RESEARCH ARTICLE

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Efficacy of some bioinsecticides against the Colorado potato beetle *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) under laboratory conditions

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ABSTRACT

The efficacy of three bioinsecticides: the microbial Naturalis (*Beauveria bassiana*), the botanical Pyrethrum FS EC (pyrethrin + sesame oil + soft potassium soap) and NeemAzal T/S (azadirachtin), allowed for application in organic farming in Bulgaria, against larvae and adults of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) was studied under laboratory conditions. The best results from the tested products showed microbial insecticide Naturalis (*Beauveria bassiana*). At a concentration of 0.2%, the efficacy reached 78.4% and 68.4% on the 5th day for larvae and adults, respectively, and 100% on the 7th day after the treatment for both larvae and adults. The preparation showed very good action in its low concentration (0.1%) – efficacy was 88.5% and 68.5% on the 7th day after the treatment for larvae and adults, respectively. Insecticides based on plant extracts showed very good results. The botanical insecticide Pyrethrum FS EC showed a fast initial action and good effectiveness. At a concentration of 0.1%, the efficacy reached 96.5% and 86.5% on the 5th day for larvae and adults, respectively, and 100% on the 7th day after the treatment for both. The preparation showed good action in its low concentration (0.05%) – efficacy was 84.3% and 68.4% on the 7th day after the treatment for larvae and adults, respectively. The botanical insecticide NeemAzal T/S at a concentration of 0.3% the efficacy was 81.8% and 63.7% for larvae and adults, respectively on the 7th day after the treatment. At its low concentration of 0.1%, the NeemAzal T/S had insufficient effect and efficacy was only was 59.3% and 29.8% on the 7th day after the treatment for larvae and adults, respectively.

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Introduction

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) is one of the most destructive insect pests of potato (*Solanum tuberosum* L.). The origin and history of the spread of the pest have been well documented by Alyokhin et al. (2013). It was first reported as a pest on potato in Nebraska in 1859 (Kennedy, 2009). Later, it became the main insect pest of potato plants in the central and north-eastern United States (Radcliffe et al., 1993) and Canada (Boiteau & Le Blanc, 1992), as well as in many European and some Asian Countries (Cassagrande, 1990; Jolivet, 1991; Liu et al., 2012). Both adults and larvae feed on leaves, stems, and exposed tuber (Weber and Ferro, 1993; Alyokhin, 2009). Defoliation of potato plants can significantly decrease yield (Kennedy, 2009; Alyokhin et al., 2013). Without the use of insecticides, Colorado potato beetle can cause 40-80 % yield losses in potato crops and a loss of more than 75 % of the foliage can cause a total crop loss (Hare, 1980; Shields & Wyman, 1984). The main control strategies of the *L. decemlineata* rely on the use of pesticides (Zabel et al., 2002; Grafius & Douches, 2008). Although the use of insecticides resulted in a drastic reduction of pest populations, resistance development against the active substances has been observed. The Colorado potato beetle, through genetic adaptation, has been able to develop resistance to most of the registered insecticides (Grafius, 1997; Stankovic´ et al., 2004; Alyokhin et al., 2008; Sladan et al., 2012; Szendrei et al., 2012). Increasing the dosage provides only short-term relief, and besides that, large scale application of chemical pesticides can lead to serious health and environmental problems (Dik et al., 2000; Wustman & Carnegie, 2000; Alyokhin, 2009). Recent concern about the effect of chemical pesticides on the environment has encouraged scientists to consider alternative, safer, and more effective control agents (Alyokhin et al., 2015). Biological control is often considered as the most environmentally friendly way to control the pest. The main factor in biological control is the use of natural enemies and biopesticides. Strains of *Bacillus turingiensis (Bt)* have been used as a foliar spray to combat *L. decemlineata* (Walker et al., 2003; Whalon & Wingerd, 2003). Besides *Bt* sprays, also *Beauveria bassiana* sprays resulted in a significant reduction of the pest population in the field (Wraight & Ramos, 2015). 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; Miller & Uetz, 1998).

The products, allowed for use in organic agriculture in Bulgaria are Naturalis, Pyrethrum FS, and NeemAzal T/S (BFSA, 2019).

Naturalis is a microbial insecticide based on living spores of a naturally occurring proprietary strain (ATCC 74040) of the entomopathogenic fungus *Beauveria bassiana*. The formulated product is a concentrated suspension of at least 2.3 x 10^7 spores/ml. It is a suspension of conidiospores in vegetal oil, which improves spore germination and UV protection, enhancing the efficacy of the antagonist in the field. *B. bassiana* can affect a wide range of arthropod pests, such as whiteflies, thrips, mites, aphids, etc. infesting numerous crops (vegetables, cucurbits, solanaceous fruits, strawberry, flowers and ornamentals, grapevine, citrus, pome, stone fruits, etc.). Recent studies have shown that the antagonistic fungus can effectively control also nut-weevils, wireworms (*Agriotes* spp.), and Tephritid flies, such as the Mediterranean fruit fly, *Ceratitis capitata*, the cherry fruit fly, *Rhagoletis cerasi*, and the olive fly, *Bactrocera oleae* (Biogard, 2019). The registered concentration is between 0.1% - 0.2% according to the crop (BFSA, 2019).

Pyrethrum FS is a botanical insecticide that is extracted from a species of daisy flower (*Tanacetum cinerariaefolium*). It has shown high efficacy and is used against a wide range of pest insects as aphids, thrips, leafhoppers, fruit flies, flea beetles, and many others. It is also one of the few insecticides registered for use in Certified Organic Production of crops in the USA, Europe, Australia, and New Zealand. Pyrethrum FS is a fast-acting contact insecticide. Sesame oil is included as a synergist to increase effectiveness. The active ingredients are rapidly broken down by sunlight and are only effective for a short time (McLaughlin Gormley King Compony, 2010). In Bulgaria, Pyrethrum FS is registered for control of aphids on vegetables at a concentration of 0.05% (BFSA, 2019).

NeemAzal T/S is another botanical pesticide with action against small insects and spider mites. Azadirachtin is the main active substance extracted from the seeds and leaves of the neem tree (*Azadirachta indica*). The effects of azadirachtin on insects include feeding and oviposition deterrence, growth inhibition, fecundity, and fitness reductions (Schumutterer 1990; Ascher, 1993; Mordue & Blackwell, 1993). Laboratory and field trials with formulated neem seed oil and neem seed extract demonstrated that these materials are effective aphicides (Lowery et al., 1993). In our country, this active substance is used as the registered botanical insecticide NeemAzal T/S to control spider mites primarily on vegetables in greenhouses in a concentration of 0.3% (BFSA, 2019).

The present study aimed to establish the efficacy of these three bioinsecticides against the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) under laboratory conditions.

Materials and Methods

The experiments were carried out in the laboratory of Department of Entomology at the Agricultural University of Plovdiv, at a temperature of 24°C and 75% relative air humidity. The efficacy of three bioinsecticides: the microbial Naturalis (*Beauveria bassiana*), the botanical Pyrethrum FS EC (pyrethrin + sesame oil + soft potassium soap) and NeemAzal T/S (azadirachtin), allowed for application in organic farming in Bulgaria, against larvae and adults of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) was studied under laboratory conditions.

The concentrations of bioinsecticides were established according to their registration for other pests (Table 1).

Table 1. *Bioinsecticides for control of Leptinotarsa decemlineata under laboratory conditions.*

Active substance	Trade name	Concentration
Beauveria bassiana, strain ATCC 74040, 2.3 x 10^7 spores/ml	Naturalis	0.1% and 0.2%
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*	NeemAzal T/S	0.1% and 0.3%

The adults (30 number) and larvae (30 number) of *Leptinotarsa decemlineata* were placed on potato plants growing in pods and covered with plastic cage isolations. The treatment was carried out by spraying directly on the adults and larvae 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 1^{st}, 3^{rd}, 5^{th}, and 7^{th} days after the treatment. The efficacy was estimated according to Henderson and Tilton formula (1955).

Results and Discussion

The best results from the tested products showed microbial insecticide Naturalis (*Beauveria bassiana*). The action of the preparation is expected to be slower, due to the need for the development of the entomopathogenic fungi in the host body. On the 1st day after the treatment the efficacy was 0% at both concentrations and on the 3rd day at a concentration of 0.2% efficacy was extremely low - only 12.9% and 2.9% for larvae and adults, respectively. In the higher concentration (0.2%) the efficacy quickly increased and reached 78.4% and 68.4% on the 5th day for larvae and adults, respectively, and 100% on the 7th day after the treatment for both larvae and adults. The preparation showed very good action in its low concentration (0.1%) – efficacy was 88.5% and 68.5% on the 7th day after the treatment for larvae and adults, respectively (Figure 1).

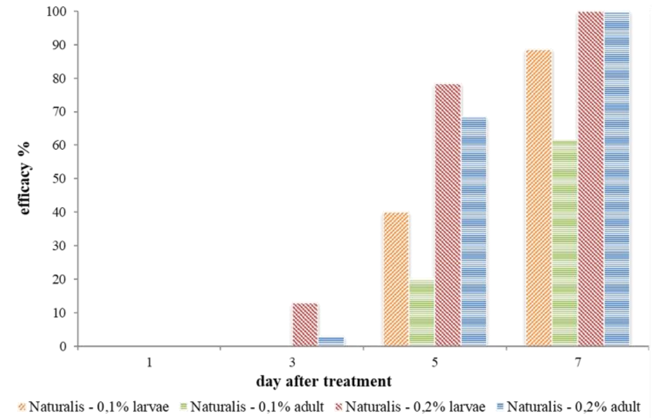

Figure 1. *Efficacy of bioinsecticide Naturalis (Beauveria bassiana) against Leptinotarsa decemlineata.*

Insecticides based on plant extracts showed very good efficacy against *L. decemlineata.*

The botanical insecticide Pyrethrum FS EC, based on natural pyrethrins showed a fast initial action and better effectiveness than azadirachtin based product NeemAzal T/S. Efficacy of Pyrethrum FS EC, applied at a concentration of 0.1% reached 96.5% and 86.5% on the 5th day for larvae and adults, respectively, and 100% on the 7th day after the treatment for both larvae and adults. The preparation showed good action in its low concentration (0.05%) – efficacy was 84.3% and 68.4% on the 7th day after the treatment for larvae and adults, respectively (Figure 2).

The action of botanical insecticide NeemAzal T/S with the active substance azadirachtin was delayed. The efficacy at a concentration of 0.3% was 81.8% and 63.7% on the 7th day after the treatment for larvae and adults, respectively. At its low concentration of 0.1%, the NeemAzal T/S had insufficient effect and efficacy was only was 59.3% and 29.8% on the 7th day after the treatment for larvae and adults, respectively (Figure 3).

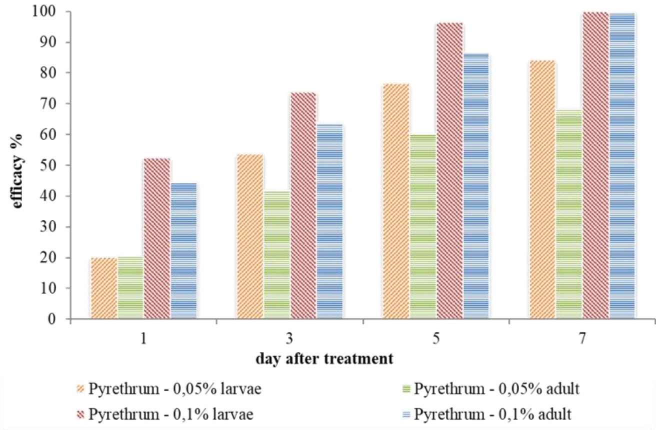

Figure 2. *Efficacy of bioinsecticide Pyrethrum FS EC (pyrethrin) against Leptinotarsa decemlineata.*

The results indicate that the larvae of the Colorado potato beetle are more susceptible to all of the tested bioinsecticides than the adults due to their soft body cover.

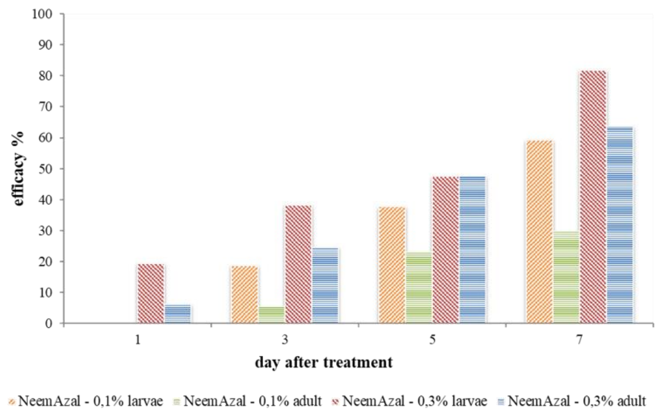

Figure 3. *Efficacy of bioinsecticide NeemAzal T/S (azadirachtin) against Leptinotarsa decemlineata.*

Conclusion

The best results from the tested products showed microbial insecticide Naturalis (*Beauveria bassiana*) against *Leptinotarsa decemlineata.* At a concentration of 0.2%, the efficacy reached 78.4% and 68.4% on the 5th day for larvae and adults, respectively, and 100% on the 7th day after the treatment for both larvae and adults.

The botanical insecticide Pyrethrum FS EC, based on natural pyrethrins showed a fast initial action and better effectiveness than azadirachtin based product NeemAzal T/S. Efficacy of Pyrethrum FS EC, applied at a concentration of 0.1% reached 96.5% and 86.5% on the 5th day for larvae and adults, respectively, and 100% on the 7th day after the treatment for both larvae and adults.

The action of botanical insecticide NeemAzal T/S with the active substance azadirachtin was delayed. The efficacy at a concentration of 0.3% was 81.8% and 63.7% on the 7th day after the treatment for larvae and adults, respectively. At its low concentration of 0.1%, the NeemAzal T/S had insufficient effect and efficacy was only was 59.3% and 29.8% on the 7th day after the treatment for larvae and adults, respectively.

These results indicate that microbial product Naturalis (*Beauveria bassiana*) and natural pyrethrin-based product Pyrethrum FS EC, applied at both concentrations and the azadirachtin based insecticide NeemAzal T/S, applied at the registered concentration for other pests of 0.3% could successfully controlling the Colorado potato beetle *Leptinotarsa decemlineata*.

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