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BIOLOGICAL EFFICIENCY OF THE EXTRACT OF *Haplophyllum perforatum* AGAINST *Tuta absoluta* AND ITS INFLUENCE ON THE PHYSIOLOGICAL PROPERTIES OF TOMATO PLANTS

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Abstract

The tomato leaf miner *Tuta absoluta* (Meyrick) (*Lepidoptera: Gelechiidae*), whose natural range includes the countries of South America, has become widespread in Africa and Europe. Since 2015, the pest has been registered in the Republic of Uzbekistan. It is known to develop resistance to many chemical plant protection products. In addition, pesticides can significantly change the intensity of plant metabolic processes, in particular photosynthesis, which reduces the productivity and quality of the crop. In this regard, plant substances are of interest, the use of which is promising in plant protection. The present work, for the first time, shows the biological effectiveness of the application of the extract of the plant *Haplophyllum perforatum* against the larvae of the tomato leaf miner in the field. The extract had a positive effect on the content of chlorophylls in tomato leaves and increased the total leaf area of the plant. The aim of our work was to study the biological effectiveness of extract of the plant *Haplophyllum perforatum* and its complex with the growth regulator Uchkun against larvae *Tuta absoluta*, as well as their effect on the physiological parameters of tomato. The field experiment was carried out on the area farming facilities "Super Garden" (Tashkent region, Kibray district). Tomato plants of the TMK-22 variety were sprayed with an extract of *H. perforatum* and its composition with growth regulator Uchkun, which was developed on the basis of polyprenols isolated from cotton leaves. We also used the insecticide of natural origin Proclaim, the active substance of which is 5% emamectin benzoate (Syngenta Crop Protection AG). The seedlings were sown on April 24, 2020. The placement of the plots is randomized, in four repetitions. Spraying was carried out in the phase of the beginning of flowering of tomatoes in the presence of pest numbers not lower than the economic threshold of harmfulness. The design of the experiments was as follows: control (option without treatment), standard Proclaim, 5% (W.S.G-water-soluble granule, 0.4 kg/ha), extract of *H. perforatum*, 1.0% (W.E-water emulsion, 0.4 kg/ha), composition — *H. perforatum* extract, 1.0% + Uchkun, 0.0001% (W.S.E, 0.4 kg/ha). The biological effectiveness of the extracts was evaluated by reducing the number of pest larvae. For 1 day before treatment, on days 3, 7 and 14 after treatment, larvae of the 1st-2nd and 3rd-4th instars were counted. The content of chlorophylls was estimated by the spectrophotometric method. During the growing season, biometric indicators were recorded: leaf surface area, plant height, number of leaves, flowers and fruits. The total leaf surface area was determined by the gravimetric method. We have revealed high toxicity of the *H. perforatum* extract and its composition with the Uchkun preparation against the tomato leaf miner. The greatest decrease in the number of pest larvae occurred on day 7 after spraying tomatoes. Against an increase in the number in the control, the effectiveness of the extract against larvae of the 1st-2nd instar was 87.1%, of the 3rd-4th instar — 77.5%. For the treatment with the composition (84.1 and 70.0%) and the insecticide (87.0 and 70.0%), the efficiency was almost comparable. Treatment of plants with the studied solutions contributed to an increase in the content of photosynthetic pigments in damaged leaves. After exposure to the extract, the content of chlorophyll a was higher than the control by 1.1 times, chlorophyll b — by 1.8 times, their sum — by 1.5 times, after treatment with the composition, the amount of chlorophyll a and b exceeded the control by 2.2 and 2.1 times, their sum — by 1.7 times. An increase in leaf surface area was also observed: after treatment with *H. perforatum* extract, the indicators were higher

than the control by 75.0 %, with the composition — by 58.3 %, the number of leaves increased by 85.1 and 89.9 %, respectively. In the variant with the use of the extract, the number of flowers was 8.3 pcs/plant, fruits — 3.8 pcs/plant, when treated with the composition — 8.9 and 4.1 pcs/plant, with Proclaim — 7.8 and 3.5 pcs/plant, in the control — only 2.2 and 1.2 pcs/plant. Thus, we have shown the possibility of using the plant extract of *H. perforatum* against the tomato leaf miner. It has been established that treatment with a composition of *H. perforatum* extract with a growth regulator improves the physiological and biochemical parameters of tomato plants.

Keywords: *Haplophyllum perforatum*, extract, *Tuta absoluta*, biological effectiveness, photosynthetic pigments, leaf area, biometric parameters

The tomato (*Lycopersicon esculentum* Mill., family *Solanaceae*) is one of the most widely cultivated vegetables in the world. More than half of world production (56.71%) is concentrated in four countries. China remains the world's leading tomato producer (31.81%), followed by India (10.39%), the US (7.36%) and Turkey (7.12%). The growth of tomato production in the period from 2005 to 2016 was 29.08% with an average annual growth rate of 3.14% [1]. In the Republic of Uzbekistan, approx. 40% area is allocated for the cultivation of tomatoes from the crops of other vegetable crops. In a large volume, products are exported abroad [2].

In recent years, the mass distribution of the tomato leaf miner *Tuta absoluta* (Meyrick) (*Lepidoptera: Gelechiidae*) has been observed in the republic, which leads to significant losses in tomato crops. This species is included in the EPPO European and Mediterranean Plant Protection Organization list of quarantine pests [3]. After the invasion of Spain in 2006, it quickly spread throughout Afro-Eurasia and became a major threat to global tomato production [4]. The emergence of *T. absoluta* in the republic has been confirmed in greenhouses and open fields since 2015 [5].

Mining-type damage caused by *T. absoluta* larvae in the mesophyll of leaves, young shoots and fruits sharply reduces their photosynthetic ability, which leads to a reduction in the number of ripe fruits formed, their size and quality. In addition, due to damage, secondary pathogenic microorganisms, including putrefactive ones (saprophytic fungi and bacteria), penetrate into plant tissues, which leads to shedding of unripe fruits and a sharp decrease in the quality of harvested fruits, their commercial value and a general drop in yield [6].

T. absoluta has been reported to develop resistance to chemicals [7, 8], so while there is a need to improve plant protection against this pest, the use of synthetic insecticidal compounds needs to be reduced [9].

Agricultural intensification, especially in exporting developing countries, is leading to the widespread use of pesticides, which affects soil quality, populations of non-target organisms and human health [10, 11]. Pesticides can significantly change the intensity of plant metabolic processes, in particular photosynthesis, which leads to a decrease in productivity and crop quality [12-14]. There are plant species that have a toxic effect on insect pests that can be used to obtain biological products [15, 16]. Previously, we have established a high insecticidal activity of the extract of *Haplophyllum perforatum* A. Juss [17, 18].

It is known that plant growth regulators are used to eliminate the negative impact of various stress factors. So, the biostimulator Uchkun, developed at the Yunusova Institute of Chemistry of Plant Substances of the Academy of Sciences of the Republic of Uzbekistan, has a stress-protective activity when cultivating plants in conditions of salinity and water deficiency [19-21].

In this work, for the first time, the biological effectiveness of the extract of the plant *Haplophyllum perforatum* against the larvae of the tomato leafminer in the field is shown. The extract had a positive effect on the content of chlorophylls in tomato leaves and increased the total area of the leaf surface.

The aim of our work was to study the biological effectiveness of the plant extract of *Haplophyllum perforatum* and its complex with the growth regulator Uchkun against *Tuta absoluta* larvae, as well as their effect on the physiological parameters of tomato.

Materials and methods. The field experiment was carried out at the site of the Super Garden farm (Tashkent region, Kibray district). Tomato plants of the TMK-22 variety were sprayed with an extract of *H. perforatum* and its composition with growth regulator Uchkun, which was developed on the basis of polyphenols isolated from cotton leaves [19]. We also used the natural insecticide Proclaim (Proclaim®), the active ingredient of which is 5% emamectin benzoate (Syngenta Crop Protection AG, Switzerland) [22].

For experiments, tomato plants of the TMK-22 variety were planted on plots by the nesting method [23]. The soil of the site is medium loamy gray. The seedlings were sown on April 24, 2020. Placement of plots was randomized, with a 4-fold repetition. The plants were sprayed with a knapsack pneumatic sprayer, the consumption of working solutions was 3 l/100 m². Spraying was carried out at the beginning of flowering of tomatoes with the pest population not lower than the economic threshold of harmfulness. During the experiments, the temperature was 30-34 °C, air humidity 23-28%, daylight for 15 h.

The scheme of experiments was as follows: control (without treatment), standard Proclaim, 5% (WSG — water-soluble granule, 0.4 kg/ha), extract of *H. perforatum*, 1.0% (WE — water emulsion, 0.4 kg/ha), and a composition of 1.0% *H. perforatum* extract + 0.0001% Uchkun (WE, 0.4 kg/ha).

The biological effectiveness of the extracts was evaluated by reducing the number of pest larvae according to the formula C.F. Henderson and E.W. Tilton (W.S. Abbot formula adjusted for control) [24]:

$$C = \frac{nK \text{ (prior treatment)} \times nB \text{ (after treatment)}}{nK \text{ (after treatment)} \times nB \text{ (prior treatment)}} \times 100 \%,$$

where n is the number of pest larvae, B is the experiment, K is the control.

One day before treatment, on days 3, 7, and 14 after treatment, larvae of 1-2 and 3-4 instars were counted in accordance with the guidelines of V.I. Dolzhenko [25] and the working program drawn up before the treatment of plants with preparations.

Chlorophyll content was assessed spectrophotometrically using a V-5000 spectrophotometer (Metash Instruments Co., Ltd., China) [8]. During the growing season, biometric indicators were taken into account: leaf surface area, plant height, number of leaves, flowers, and fruits. The total leaf surface area was determined by the gravimetric method [26].

Mathematical processing of the obtained data and calculation of statistical parameters were carried out using the Microsoft Excel 2016 software package. Average values of the indicators (M) and standard errors of the means (\pm SEM) are presented. To compare data, combined on the same trait, we used a one-dimensional one-way analysis. When evaluating the ratio of intergroup variability, Student's t -test was used to assess the null hypothesis of the equality of means for the treatments for a significance level of $p < 0.05$.

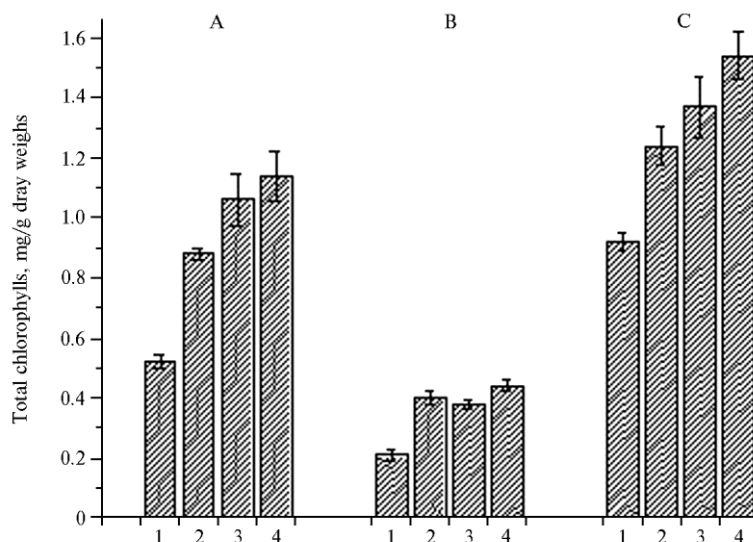
Results. The biological effectiveness of the extract of *H. perforatum* on the day 3 against the larvae of the tomato miner moth *T. absoluta* of the 1st-2nd age was 81.0%, of the 3rd-4th age 83.6%. The effectiveness of the composition was somewhat lower, 73.3 and 69.6%, respectively, of the insecticide Proclaim 78.0 and 76.0% (Table 1).

1. Biological efficiency of the *Haplophyllum perforatum* A. Juss extract and its composition with growth regulator Uchkun against *Tuta absoluta* (Meyrick) larvae of 1-2 and 3-4 instars on tomato *Lycopersicon esculentum* Mill. cv. TMK-22 ($n = 4$, $M \pm SEM$; Tashkent region, Kibray district, 2020)

Treatment	Prior treatment			Days after extract of								
	1-2 instars	3-4 instars	total	3-и			7-е			14-е		
				1-2 instars	3-4 instars	total	1-2 instars	3-4 instars	total	1-2 instars	3-4 instars	total
The number of larvae per plant												
Control (without treatment)	5.7±0.3	6.5±0.5	12.2±0.6	9.7±1.2	8.8±0.6	18.5±0.7	12.7±0.8	10.7±0.8	23.4±1.3	17.7±1.3	14.0±0.5	31.7±1.5
Proclaim (standard), 0.4 kg/ha	6.5±0.9	4.0±0.2	10.5±0.9	2.4±0.5	1.3±0.2	3.7±0.4	1.9±0.5	1.8±0.3	3.9±0.5	3.2±0.5	2.9±0.8	6.1±0.9
Extract, 0.4 kg/ha	6.2±0.9	5.4±0.6	11.6±0.5	2.0±0.4	1.2±0.0	3.2±0.3	1.8±0.2	2.5±0.4	3.8±0.3	3.4±0.5	3.0±0.6	6.4±0.1
Composition, 0,4 kg/ha	5.5±0.6	3.4±0.5	8.9±0.9	2.5±0.6	1.4±0.5	3.9±0.5	2.0±0.3	1.7±0.3	3.7±0.6	2.9±0.5	3.4±0.6	6.3±1.0
Biological efficiency, %												
Control (without treatment)												
Proclaim (standard), 0.4 kg/ha				78.0	76.0	77.0	87.0	72.6	81.0	84.1	66.3	77.6
Extract, 0.4 kg/ha				81.0	83.6	82.0	87.1	71.9	83.0	82.3	74.2	78.7
Composition, 0,4 kg/ha				73.3	69.6	71.1	84.1	70.0	78.3	83.0	53.6	73.0

We observed the greatest decrease in the number of pest larvae on day 7. With an increase in the number in the control, the effectiveness of the extract against larvae of the 1st-2nd age was 87.1%, of 3-4th age 77.5%. With the use of the composition and the insecticide, the efficiency was practically comparable (see Table 1). On day 14 after spraying, the biological effectiveness of the extract remained (82.3 and 74.2%), the biological effectiveness of the composition against larvae of the 1st-2nd age remained high (83.0%), of the 3rd-4th age decreased (53.6%).

Treatment of tomato plants with *H. perforatum* extract contributed to a significant increase in the amount of photosynthetic pigments: 7 days after treatment, the content of chlorophyll a in the leaves was 1.1 times higher (1.06 mg/ml, $p < 0.05$) than in the control, chlorophyll b was 1.8 times higher (0.38 mg/ml, $p < 0.05$), their sum was 1.5 times higher (1.37 mg/ml, $p < 0.05$) (Fig.).



The content of chlorophyll a (A), chlorophyll b (B) and total chlorophyll (C) in the leaves of tomato *Lycopersicon esculentum* Mill. cv. TMK-22 after treatments: 1 — control (without treatment), 2 — Proclaim (standard), 0.5 %, 3 — *Haplophyllum perforatum* A. Juss extract, 1.0 %, 4 — composition of the *H. perforatum* extract, 1.0 % + Uchkun, 0.0001 % ($n = 4$, $M \pm SEM$; Tashkent region, Kibrai district, 2020).

When using the composition, the amount of photosynthetic pigments was significantly higher. The content of chlorophyll a (1.14 mg/ml) and chlorophyll b (0.44 mg/ml) exceeded that in the control variant by 2.2 and 2.1 times, respectively ($p < 0.05$), their sum (1.54 mg/ml) by 1.7 times. The high content of photosynthetic pigments in the experiment with the use of the composition may be due to the synergistic effect of the biologically active substances of the Uchkun preparation in combination with the substances of the *H. perforatum* extract. Due to the decrease in the number of the pest after treatment with the insecticide Proclaim, an increased content of chlorophyll was also observed compared to the control, for chlorophyll a (0.88 mg/ml) by 1.7 times ($p < 0.05$), for chlorophyll b (0.40 mg/ml) by 1.9 times, for their sum (1.24 mg/ml) by 1.3 times ($p < 0.05$) (see Fig.).

Also, due to the decrease in the number of larvae of *T. absoluta*, the growth rate of the leaf surface area in the budding phase increased after spraying the plants

with the extract of *H. perforatum* (105 cm² per plant) and the composition (95 cm²/plant), while in the control, it was 60 cm² per plant (Table 2). The most intensive growth rates occurred at the beginning of fruit formation, with the use of the extract 850 cm² per plant, with the composition 855.3 cm² per plant. In these options, these values significantly exceeded the control (440 cm² per plant) and standard (780 cm² per plant). The decrease in the leaf surface area before the mass ripening of fruits occurred mainly due to the death of the main part of the leaves of the lower tier (see Table 2).

2. Total leaf area (cm²) in tomato *Lycopersicon esculentum* Mill. cv. TMK-2 plants treated with an extract of *Haplophyllum perforatum* A. Juss. and its composition with growth regulator Uchkun ($n = 4$, $M \pm SEM$; Tashkent region, Kibrai district, 2020)

Treatment	Stage of growth				
	intensive growth	budding	blossoming	fruit formation	ripening
Control (without treatment)	45.3±3.0	60.0±2.5	99.0±1.5	440.3±3.2	310.3±0.5
Proclaim (standard), 0.4 kg/ha	49.0±2.0	89.0±1.0	165.0±3.7	780.0±3.2	615±3.0
Extract, 0.4 kg/ha	55.3±1.5	105.0±2.5	255.0±3.0	850.0±3.7	650.3±1.5
Composition, 0.4 kg/ha	50.0±1.0	95±1.0	230±2.5	855.3±2.5	640.0±2.6

Spraying plants with *H. perforatum* extract and its composition with a growth regulator had a significant effect on the biometric parameters of tomato plants. When treated with the extract, the height of plants was 1.7 times ($p < 0.05$) higher than in the control; with the composition, it was 1.8 times higher ($p < 0.05$) (Table 3). In terms of the number of leaves, these variants exceeded the control one by 1.8 and 1.9 times, respectively ($p < 0.05$), and the values were comparable to the results of insecticide application. By reducing the negative impact of pests on plant development, the number of flowers and fruits increased in the experimental variants (see Table 3).

3. Biometric parameters in tomato *Lycopersicon esculentum* Mill. cv. TMK-2 plants when treated with an extract of *Haplophyllum perforatum* A. Juss. and its composition with growth regulator Uchkun ($n = 4$, $M \pm SEM$; Tashkent region, Kibrai district, 2020)

Treatment	Plant height, cm	The number of leaves	The number of flowers	The number of fruits
Control (without treatment)	28.5±0.1	31.6±0.1	2.2±0.1	1.2±0.1
Proclaim (standard), 0.4 kg/ha	35.6±0.1	58.7±0.0	7.8±0.1	3.5±0.0
Extract, 0.4 kg/ha	49.5±0.1	58.5±0.1	8.3±0.0	3.8±0.1
Composition, 0.4 kg/ha	50.2±0.0	60.0±0.1	8.9±0.1	4.1±0.1

The resistance of the tomato miner moth to chemicals occurs worldwide. In Brazil, Chile and Argentina, the effectiveness of Vertimec, as well as some organophosphorus compounds and pyrethroids, against the pest has decreased [27]. Successful pest control requires multiple pesticide treatments, which speeds up the selection of the most resistant individuals in the pest population. Preparations based on carbamates (Lannat) and pyrethroids (Decis) can cause rapid death of adults and caterpillars [28]. Currently, effective agents include Tracer (spinosad), Pirate (chlorfenapyr), Aktara (thiamethoxam), Emperor (chlorpyrifos), cypermethrin, Koragen (chlorantraniliprole) [29, 30]. In Spain, LIDA Plant Research's new organic products, Ecothrin® and Acaridoil®, are used to control a wide range of pests, including *Tuta absoluta*. This unique pyrethrin-based product is produced in microcapsules for greater persistence and greater effectiveness in pest control. Acaridoil is a natural product based on oleic acids from olive oil [31].

An example of an effective pesticide based on azadirachtin from neem seeds is NimAtzal T/S. The biological, as a contact and at the same time systemic insecticide, has a good effect on the tomato mining moth. The biological effectiveness of azadirachtin (active ingredient) against *Tuta absoluta* was 80.0% [32-35].

In this work, we found that the biological effectiveness of the extract of the plant *Haplophyllum perforatum* against the tomato leaf miner in open ground conditions reached 87.1%. To a greater extent, the extract was toxic against larvae of the 1st-2nd age. Similar data were obtained with Fame (flubendiamide), Divipan, Talstar EC (bifenthrin) and Tracer, which are effective against young larvae up to 5 mm in size [31].

It is known that plant growth regulators can influence all processes of growth and development. We have shown the effectiveness of using the composition of the extract of *Haplophyllum perforatum* with growth regulator Uchkun: spraying tomato plants with this composition contributed to an increase in the content of photosynthetic pigments, leaf surface area and the number of fruit elements.

Thus, our findings indicate the high toxicity of the *Haplophyllum perforatum* extract and its complex with the growth regulator Uchkun against the tomato leaf miner *Tuta absoluta*. On day 7 after spraying the tomatoes with the extract, the biological effectiveness against larvae of the 1st-2nd age was 87.1%, of the 3d-4th age 77.5%, for composition, it counted for 84.1 and 70.0%, respectively. In damaged leaves, the content of chlorophyll a after exposure to the extract was 3.8% higher than the control, of chlorophyll b by 80.9% higher, their total amount by 48.9% higher, after treatment with the complex, the values exceeded by 119.2, 109.5 and 67.3%, respectively. With reducing the leaf damage, an increase in leaf area occurs, after treatment with *H. perforatum* extract, it was by 75.0% higher than the control, after treatment with the composition by 58.3% higher, the number of leaves increased by 85.1 and 89.9%, respectively. With the use of the extract, there was 8.3 flowers per plant and 3.8 fruits per plant, when treated with the composition, these values were 8.9 flowers and 4.1 fruits per plant, respectively, for Proclaim, 7.8 flowers and 3.5 fruits per plant, while in control only 2.2 flowers and 1.2 fruits per plant.

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