UDC:634.22:632.775:632.937

EVALUATION OF EFFICACY OF BACILLUS THURINGIENSIS STRAINS AGAINST PLUM APHIS - *HYALOPTERUS PRUNI* (GEOFFR) (HEMIPTERA: APHIDIDAE) IN LABORATORY AND FIELD CONDITIONS

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https://doi.org/10.5281/zenodo.8092393

Abstract. *In the conditions of currently observed environmental changes, biological control is considered as the most promising tool and strategy of sustainable agriculture, because it has economic and ecological advantages. Therefore, Bacillus thuringiensis bacteria isolated from infected and dead insects against Hyalopterus pruni were tested in laboratory and field conditions. In laboratory conditions, strains Bt-26 and Bt-1Fo killed 100% of H.pruni by day 14, while strain Bt-1 showed 86,7% efficiency. In our field experiments, strains Bt-26, Bt-1, Bt-1Fo showed efficiency higher than 80,0%. In the article, the use of microorganism strains with insecticidal effect for biological control in agriculture can be a scientific and practical solution against Diptera pests.*

Keywords: *plum sap, pest, leaf, parthenogenesis, imago, larva, Bacillus thuringiensis, bacterium, spore, crystal, toxin.*

Introduction. Development and application of effective, ecologically safe methods of protecting crops from pests is one of the urgent problems of today. This is one of the most important measures aimed at ensuring the implementation of the food safety program. *Aphids* (*Hemiptera: Aphididae*) are a diverse group of specialized insects recognized as organisms that feed on the sap of plant leaf tissue [9].

More than 4000 species of aphids have been identified, of which about 250 species are pests of crops and ornamental plants. They are mainly oligophagous and monophagous, only some representatives are polyphagous species [4,17].

The plum aphid, *Hyalopterus pruni Geoffroy* (Hemiptera: Aphididae), is a serious pest of plum (*Prunus domestica*) and was first recorded in 1881 [11]. Hyalopterus pest initially damages pome fruits such as almond (P. dulcis), apricot (*P. armeniaca*), blackthorn (*P. spinosa*), peach (*P. persica*) and plum (*P. damestica*), followed by unfavorable weather conditions. upon emergence, it migrates to the reed (*P. australis*) plant [8.10].

In Europe and North America, plum aphid *Hyalopterus pruni* (Homoptera: Aphididae) is the most common insect species in terms of number and biomass, plum aphid is a Eurasian species originating in North America[5].

The use of microbiological biopreparations against pest insects gives positive results. Especially suitable for bacterial preparations based on *B.thuringiensis* Berliner. *B.thuringiensis* species produce toxic insecticidal crystal proteins that are used on more than 3000 different insects [2.3.13]. Insecticidal microorganisms were first identified in 1901 by Shigetane Ishiwatari and were used commercially in the 1920 [7]. *B.thuringiensis* accounts for 95% of the biopesticide

market worldwide. Bacteria play an important role in biological control because it is the most widely used microbial control agent [6].

B.thuringiensis is an entomopathogenic, aerobic, gram-positive soil microorganism, capable of producing crystal-like inclusions during sporulation, called entomocidal proteins-delta-endotoxins (also *Cry* proteins). Crystals are bipyramidal, cubic or round in shape and are located at the end of the cell opposite the spore in the sporangium [18].

More than 60 subspecies of the bacterium *B.thuringiensis* have been described so far. The toxins produced by them differ in the specificity of their insecticidal action. The toxins are known to kill individual members of the Diptera family (*Cry*4 and *Cryl*1) at the larval stage with high specificity. Recently, much attention has been paid to the effects of *B.thuringiensis* exotoxin-containing bacterial strains on sucking pests [12].

Research materials and methods.

Our research 2022 "Laboratory for the fight against harmful organisms of grain and leguminous crops" of the Scientific Research Institute of Plant Protection and "Scientific Research Institute of Horticulture, Viticulture, Winery" named after Akademik Mahmud Mirzayev at the Tashkent scientific-experimental station in 2010 6× We took different varieties of plums planted in 5 schemes in a 0.6-hectare local Hungarian variety of a 5.4-hectare collection garden. We used *B.thuringiensis* Bt-26, Bt-1, Bt-1Fo and Bt-91 strains available in the institute's collection. Toxins of *B.thuringiensis* bacterium adhere to specific binding sites in the midgut of insects. As a result, the insect stops eating, becomes infected with bacteriosis and eventually dies.

In the field experiment studies were conducted in the form of experimental variants, model and control (untreated variant). Strains were sprayed using an OPRD-10 motorized handheld device. The consumption of the working fluid was taken at the rate of 1000 liters per hectare according to the size of the garden. Pest counts before treatment were counted as the number of pests on one (1) leaf in the control and experimental plots.

In laboratory conditions bacteria were grown in liquid nutrient medium by adding 5% inoculum to liquid peptone nutrient medium in 750 ml Erlenmeyer flasks and incubating at 28-30ºC on a shaker at 160 rpm. Full sporulation of the strain occurred in 72–80 h when cultured in liquid medium, with 90–95% of cells forming spores.

The leaves infested with the pest were collected from plum orchards and the number of aphids on each leaf was counted. So that the leaves do not dry out, the leaf band is wrapped with cotton and moistened with water. Petri dishes were autoclaved with filter paper, and then one infected leaf was placed in each petri dish.

In order to determine the effect of insecticidal strains, 10 ml of 1.0% concentration was taken and suspended on leaves in 5 variants, 4 repetitions. The concentration of the solution of bacterial strains used in the experiment was $2x10^9$ KHB in 1 ml. In the control option, it was treated with water.

Pests in Petri dishes were placed in thermostats and held at the same temperature and humidity. Experiments were kept at a temperature of 25-28 ^{0}C and a humidity of 60-65%.

The dynamics of aphid dying (death) was recorded every 48 hours. Those that could not move when tested by touching the pest with a blunt needle were considered dead.

In the course of our research, laboratory and field experiments were used in generally accepted entomological (Dospekhov, 1979; Fasulati, 1971) methods [16.20].

Determining the number of aphids during the growing season of plum trees was carried out according to the methods of Dolgovoy (1979), collection of aphids by Folkina (1978) [15.21]. The method proposed by Storchevaya E (2002) was used for determining the degree of damage by aphids in plum varieties [19].

Aphid mortality in the experimental and control variants was recorded before the experiment and 3, 7, 14 and 21 days after the experiment. Accounting works by Guliy V and Pamujak N (1992). according to the method proposed by [14], the methods of Khojayev (2004) were used to test the strains in the field [22].

Biological efficiency in laboratory and field experiments was determined according to Abbot's formula (1925) [1].

Bs=(Ab-Ba)/Ab) x 100%

Bs - biological efficiency,

A is the number of pests before treatment in the experimental option;

a- the number of pests observed in the days after treatment;

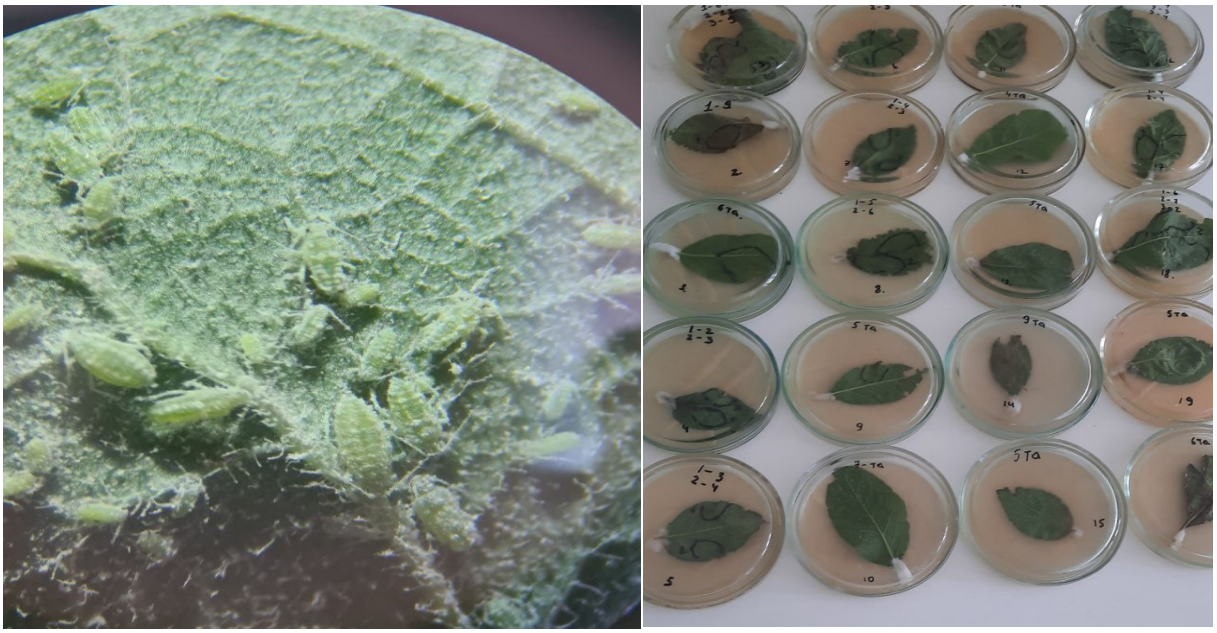
B- the number (density) of the pest before treatment in the control (unsprayed) option;

b- in the control option, the number of pests observed in the following days.

Research results and their discussion. In April 2022, the bacterial strains of B. thuringiensis were used as a suspension on plum leaves in laboratory conditions against plum sap in the state of culture liquid solution.

Plum aphid-infested leaves were collected from the field and the number of aphids per leaf was counted. Petri dishes were autoclaved with filter paper, and to prevent the leaves from drying, the leaf band was wrapped in cotton and moistened with water, and one infected leaf was placed in each petri dish.

10 ml of 1.0% concentration of Bt strains were suspended on the leaves. The dynamics of the death of aphids was recorded every 48 hours (pictures A-B).



A. B.

Bt-26, Bt-1, Bt-1Fo and Bt-91 strains showed high insecticidal activity against plum aphid on the 14th day. That is, the efficiency was 100%, 86,7%, 100% and 78,2%. As a model, Bioslip Bt biopreparation was 76,0% effective on day 14 of the count (Table 1).

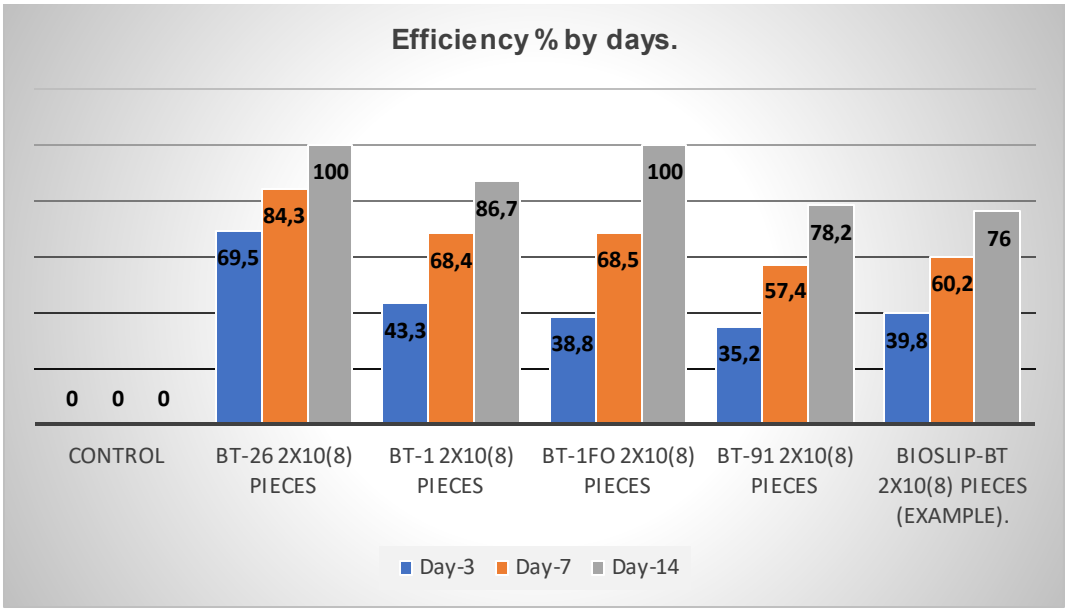


Diagram 1. Insecticidal effect of *B.thuringiensis* bacterial strains against Plum sap
(in laboratory conditions)

Bt-26, Bt-1, Bt-1Fo strains with high insecticidal activity against plum aphid were obtained in the field experiments of the Tashkent scientific research station "Scientific Research Institute of Horticulture, Viticulture and Winemaking" named after Akademik Mahmud Mirzayev. These strains were processed 2 times with the help of OPRD-10 motorized manual device at the rate of 6,0 liters per hectare. After treatment, the biological efficiency against the pest was 82,7%, 81,4% and 82,0% on the 14th day of the calculation. The model strain Bt-91 was 67,2% effective on day 14 of the count (Table 2).

Strains with high activity are an important source for the preparation of bioinsecticidal preparations against sucking pests in the future in the agricultural sector.

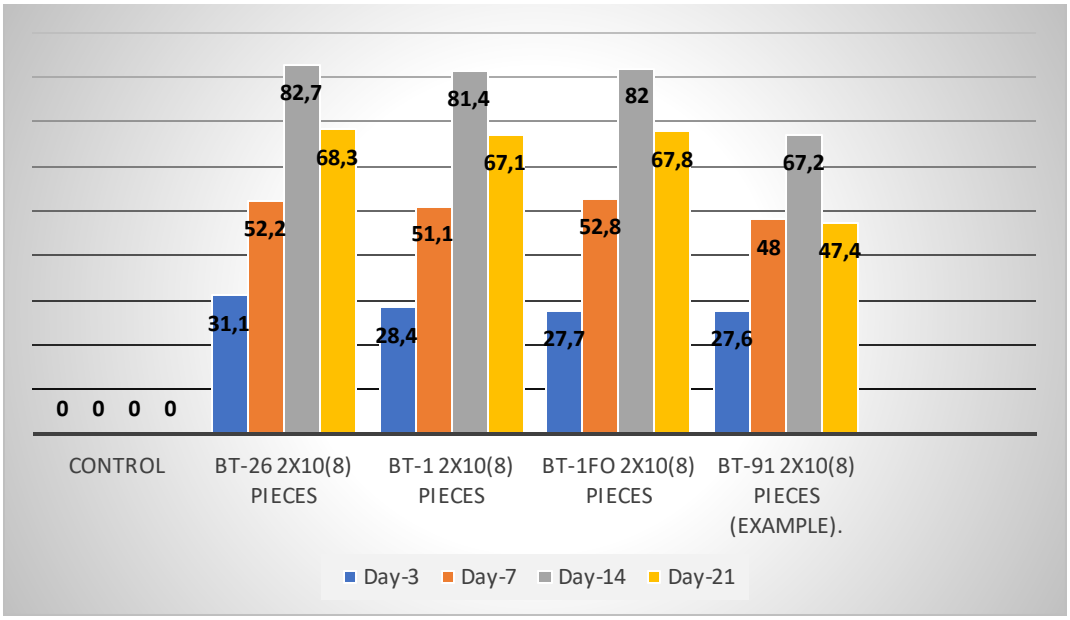


Diagram 2. Insecticidal effect of B. thuringiensis bacterial strains against Plum sap
(in field conditions)

Conclusion. In our laboratory and field experiments, strains Bt-26, Bt-1, Bt-1Fo showed efficiency higher than 80,0%. We believe that the high level of synthesis of β-exotoxin in these

strains affected the increase of insecticidal activity and depends on the activity of crystalline d-endotoxin, which has a specific effect on Diptera insects.

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