RESEARCH ARTICLE

Daniela Atanasova[1]
Pavlin Vasilev[1]

Biological control of *Panaphis juglandis* (Goeze, 1778) with some bioinsecticides in Bulgaria

Authors' addresses:
[1]Agricultural University,
Department of Plant Protection and Agroecology, Plovdiv, Bulgaria

Correspondence:
Daniela Atanasova
Agricultural University,
Department of Plant Protection and Agroecology, Plovdiv, Bulgaria
Tel: +35932654246
e-mail: daniat88@yahoo.com

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ABSTRACT

The aim of the study was to establish the efficacy of some bioinsecticides, for the control of the large walnut aphid *Panaphis juglandis* (Goeze, 1778). The experiments were conducted under laboratory conditions. Two botanical insecticides with active ingredients natural pyrethrins (Pyrethrum FS EC - in concentration 0.05% and 0.1%) and azadirachtin (NeemAzal T/S - in concentration 0.2% and 0.3%) were used, as well as one microbial product based on *Beauveria bassiana* (Naturalis - in concentration 0.1% and 0.2%). All insecticides are registered in Bulgaria for application in organic farming. The best results from the tested products showed the botanical insecticide Pyrethrum FS EC with a knock-down effect and the microbial insecticide Naturalis (*Beauveria bassiana*) with slower action and 100% efficacy on the 3rd and 5th day after the treatment, respectively. The slowest action showed the product NeemAzal T/S. The efficacy reached 100% at both concentrations on the 7th day after the treatment. These results indicate that tested products, applied at an appropriate concentration could successfully control the large walnut aphid *P. juglandis.*

Key words: *Panaphis juglandis*, large walnut aphid, bioinsecticides, walnut.

Introduction

The walnut (*Juglans regia* L.) is widespread throughout the world and has been cultivated by people from very ancient times due to the high nutritional properties of the nuts and the great importance of wood in the furniture industry and decorative appearance. The walnut takes first place in the group of nut crops both globally and in Bulgaria. This is due to the rich food and valuable dietary and medicinal properties of kernels. Its oil is rich in unsaturated fatty acids, phytosterols, and tocopherols (Amaral *et al.* 2003, 2005), whose consumption gives many health benefits (Sabaté *et al.* 1993; Anderson *et al.* 2001; Fukuda *et al.* 2004). Moreover, its non-edible parts such as leaves, husks, and wood also find broad application as flavour liqueurs (Jakopic *et al.* 2007), cosmetics (Tsamourisa *et al.* 2002), dyes (Park *et al.* 2005), furniture and in traditional medicine products (Amaral *et al.* 2008).

Depending on the variety, the nut occupies between 35 and 60% of the fruit weight and has a high energy value (525-622 kcal/100 g). The soil and climatic conditions of Bulgaria are suitable for growing walnuts, and in recent years there has been an increase in the area in the country occupied by this crop. The increased interest is due to the undertaken state policy, providing high subsidies and stimulation of projects for organic cultivation of this fruit species (Gandev 2007). According to Agrostatistics of the Ministry of Agriculture, Food and Forestry (2020) in 2019 the total harvested area with walnut trees in Bulgaria was 18 601 hectares with an average yield of 760 kg per hectare.

More than a hundred species of insect pests attack walnut. Alford (2007) lists 27 arthropod species associated with *J. regia*, which all are found in Europe. This list included some highly monophagous species, e. g. two gall-forming eriophyoid mites: *Aceria erinea*, *Aceria tristriata*; two aphids: *Chromaphis juglandicola*, *Panaphis juglandis*; and one fly, *Rhagoletis completa*. Atlihan *et al.* (2011) studied the insect fauna of walnut orchards, in the Van lake river basin in Turkey and found 29 phytophagous species belonging to 11 different families. The economically important pests were *Cydia pomonella* (L., 1758) (Lepidoptera: Tortricidae) and *Panaphis juglandis* (Goeze, 1778) (Hemiptera: Aphididae).

According to recent research on walnut pests (Riedl *et al.* 1979; Sharma *et al.* 2012), aphids (Hemiptera: Aphididae) are categorized as one of the most serious pests. Blackman & Eastop (1994) list about 20 species of aphids feeding on walnut species worldwide, and provides formal identification keys for aphids on *Juglans*.

Panaphis juglandis, and *Chromaphis juglandicola* were all reported for the first time in Europe between 1800 and 1849, but they were probably introduced long before along with their host plants (Huntley & Birks 1983).

Tasheva-Terzieva (2004) listed *Panaphis juglandis*, and *Chromaphis juglandicola* in a checklist of all recorded aphid species from the city of Sofia, Bulgaria.

According to many authors, two species of aphids colonize walnut, large walnut aphid *P. juglandis* (Goeze 1778) and small walnut aphid *C. juglandicola* (Kaltenbach 1843) (Jaśkiewicz & Kmieć 2007; Karczmarz 2012; Wani & Ahmad 2014).

Pest management is really a big constraint in the walnut because of the difficulty faced in spraying the giant trees. In fact, walnut (*J. regia*) competes with many insect and disease pests to produce a marketable and acceptable crop. Traditional control strategies work reasonably well for most pests.

The farmers usually control aphids with chemical insecticides and this has stimulated the development of resistant aphid genotypes. In recent years with the development of organic agriculture in Bulgaria, there has been an increased interest in bioinsecticides as alternatives to chemical products.

The bioinsecticides are an important component in modern plant protection because they are selective and relatively safe for the environment and human health and at the same time effective measures for controlling many pests (Stiener & Elliot 1987; Stauffer & Rose 1997). Three bioinsecticides suitable for the control of aphids have been registered in Bulgaria: Naturalis, Pyrethrum FS, and NeemAzal T/S (BFSA 2020). Experiments with these products already have been conducted in Bulgaria to control aphids on cherry, apple, plum, and roses (Andreev *et al.* 2008, 2012; Atanasova *et al.* 2014; Andreev & Vasilev 2018).

The present study aimed to establish the efficacy of three bioinsecticides, which are allowed for application in organic farming in Bulgaria, against the large walnut aphid *P. juglandis* (Goeze, 1778) under laboratory conditions.

Materials and Methods

The experiments were carried out in the laboratory of the Department of Entomology at the Agricultural University of Plovdiv, at a temperature of 24^0C and 75% relative air humidity. The efficacy of three bioinsecticides against the large walnut aphid *P. juglandis* was tested. The concentrations of bioinsecticides were established according to their registration for other pests (Table 1).

Natural colonies with a minimum of 50 nymphs and wingless adults of the large walnut aphid *P. juglandis* were collected from infested leaves of walnut (*J. regia*) in the field of Agroecology at the Agricultural University, Plovdiv, and transported to the laboratory. The leaves with aphids were dipped in small bottles with water to keep them fresh for a long time. The treatment was carried out by spraying directly on the aphids colonies with tested concentrations of bioinsecticides and the control was treated with water. Each variant was implemented with three replicates. The number of surviving individuals was recorded on the 1st, 3rd, 5th, and 7th days after the treatment. The efficacy was estimated according to the formula of Henderson & Tilton (1955).

Results and Discussion

In our field survey on walnut at the end of June 2020, only the large walnut aphid *P. juglandis* was found. The adults and nymphs were feeding on the top surface of the leaf along the main vein in large colonies. The aphids usually gather on one side of the vein or on both sides of it beginning from the base of the leaf to the end of it. Sometimes, there are some nymphs on the vein. Sucking juice flowing from the main vein makes the nutrition more effective for aphids and helps to stay close to the center and protect them from the wind. As a result of nutrition, the tree loses its plastic materials and is "dehydrated". Feeding a large number of nutrients on the leaf body causes them to sprout and prematurely spill. The large walnut aphid has a yellow and dark brown preventive color that replaces each other. This is for the protection of the predators from feeding them. The nymphs have large dark brown spots on bright yellow on their body, and in adult flies, they are clearly visible in the form of yellow and brown stripes (Picture 1).

Picture 1. *Panaphis juglandis wingless adult parthenogenetic females, nymphs and winged adult nourishing on the upper surface of the walnut leaf (photo D. Atanasova)*

The preventive color is typical for the aphids living in visible places. But aphids living under the leaf do not need to be hidden from the potential predator, so they don't have such a preventive color. The fact that these aphids on walnut are not eaten by the vertebrate animals is related to the

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Table 1. *Bioinsecticides for control of Panaphis juglandis under laboratory conditions*.

Active substance	Trade name	Concentration
Natural extract with contact containing 32% pyrethrum extract (25% pyrethrin) + 32% sesame oil + 36% adjuvants - soft potassium soaps - 0.05% and 0.1%	Pyrethrum FS EC	0.05% and 0.1%
1% Azadirachtin A + 0.5% Azadirachtin B, C, D, + 2.5% Plant Extract from *Azadirachta indica* (Neem tree)	NeemAzal T/S	0.2% and 0.3%
Entomopathogenic fungi *Beauveria bassiana*, strain ATCC 74040, 2.3 x 10^7 spores/ml	Naturalis	0.1% and 0.2%

toxicity of them or there are other reasons, which should be clarified (Nuriyeva & Nadirova 2018).

Therefore, it is not possible to rely entirely on bio-agents to regulate the density of the aphids and the use of bioinsecticides is necessary.

The botanical insecticide Pyrethrum FS EC, based on natural pyrethrins, showed the best results and fastest action from the tested products. After the treatment, a knock-down effect was observed, and 24 hours after the treatment efficacy was 78% and 83% at concentrations of 0.05% and 0.1%, respectively. On the 3rd day after the treatment efficacy at both concentrations reached 100% (Figure 1). This is a very good result for a bioinsecticide controlling the aphid density,

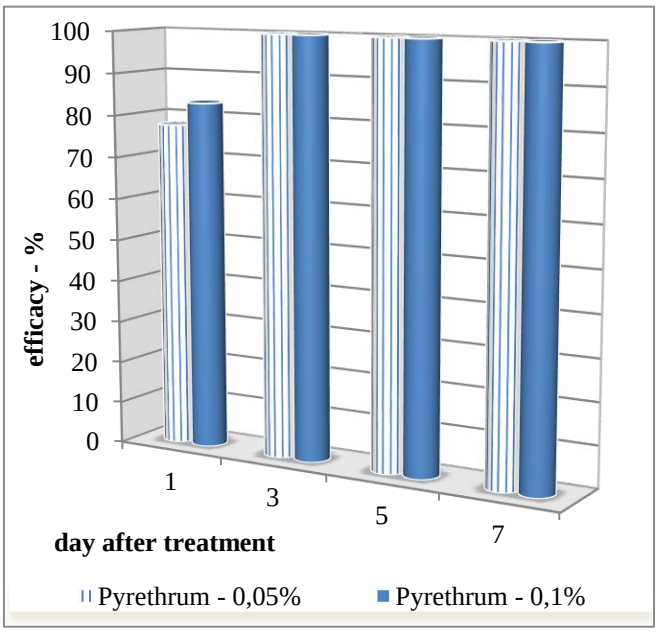

Figure 1. *Efficacy of bioinsecticide Pyrethrum FS EC (pyrethrin) against Panaphis juglandis*

and the product can be used even under very strong attack by *P. juglandis*.

The action of the product with the active substance azadirachtin – NeemAzal was delayed and the efficacy was low at the beginning (Figure 2). On the 3rd day after the treatment the efficacy was only 13.6% and 33.3% and on the 5th day 62.3% and 77% at concentrations of 0.2% and 0.3%,

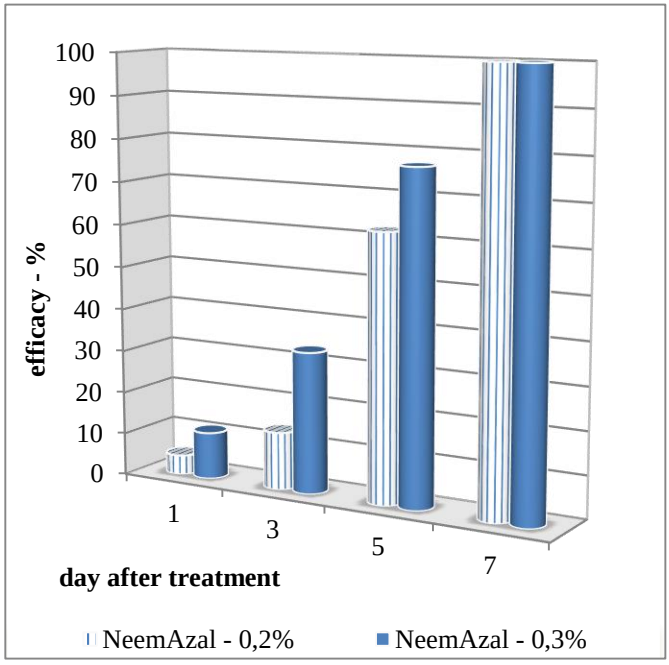

Figure 2. *Efficacy of bioinsecticide NeemAzal T/S (azadirachtin) against Panaphis juglandis*

respectively. The efficacy reached 100% at both concentrations on the 7th day after the treatment.

Despite this slower action, it can be concluded that azadirachtin-based products, applied at an appropriate concentration, are also capable to suppress the attack of the

large walnut aphid. They could be used at the beginning of aphid development and subsequent regular treatments every 7-10 days.

The action of the microbial insecticide Naturalis (*Beauveria bassiana*) is expected to be slower and on 24 hours after the treatment the results are extremely low - only from 3 to 6% efficacy at both concentrations, due to the need for the development of the entomopathogenic fungi in the host body - treated aphids. In the higher concentration (0.2%) the efficacy quickly increased and on the 3rd day reached

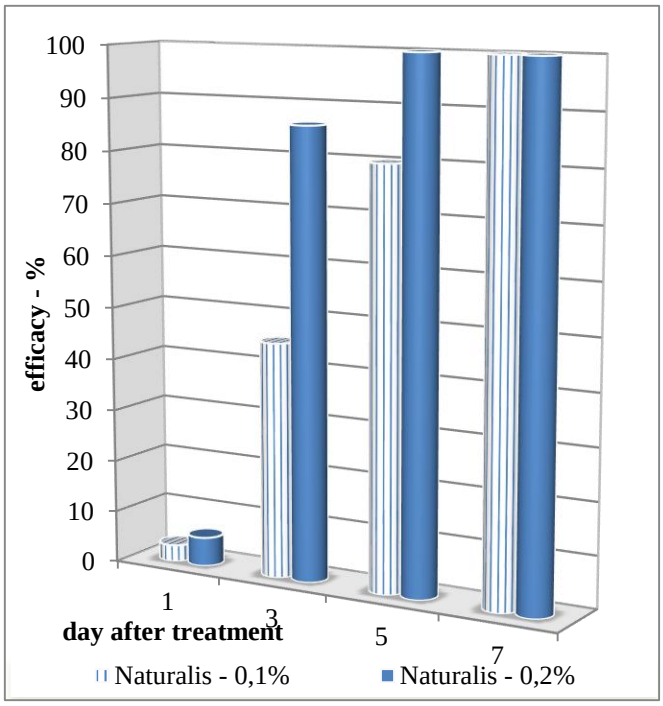

Figure 3. *Efficacy of bioinsecticide Naturalis (Beauveria bassiana) against Panaphis juglandis*

86% and 100% on the 5th - 7th day after the treatment. The preparation showed very good action in its low concentration (0.1%) where the efficacy was 80% on the 5th day and 100% on the 7th day after the treatment (Figure 3).

This is a very good result and such as botanical insecticide Pyrethrum FS EC, the product can be used even under very strong aphid attack.

Similar results were obtained by Andreev & Vasilev (2018) in their study of *Hyalopterus pruni*. They found that Naturalis has an excellent effect against *H. pruni* and at concentrations of 0.1% and 0.2% the efficacy reached 90% and 95%, respectively on the 3rd day and 98.6% and 100%, respectively on the 7th day after the treatment.

According to Nuriyeva & Nadirova (2018), *P. juglandis* feeding causes the core of the leaf to darken, as the ball dew is toxic, and in some varieties of walnut, the leaves were darkened. In our survey, we also observed darkening of the main vein of the leaf due to aphid feeding (Picture 2).

Picture 2. *Action of Naturalis against Panaphis juglandis nymphs and winged adults on the upper surface of the walnut leaf and darkened the main vein causes of aphids feeding (photo D. Atanasova)*

Conclusions

The best results from the tested products showed the botanical insecticide Pyrethrum FS EC with a knock-down effect and the microbial insecticide Naturalis (*Beauveria bassiana*) with slower action and 100% efficacy on the 3rd and 5th day after the treatment, respectively. The slowest action showed the product NeemAzal T/S. The efficacy reached 100% at both concentrations on the 7th day after the treatment.

These results indicate that tested products, applied at an appropriate concentration could successfully control the large walnut aphid *P. juglandis.*

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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