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Study for bioinsecticidal activity of tobacco extracts against *Helicoverpa armigera* Hübner (Lepidoptera: *Noctuidae*)

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Abstract

Radev, R. & Stoyanova, L. (2024). Study for bioinsecticidal activity of tobacco extracts against *Helicoverpa armigera* Hübner (Lepidoptera: *Noctuidae*). *Bulg. J. Agric. Sci., 30* (3), 486–490

Bioinsecticidal toxicity of tobacco leaf and seed extracts against 2nd and 3rd larva instar of *Helicoverpa armigera* Hübner (Lepidoptera: *Noctuidae*) was studied in laboratory conditions. There was found higher toxicity of tobacco leaf extract against *H. armigera* 2nd and 3rd larva instar compared to the tobacco seed extract. These differences are probably owing to the nicotine content in the leaves, while it is missing in the seeds. There was found difference in insecticidal toxicity between *H. armigera* 2nd and 3rd larva instar. The highest effectiveness of tobacco extracts against cotton bollworms were found in 2nd larva instar. When applied concentration of 5% leaf extract there was found 93.3% mortality in 2nd instar and 56.7% mortality in 3rd instar. They were similar results when compared 5% seed extract, 80% mortality in 2nd instar and 43.3% mortality in 3rd instar. No phytotoxicity of tobacco plants was found with both types of extracts. This research showed the possibility for the applying of non-nicotine extracts in the pest control of tobacco plants.

Keywords: tobacco; bioinsecticide; cotton bollworm; *Helicoverpa armigera*

Introduction

The cotton bollworm *Helicoverpa armigera* Hübner (Lepidoptera: *Noctuidae*) is a pest, which atacking more than several hundreds of plant species and causes severe damages. The species is spread worldwide (Chari et al., 1990; Liu et al., 2004; Mehrvar, 2009), shows a high level of polyphagia (Jallow & Zalucki, 1996), and is one of the major agricultural pests (Reddy et al., 2004; Yu et al., 2008) as it does a huge crops damage (Fitt, 1989). There is an impact of different host crops over: the *H. armigera* development (Gomes et al., 2017); on the intensity of food consumption of the larvae (Suzana et al., 2015) and the adults' longevity (Ruan & Wu, 2001; Kulkarni et al., 2004).

Caterpillars of *H. armigera* attack the host species at different phenological stages but they prefer feeding on the reproductive organs of their hosts (Wackers et al., 2007; Rogers & Brier, 2010; Bortolotto et al., 2014). According to Cunningham & Zalucki (2014) the *H. armigera* is the economically most important pest of the genus *Helicoverpa*. This insect species does an enormous damage of tobacco plants (Annecke & Moran, 1982), and primarily in the seed tobacco plantations (Dimitrov et al., 2005).

A promiscuous using of pesticides, during 80s and 90s in the last century was giving severe outbreaks of *H. armigera* (Ahmad et al., 1997). An according to evidences the cotton bollworm increasing its stability to chemical insecticides (Qayyum et al., 2015). There are integrated programs for *H. armigera* control with aim to limit the use of pesticides and rising the impact of natural enemies (Kuklinski & Borgemeister, 2002; Mensah, 2002). It seems that plant pesticides are effectiveness against pests and safety to the natural enemies (Srinivasa et al., 1999).

Many extracts of neem have given satisfactory to control of *H. armigera* (Sachan & Lal, 1990; Schmutterer, 1990; Dubey et al., 1991). More than ten years the essential oils

from plants have an increasing adoption (Koul et al., 2008). The thymol could be applied as alternatively control of bollworms (Bovornnanthadej et al., 2013). There is an interest of biological production of tobacco (McNeil et al., 2010), and tobacco could find application as botanical pesticide (Dawitri, 2013), as it been used since very long term for pest control in the farming (McIndoo, 1943).

The purpose of the study was to determine the ability of the bioinsecticidal efficacy of tobacco of leaf and seed extracts from oriental tobacco grown under organic production against *Helicoverpa armigera* Hübner.

Material and Methods

Plant material

Tobacco plant from Bulgarian variety Krumovgard 58 was used. Plant material – tobacco seeds and leaves were collected from certified organic field Gotse Delchev. 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.

Extraction procedure was described by Richa et al. (2020). Some modifications were made. Ultrasound extraction was performed with an ultrasonic bath at 60°C for 60 min. The extracts were obtained from 2.5 g of the tobacco material (seeds and leaves) and 25 mL of ethanol. They were put into 50 mL flask and after sonication were filtered through a simple filter to remove organic matter. 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 min. 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:

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

where C, % – nicotine content;

1.059 – correction factor;

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

Collection of cotton bollworms

The *Helicoverpa armigera* larvae collection was done according to Papulwar et al. (2018) methodology from tobacco plants at the Tobacco and tobacco products institute, Markovo.

Treatment

The *in vitro* research of the bioinsecticidal activity of tobacco seeds and leaves oil was done under laboratory conditions during 2022. The extracted oils were studied in three concentrations 1%, 3% and 5%. The 2nd and 3rd larvae instars were determined according to DPI&F (2005) methodology. 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 tobacco plants under laboratory 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

Ultrasound – assisted extraction was used as a method for tobacco extracts preparation. It is fast, cheap and accessible method. 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). Tobacco extracts were obtained with 95% ethanol as a solvent.

Seeds, leaves and obtained extracts were analyzed for nicotine content. 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. Nicotine content in leaves as a row material was 1.18 % and the nicotine content in the leaves extract was 0.21% (Figure 1). The obtained results show different qualitive and quantitative composition of the extracts.

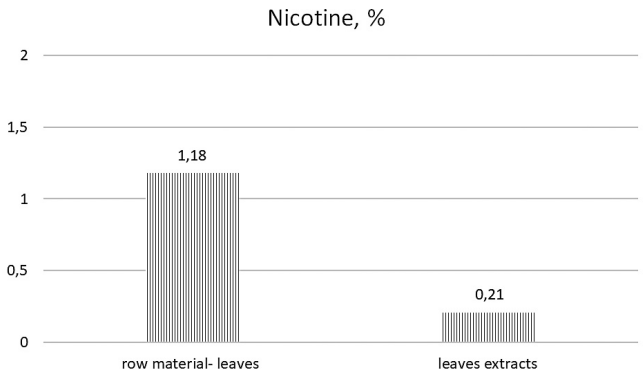

Fig. 1. Nicotine content in samples from Oriental Tobacco, variety Krumovgrad 58

The obtained data of the research showed insecticidal effectiveness of tobacco leaf and tobacco seed extracts. There was found higher toxicity of tobacco leaf extract against *H. armigera* 2nd and 3rd larva instar compared to the tobacco seed extract (Figure 2 and Figure 3). These differences are probably owing to the nicotine content in the leaves, while

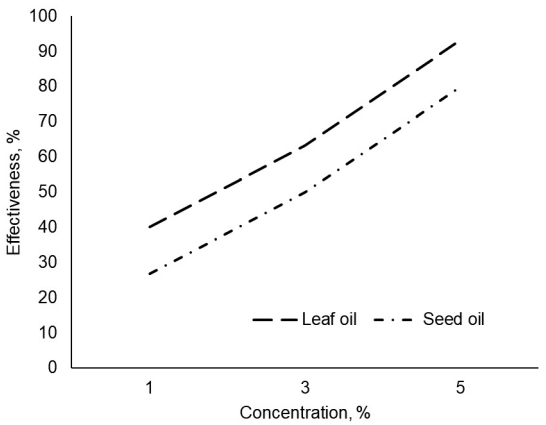

Fig. 2. Insecticidal activity of tobacco leaf and tobacco seed extracts against *H. armigera* 2nd larva instar at different concentrations

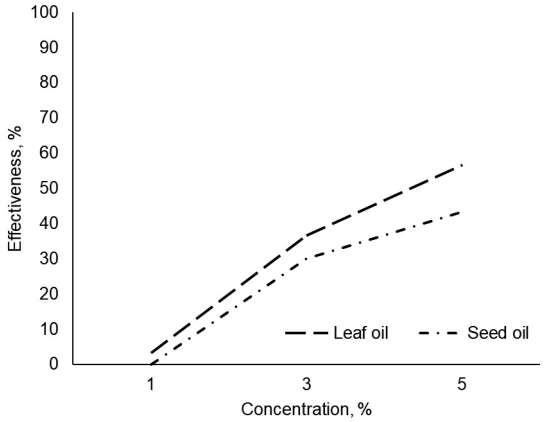

Fig. 3. Insecticidal activity of tobacco leaf and tobacco seed extracts against *H. armigera* 3rd larva instar at different concentrations

it is missing in the seeds (Figure 1). Leaf extract of tobacco indicate larvicidal activity as it contains nicotine and some toxic compounds (Ekapratiwi et al., 2019).

Also there was found difference in insecticidal toxicity between *H. armigera* 2nd and 3rd larva instar. The highest effectiveness of tobacco extracts against cotton bollworms were found in 2nd larva instar. When applied concentration of 5% leaf extract there was found 93.3% mortality in 2nd instar and 56.7% mortality in 3rd instar. They were similar results when compared 5% seed extract, 80% mortality in 2nd instar and 43.3% mortality in 3rd instar (Figure 2 and Figure 3).

The insecticidal activity at 3% tobacco leaf and seed extracts showed very good efficacy when applied to 2nd larva instar, respectively 63.3% and 50% compared to 3rd larva instar respectively 36.7% and 30%. At 1% concentration of tobacco leaf and seed extract mortality of 2nd larva instar was 40% and 26.7%, while in 3rd instar mortality was only 3.3% when applied tobacco leaf extract, and no mortality was recorded of tobacco seeds extract of the same concentration. They were found differences when compared the efficacy of the different concentrations of the extracts ($F > F_{crit}$) (Figure 2 and Figure 3). The control groups were treated with 1%, 3% and 5% ethanol, and was found no mortality, and also no phytotoxicity of tobacco plants was found with both types of extracts.

That research displays the potential for control of cotton bollworm of tobacco plants by using of tobacco as a feedstock. It was found, that the tobacco seed extract not contains nicotine, and has very good bioinsecticidal toxicity on 2nd larva instar of *H. armigera*. It is need more deep research of the chemical composition of tobacco seed oil to identify the compounds liable for its insecticidal activity. The applying

of biological methods of pest control in the agriculture need to be use.

Conclusions

The highest effectiveness of tobacco leaf and seed extracts against cotton bollworms were found in 2nd larva instar.

It was found, that the tobacco seed extract not contains nicotine, and has very good bioinsecticidal toxicity on 2nd larva instar of *H. armigera.*

No phytotoxicity of tobacco plants was found with both types of extracts.

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