus* and 4 species belonging to the family *Proteus*.

Of the 21 species of bacteria identified, 16 have relatively high virulent to alfalfa weevil larvae, including *Bacillus subtilis*, *Bacillus vulgatus*

Bac. megaterium has the most active pathogenicity potential.

Keywords: Phytonomus, alfalfa, bathyplectes, parasite, epizootic, plant protection, bacterium.

Introduction

Various biotic stressors exist in agricultural ecosystems yet insect pests become the primary factor that diminishes crop yields the most. Alfalfa cultivation suffers the major economic damage from *Phytonomus variabilis* Hbst also known as the alfalfa weevil. [1] The high consumption behavior of the pest results in enormous yield reductions of up to 60–65% during the first alfalfa harvest. This species poses management challenges to farmers who need to adopt sustainable pest management techniques that protect the environment. This research examines *Phytonomus variabilis* bioecology specifically in the Uzbekistan Karshi desert by exploring *Bathyplectes* species along with epizootic bacterial pathogens that serve as natural enemies.[2]

Integrated pest management draws biological control as its essential element since it works toward lowering dependency on chemical pesticides. Synthetic insecticides can be replaced effectively by natural enemies consisting of parasitoids together with microbial pathogens that defend both target pest populations and ecosystem diversity.[3] Different agroecosystems show that multiple predators and microbial agents control *Phytonomus* populations based on previous scientific research finding. Research about the precise relationships between *Bathyplectes* parasitoids and bacterial epizootics and alfalfa weevils in Central Asia remains limited. Knowledge deficits rate high importance in places like Karshi steppe because environmental elements deeply affect how pests behave and biological control functions.[4]

This research combines field and laboratory methods in order to fully understand how *Bathyplectes* parasitoids and bacterial pathogens affect *Phytonomus variabilis* population control. Research teams performed field surveys in Kashkadarya agrocenoses to track the population numbers of alfalfa weevils together with natural control agents.[5] The evaluation of pathogenic bacteria demanded an investigation of bacterial strains derived from *Phytonomus* larvae conducted through microbiological analysis. The research investigated how biological control agents performed across seasons while establishing relationships between their activity levels and weather factors and host population densities.[6]

The research will produce better insights into the ecological relationships that exist between *Phytonomus variabilis* and both *Bathyplectes* parasitoids and bacterial pathogens.[7] Scientific data obtained from this study will establish the best circumstances for biological control so sustainable pest management methods specific to the Karshi desert environment can be developed. The research seeks to determine which bacterial strains demonstrate the greatest pathogenicity against *Phytonomus* as part of developing targeted bioinsecticide remedies.[8]

Methodology

The investigation utilized both laboratory and field study methods to research *Phytonomus variabilis* bioecology along with its natural enemies *Bathyplectes* and bacterial pathogens within Karshi desert alfalfa ranges. The Kashkadarya region farms served as the research sites for field investigations because they represent the primary area for alfalfa cultivation in the region. Survey researchers examined *Phytonomus* populations in alfalfa fields of different ages with special attention on fields beyond their first year since pest problems reached their peak levels during this period. [9]Observations within the fields were performed on a regular basis in order

to monitor pest population densities and observe larval growth stages and natural control organisms like parasitoids and epizootic bacteria. Field collectors obtained their specimens manually before storing them for analysis.

Detailed microbiological together with parasitological studies took place within the Scientific Research Institute of Plant Quarantine and Protection's laboratories. The scientists analyzed more than 6000 *Phytonomus* specimens to detect *Bathyplectes* parasitoid presence alongside specific bacterial pathogens. The techniques outlined by V.A. Megolev and Artokhin served to both tally weevil larvae and adult counts in an established manner. The detection of bacterial strains required researchers to obtain bacteria from infected larvae followed by controlled lab cultures of pathogenic microorganisms. Microbiologists obtained bacterial colonies from nutrient culture media before conducting Berger bacterial identification system analysis to study morphological and cultural and biological aspects of the bacteria. The microscopic analysis of infected larvae involved observing bacterial and fungal spores through hemolymph examination.

The examination of environmental factors included humidity and temperature assessments in order to monitor their influence on bacterial epizootics spread and *Bathyplectes* parasitoid effectiveness. Research analysts determined *Phytonomus* infection rates by *Bathyplectes* parasitoids through larval dissection to detect parasitoid cocoons. The research analyzed biological control effectiveness across different seasons together with the connection between pest population control and natural enemy prevalence. The integrated research method delivered a complete understanding about natural regulatory mechanisms for *Phytonomus variabilis* management in alfalfa-based agroecosystems.[10]

Results and discussion

In the Republic of Uzbekistan in the program on modernization of agriculture mikrobiopreparations against pests and diseases on cotton, wheat, legumes, garden, vegetable crops, vineyards, ornamental plants.

High productivity, record yield of deficient protein per unit area, ability to give high yields without large amounts of fertilizers make alfalfa one of the most important crops. The practical value of alfalfa is not limited only to its fodder qualities. It enriches the soil with nitrogen, is a good precursor for many agricultural crops, reduces the effect of water and wind erosion.[11]

Alfalfa is one of the most important among the main crops and in cotton crop rotation, but it suffers great losses of yield from damage by pests. One of the dangerous pests of alfalfa of the second and subsequent years of standing is alfalfa leaf elephant phytonomus (*Phytonomus variabilis* Hbst). This vicious pest causes the greatest damage to the first cutting, and the productivity of alfalfa crop decreases up to 60-65%. [12]

In the history of phytonomus study, many facts about its harmfulness in different regions, about natural regulators of abundance, as well as about control measures have been given. At the same time, a critical analysis of the national literature shows that phytonomus has been insufficiently studied in the conditions of Central Asia in recent years. The correction of the existing situation prompted us to conduct the present research.[13]

The studies related to microorganisms were carried out

at the Scientific Research Institute of Plant Quarantine and Protection, Laboratory of Pest Control of Pasture, Forage, Oilseed Crops and Medicinal Plants. Field experiments were conducted in alfalfa agrocenoses of different farms of Kasbin district, which is considered to be the center of Karshi steppe. Visual observations of insects in nature, photographing of material, collection and keeping of potential hosts in laboratory and field conditions by rearing larvae and adults to study the trophic spectrum and obtaining information on polychinous feeding were applied. During the research period, more than 6000 specimens of phytomonas of different instars were collected for the purpose of parasite rearing.

The number of phytonomas larvae and beetles was counted according to the method proposed by V.A. Megolev Artokhin.

Microbiological studies and observations were conducted based on the methodological guidelines of A.N. Evlakhova, O.I. Shvetsova

Isolation of microorganisms and their biological characteristics were studied using the methods of Gerhard F.R. et al. and A.S. Labinskaya .

Identification of bacteria isolated during the studies was carried out based on the results of studying their morphological, cultural and biological characteristics using the Berger bacteria identifier.

Numerous predators, parasites and pathogens - causative agents of diseases - are of great importance in the natural regulation of the number of phytonomus. The most effective action on reducing the number of pests is provided by the parasite bathyplektes *B. curculionis* and *B. anurus*. See Fig. 1.

In order to clarify the role of the parasite and pathogens in the natural regulation of the number of phytonomus, studies were conducted at stationary sites and route surveys in alfalfa fields in the Kashkadarya region. As a result of the studies, it was found that in the conditions of the Karshi steppe, epizootics of bacterial infections affecting phytonomus in alfalfa fields develop with a high number of phytonomus. Then the spread of infection accelerates. With a lack of food, the physiological state of the larvae worsens, and relatively high humidity and a sharp increase in air temperature create favorable conditions for the development of the disease. As a result of studying the infection of phytonomus larvae with bathyplektes and bacterial pathogens, certain patterns were revealed. Pathogens are noted as the main regulator of the decrease in the number of phytonomus, and in subsequent years, bathyplektes has the main influence on the decrease in its numbers. The increase in the number of the pest and its natural regulators is explained by favorable conditions for each of them. As a result of dissection of the sedentary, sluggish larvae, white thick larvae of the parasite were found under the skin. (Fig. 2) According to our research, the infection of phytonomus larvae with bathyplektes pathogens in the farms of the Kashkadarya region on alfalfa fields of the 2nd year of standing was: up to 30% of larvae were infected with bathyplektes, 20-25% died from diseases; on alfalfa fields of the 3rd year of standing, the death of larvae occurred in 38-39% of cases - from parasites, in 35-37% - from diseases.

Bathyplektes curculionis – *B. exigua* *Bathyplektes curculionis*, *B. anurus* are the main parasites of the larvae, and when they meet together, *B. anurus* dominates. The difference between them is that *B. anurus* has a flat, thin belt with white tubercles, and *B. curculionis* has a widely flattened belt and the edges of the white lines are unclearly outlined. (Fig. 1.) *Bathyplektes* larvae greatly damage the larvae of the phytonomus and the infestation was up to 65%. It is promising to use it against the phytonomus in the future.





Fig.-1. Cocoons of Bathyplectes curculionis and B. Anurus. Fig.-2. Larva of Bathyplectes

To isolate pathogens and accurately identify them, hemolymph of patients, contents of corpses and intestines of insects were inoculated onto nutrient media. The inoculations were then examined using a microscope. According to the bacteriological analysis, both spore-forming and non-spore-forming rod-shaped bacteria, cocci, and fungal spores and conidia were always present in the studied biological material. Rod-shaped bacteria were most common (19.6-29.6%), while fungal spores were much less common (1.4-3%). (Fig. 3). In the course of bacteriological studies, 59 bacterial strains were isolated from the biological material, which, based on the determination of morphological, cultural and biological properties, were assigned to 4 genera and 21 species. (Table 1). Among those identified, 13 species were assigned to the genus *Bacillus*; 1 to the genus *Aerobacter*; 3 to the genus *Micrococcus*; and 4 to the genus *Proteus*. The frequency of these microorganisms in the studied samples varied, and along with the widespread species, relatively rare ones were identified, for example, the following are rarely found: *Bacillus cereus*. *Bac. megatherium*. *Micrococcus saccatus*, isolated from the corpses of larvae.[14]

Table 1 Isolated bacteria from phytonomus larvae

№	Type of bacteria
1	<i>Aerobacter oxytocum</i> (Migyla Bergey et all)
2	<i>Bacillus aminovorans</i> (deen Dooren de Jong)
3	<i>Bac. amaylolyticus</i> (Mermany Me Beth)
4	<i>Bac. cereus</i> (Fr. Et Fg)
5	<i>Bac. cohaerens</i> (Gottheil)
6	<i>Bac. danicus</i> (Iohnis u Wet)
7	<i>Bac. evanidus</i> (Grohman)
8	<i>Bac. felexus</i> (Batchelor)
9	<i>Bac. megatherium</i> (De Bary)
10	<i>Bac. silvatikus</i> (Weide)
11	<i>Bac. sp.</i>
12	<i>Bac. subtilis</i> (Cohn)
13	<i>Bac. thuringiensis</i> Ber
14	<i>Bac. vulgatus</i> (Trevisan)
15	<i>Micrococcus candidus</i> (Cohn)
16	<i>M. candicans</i> (Flugge)
17	<i>M. saccatus</i> (Migulla)
18	<i>Proteus americanus</i> (Pacheco)

19	<i>P. mirabilis</i> (Hauser)
20	<i>P. nostuarum</i> (White)
21	<i>P. sphingidis</i> (White)

CONCLUSION

The research delivers vital knowledge about *Phytonomus variabilis* bioecology in Karshi desert alfalfa fields by demonstrating how *Bathyplectes* parasitoids and bacterial pathogens control weevil population numbers. *Bathyplectes curculionis* together with *B. anurus* maintain dominance as parasitoids which succeed at suppressing weevil populations to 65% infestation rates during the second and third years of alfalfa cultivation. [15] Bacterial epizootics led to significant *Phytonomus* population control when species from *Bacillus*, *Proteus*, and *Micrococcus* genera occurred in the environment with a strong relationship between environmental conditions and pathogenicity forming this connection. Research findings validate the potential for biological pest control approaches as a sustainable pest management strategy which helps eliminate chemical pesticides from agricultural practices. The research implications from this study provide a control methodology for pest management in arid and semi-arid ecological zones which experience climate-dependent biological agent effectiveness. The implementation of *Bathyplectes* parasitoids and microbial control agents at scale requires additional studies which must focus on how to optimize their practical usage by developing bioinsecticides and aspect-driven field release protocols. The long-term evaluation needs to examine how biological control agents adjust to environmental changes while studying their relationships with beneficial species living in the same habitat.

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