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Test of bio-insecticidal activity of tobacco extracts against *Leptinotarsa decemlineata* Say (Coleoptera: *Chrysomelidae*)

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Abstract

Radev, Zh. & Stoyanova, L. (2023). Test of bio-insecticidal activity of tobacco extracts against *Leptinotarsa decemlineata* Say (Coleoptera: *Chrysomelidae*). *Bulg. J. Agric. Sci., 29*(6), 1075–1078

In the present study under laboratory conditions, the bio insecticidal activity of tobacco leaf and seed extracts against larvae of *Leptinotarsa decemlineata* Say (Coleoptera: *Chrysomelidae*) were tested. Higher toxicity was recorded of the tobacco leaf extract compared to the tobacco seed extract. This is most likely due to the nicotine content in the leaves, while it's absent in the seeds. The highest mortality of Colorado beetle larvae 90.5% was reported for the tobacco leaf extract at concentration of 5%. Tobacco seed extract showed 46.7% efficacy at 5% concentration, and this efficacy was similar at 3% concentration of tobacco leaf extract. Differences were found between efficacy of the different concentrations of the extracts. Phytotoxicity of eggplants *Solanum melongena* L. was not reported during experimental treatments at a concentration of 5 % with both extracts. This study demonstrated the potential for the use of non-nicotine extracts in the control of plant pests of *S. melongena* of Solanaceae family.

Keywords: tobacco; bioinsecticide; colorado beetle; *Leptinotarsa decemlineata*

Introduction

The Colorado beetle *Leptinotarsa decemlineata* Say is one of the pests of great economic importance in agriculture in plants of the *Solanaceae* family. One of the most important plant species of *Solanaceae* family are eggplant, tomato, pepper, potato and tobacco (Shah et et al., 2013). In potatoe *Solanum tuberosum* L. production, yields can decrease up to 30% (Mateeva-Radeva, 1997), and in eggplant *Solanum melongena* L. and direct varieties tomatoes *Lycopersicon esculentum* Mill. up to 60%.

The fight against Colorado beetle is difficult and is mainly carried out by using chemical substances, but its resistance to chlorinated hydrocarbons, organophosphates, carbamates and pyrethroids (Forgash, 1985) leads to the development of biological control methods and the search for alternative methods of control. The species *L. decemlineata* was attracted by the smell of host plants, the smell of non-hosts plants neither repels nor attracts it. The attractiveness of the odor of the host can be neutralized in an odor mixture of plant species and thus will deter away the pest (Thiery & Visser, 1987). All biological methods allowing the regulation of the pest and the minimum use of pesticides in order to avoid resistance should be applied (Weber, 2003). When a well manage system of measures is applied, the results are higher (Kalinina, 2017).

With the admission of Bulgaria to the European Union, it has the obligation to apply the agro-ecological schemes as part of acquits communautaire (Bozukov et al., 2013). In recent years, there has been increasing interest in the biological production of a number of crops, including tobacco (McNeil et al., 2010). Tobacco could be used as a biopesticide (Dawitri, 2013). Tobacco have been used for centuries to control agricultural pests (McIndoo, 1943). In traditional pharmacopeia, tobacco is often mentioned as an efficient pesticide. This activity is usually due to nicotine, but tobacco

contain also other alkaloids that could potentially forward this effect (Weber et al., 2019). Pesticide activity in tobacco bio-oil was studied (Bedmutha, 2008; Gozan et al., 2014). Changes in climate conditions on a global scale lead to difficulty and face plant protection to a serious test in the protection of agricultural crops. Petkova et al. (2021), found efficacy of *Beauveria bassiana* strain 339 against adults and larvae of *L. decemlineata* under laboratory conditions. An entomopathogenic effect was established on the 7th day after treatment, when 90% larval mortality and 40% adult mortality were reported. Endophytic yeast can also be used for biological control (Petkova et al., 2022).

Pesticides used to combat against *L. decemlineata* not only pollute the environment, but also increase the cost of implementing the plant protection method (Bekuzarova et al., 2005). The chemicals content in plants shows bioactivity against insects (Listiyani, 2012). Basiev et al. (2019), successfully use the plants jimson weed, white hellebore and Camelina to combat the Colorado beetle. They contain a lot of alkaloids and essential oils that frighten off potato pests. Studying a chemical composition of plants is needed (Kang & Baldwin, 2006), because biological products are not cheap and their repeated use increases the cost of production (Fernandez et al., 2007).

The study aimed to determine the ability of the bio insecticidal activity of tobacco of leaf and seed extracts from oriental tobacco grown under organic production against *Leptinotarsa decemlineata* Say.

Material and Methods

Plant material

Tobacco seeds and tobacco leaves from Bulgarian variety Krumovgrad 58 were used. The tobacco was grown in the conditions of organic production in a certified experimental field Gotse Delchev at Tobacco and Tobacco Products Institute, Markovo, Bulgaria, under "Technology for organic tobacco production" developed at the Institute of Tobacco and Tobacco Products (Bozukov et al., 2019). Seeds and leaves were dried and grounded.

Extracts preparation

96% Ethanol (CAS Number 64-17-5), Methanol (CAS Number 67-56-1), 37% hydrochloric acid (CAS Number 7647-01-0), glycerin (CAS Number 56-81-5), Activated charcoal (CAS Number 7440-44-0) were used. Ultrasonic bath "Elma transonic T 460/H" with frequency 460 H, Analytical balance "Sartorius", UV/VIS Spectroquant Pharo 300, Merck, Germany.

Ultrasound extraction of extract was performed by methodology described by Richa et al. (2020) with some modifications. 2.5 g of the tobacco material (seeds and leaves) were weighted on an analytical balance to the nearest 0.001 g, placed in a 50 mL flask and filled with 25 mL of ethanol. Extraction was performed with an ultrasonic bath at 60°C for 60 minutes. The obtained extracts were filtered through a simple filter to remove organic matter into pre-weighed 50 mL graduated flasks on an analytical balance. The filtrates were evaporated on a vacuum evaporator at 70°C. The obtained extract-fraction was diluted in 10 mL of absolute alcohol, from which the appropriate concentrations for pest treatment were obtained by dilution with water (1%, 3%, 5%).

Quantitative analysis of nicotine content

0.1 g of the sample was weighed on an analytical scale and placed in a 250 mL flask. 98 mL extraction solution (methanol/ 37% hydrochloric acid/water at ratio 1/10/39) and 2 mL activated charcoal were diluted into a flask. The extraction was performed on a mechanical shaker for 15 minutes. The nicotine content was determined after filtration and spectrophotometric determination at multi-wavelength 236, 259, 282 nm, respectively. The result is expressed in percentage according to the formula:

$$C, \% = 1.059 \times (A_{259} - \frac{A_{236} + A_{282}}{2}),$$

where C, % – nicotine content

1.059 – correction factor

A_{236}, A_{282}, A_{259} – absorption wavelength – 236 nm, 282 nm, 259 nm.

Collection of Colorado beetles

Larvae of the Colorado beetle were collected from a 0.1 ha eggplant *Solanum melongena* L. crop located in Belozem district of Plovdiv.

Treatment

The *in vitro* study of the insecticidal activity of tobacco seeds and leaves oil was done in laboratory conditions in 2022. The oils were tested in three concentrations 1%, 3% and 5%. Each concentration was tested on 10 larvae placed on filter paper in 90 × 20 mm Petri dishes. Each variant was repeated three times. The larvae of each test variant were sprayed with the respective oil concentration. Petri dishes were covered and left at room temperature of 23–24°C. Mortality was taken after 24th, 48th and 72nd hour after treatment. The efficacy of the oil concentration was calculated according to Abbott (1925):

$$E (\%) = (T - t)100)/T,$$

where: T – is number of alive larvae in control;

t – is number of alive larvae on each treatment.

Phytotoxicity tests of both extracts by spraying on eggplant plants *S. melongena* under field conditions were made.

Statistical analysis

The data were statistically processed by using one-way ANOVA in Excel at $p \leq 0.05$.

Results and Discussion

In ultrasound-assisted extraction, the plant`s raw material is immersed in water or another solvent (methanol or ethanol or anyone from the solvents) and at the same time, it is subjected to the work of ultrasound (Assami et al., 2012). This type of extraction method has been used in the extraction of essential oil from tobacco leaves and seeds with 95 % ethanol as a solvent. Plant concentration in all samples was 10 %.

The yield of extract from the seeds was 32.5 %, while from the leaves – 43.15% (Table 1). The results are higher than those reported from Puripattanavong et al. (2013) (18.15% with 95% ethanol), who also reported that ethanol is the solvent with the highest percent yield of extraction. The obtained results show different qualitive and quantitive composition of the extracts.

Table 1. Nicotine content in samples from Oriental Tobacco, variety Krumovgrad 58

Sample	Seeds	Leaves
	Nicotine, %	Nicotine, %
Row material	< LOD/LOQ*	1.18
Extract	< LOD/LOQ*	0.21

Seeds, leaves and obtained extracts were analyzed for nicotine content. The nicotine content of the study samples is presented in Table 1. The obtained results show, that the tobacco seeds grown in the organic production and the extracts obtained from the seeds do not contain nicotine. The nicotine in the tobacco leaves extract is five times higher than the content of nicotine in the extract.

The results of the study showed bio-insecticidal efficacy in both types of extracts. Higher toxicity was recorded of the tobacco leaf extract compared to the tobacco seed extract (Figure 1).

This is most likely due to the nicotine content in the leaves, while it is absent in the seeds (Table 1). According to Ekapratiwi et al. (2019), tobacco leaf extract has larvicidal activity because it contains nicotine and some toxic compounds. The highest mortality of Colorado beetle larvae 90.5% was reported for the tobacco leaf extract at concentration of 5%. Tobacco

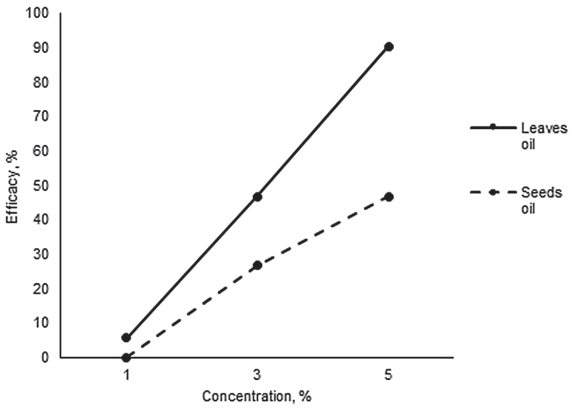

Fig. 1. Bio-insecticidal activity of tobacco leaf and tobacco seed extracts against *L. decemlineata* at different concentrations

seed extract showed 46.7% efficacy at 5% concentration, and this efficacy was similar to 3% concentration of tobacco leaf extract. At 1% concentration of tobacco leaf extract mortality was only 5.6%, and no mortality was recorded of tobacco seeds extract of the same concentration. Differences were found between the efficacy of the different concentrations of the extracts (F > F crit, Anova: Single factor, p = 0.000) (Figure 1). The control groups were treated with 1%, 3% and 5% ethanol, and showed no mortality. Phytotoxicity of eggplant plants was not reported during experimental treatments at concentration of 5% with both types of extracts.

This study shows the potential for expansion and use of tobacco as a feedstock for control of pests of eggplant from the *Solanaceae* family. Most importantly, that the tobacco seed extract not contains nicotine, but showed bio-insecticidal activity against *L. decemlineata* larvae. Due to the lack of nicotine, it showed less efficacy, but it is possible to increase the concentration in order to achieve higher efficacy. Toxic effect of tobacco bio-oil from leaves with and without nicotine content have been established on the Colorado beetle (Booker et al., 2010). Further studies of the chemical composition of tobacco seed oil are needed to determine which components are responsible for its insecticidal activity.

The use of biological approaches in agriculture will lead to a reduction in the load of chemical substances on the environment and also improve the quality of the products (Basiev et al., 2019).

Conclusions

Tobacco seed extract showed bio-insecticidal efficacy of about 50% at 5% concentration against larvae of *Leptinotarsa decemlineata* Say.

Higher toxicity was recorded of the tobacco leaf extract compared to the tobacco seed extract, probably due to the nicotine content in the leaves, while it is absent in the seeds.

Phytotoxicity of eggplant plants was not reported during experimental treatments.

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