Bulgarian Journal of Agricultural Science, 28 (No 5) 2022, 901–910

The chemical composition of the *n*-butanol fraction of the extract of *Datura stramonium* and its insecticidal activity against pests of the genus *Aphididae*

S. M. Turaeva[1*], M. M. Makhmudova[1], E. R. Kurbanova[1], I. D. Babaev[1], R. P. Zakirova[1], A. O. Berestetskiy[2], A. A. Dalinova[2], I. V. Sendersky[2] and D. T. Juraev[3]

[1]*Academy of Sciences of the Republic of Uzbekistan, Institute of the Chemistry Plant Substances "Acad. S. Yu. Yunusov", 100170 Tashkent, Uzbekistan*
[2]*All-Russian Institute of Plant Protection, 196608 S. Petersburg, Russian Federation*
[3]*Southern Agricultural Scientific Research Institute, 180100, Karshi, Uzbekistan*
[*]*Corresponding author:* saidaicps@gmail.com

Abstract

Turaeva, S. M., Makhmudova, M. M., Kurbanova, E. R., Babaev, I. D., Zakirova, R. P., Berestetskiy, A. O., Dalinova, A. A., Sendersky, I. V. & Juraev, D. T. (2022). The chemical composition of the *n*-butanol fraction of the extract of *Datura stramonium* and its insecticidal activity against pests of the genus *Aphididae. Bulg. J. Agric. Sci., 28 (5)*, 901–910

The aim of this work was that the studying the chemical composition of the *n*-butanol fraction of the extract of the aerial parts of *Datura stramonium* L. plants collected in the Fergana region of the Republic of Uzbekistan and investigation of its insecticidal activity against certain types of pests of the genus of *Aphididae*.

It was found as a result of this study that the high efficiency of the *n*-butanol fraction of the extract of *Datura stramonium* L. against sucking pests of the genus *Aphididae – Aphis pomi* and *Macrosiphum euphorbiae.* Additionally, 10 mg/mL dose of this fraction also showed the first level activity with 98.5% against *Macrosiphum euphorbiae.* The level of mortality was lower at 1 mg/mL and 5 mg/mL concentration that 81.3% and 83.3%, respectively. The index was significantly low as a follows -15.1% for 1 mg/mL of the dose, 19.5% for 5 mg/mL of the dose, and 24.0% for 10 mg/mL of the dose when exposed to *Schizaphis graminum.* It was revealed that the *n*-butanol fraction of the extract of the aerial part of the *Datura stramonium* L. plant at a concentration of 1 mg/ml did not show phytotoxicity on the leaves of wheat, tomato, and cucumbers. The first time, it was established that 500 µg / ml and 100 µg/ml concentrations of the fraction showed 100% cytotoxicity against the cell line of maize deciduous scoops (*Spodoptera frugiperda*) under *in vitro* conditions and found to be non-toxic to ciliates (*Paramecium caudatum*) in 5 µl of 10 mg/mL extract.

Keywords: Datura stramonium L.; *n*-butanol fraction; insecticidal activity; *Schizaphis graminum; Aphis pomi; Macrosiphum euphorbiae;* phytotoxicity; cytotoxic activity

Introduction

The widespread and extensive use of synthetic insecticides creates urgent problems for environmental pollution. New global trends aimed at the development of ecological farming require the creation of balanced agriculture with the reduction or complete replacement of synthetic chemicals (Ojebode et al., 2016; Volociuc, 2008). A promising direction is considered that search of new secondary plant metabolites with insecticidal properties (Mohammadhos-

seini, 2021; Benelli, 2016). In the last few years, works are underway to create drugs based on terpenoids, chromones and azadirachtins found in representatives of the tropical and subtropical flora and related to the "third-generation pesticides" – regulators of the growth and development of insects (Berenbaum, 1995; Pascual & Marco, 1990). For example, NeemAzal®-T/S was created and implemented to practice in recent years (Schmutterer, 1990). In Russia, the company "NEST M" has developed a new natural "Hardy" retardant, the main active ingredients which are a-diphenols and epibrassinolide (Pushkina et al., 2016; Kurapov et al., 1999).

Natural pesticides have several advantages over synthetic: they are cheaper, biodegradable, and minimally harmful to humans and animals. In addition, they are multi-component complexes of various chemical structures of natural compounds, which complicates the formation of resistance to pests, but at the same time contributes to the rapid utilization of these substances in the biocenosis.

The genus of *Aphididae* occupies a special place among the most dangerous pests for agricultural crops cultivated in Uzbekistan. One of the dangerous pests that inhibit the growth of potato productivity is considered that the large potato aphid (*Macrosiphum euphorbiae* Tomas). The high harmfulness of potato aphids in Uzbekistan is dependent on its predisposition to develop resistance to a wide range of pesticides.

One of the vector pests of viral diseases is considered the apple aphid (*Aphis pomi*), which inhibits the growth of new shoots of apple trees. Ordinary cereal aphid (*Schizaphis graminum* Rondani) is considered an economically important pest of winter wheat, winter rye, and barley. Aphid saliva has an enzymatic activity that destroys cell walls and chloroplasts in sensitive plants. Aphids suck spike juice, which causes partial whiteness, hollowness and the frailty of the grain (Morkunas et al., 2011).

It is known that the *Datura stramonium* L. *(*family of *Solanaceae)* plant has insecticidal properties, which are due to the presence in them of the alkaloids hyoscyamine, scopolamine, and atropine. The percentages of alkaloids in the leaves are 0.23-0.37%, in the stems 0.06-0.24%, and in the flowers from 0.13 to 1.9% (Makhmudova et al., 2018).

The chemical composition of the leaves of *D. stramonium* L. is well studied. Vitasteroids – daturalacton, vitastramonolide, alkaloids – hyoscyamine, scopolamine, phenol carboxylic acids, and their derivatives, flavonoids, tannins, carotenoids, and steroids are isolated and identified from the leaves of the plant, interest in which is due to their biological activity (Bhakta, 2005).

The species is widespread in the southern and middle strip of the European part of Russia and the Caucasus, in the south of Western Siberia, the Far East, and Central Asia-everywhere except deserts and semi-deserts (Sokolov, 1990; Vvedenskiy, 1961). In this regard, the urgent problem is the study and development of fundamentally new tools based on secondary metabolites of *D. stramonium* L. plants.

The aim of this work was that the studying the chemical composition of the *n*-butanol fraction of the extract of the aerial parts of *D. stramonium* L. plants collected in the Fergana region of the Republic of Uzbekistan and investigation of its insecticidal activity against certain types of pests of the genus of *Aphididae*.

Materials And Methods

Leaves of *D. stramonium* L. were collected in July 2018 in the Fergana region at the flowering stage. The air-dried leaves of *D. stramonium* L. were extracted 7 times with ethanol. The extract was concentrated and diluted with an equal volume of water. The obtained precipitate was removed by filtration and evaporated with ethanol. The aqueous portion was sequentially extracted with chloroform, ethyl acetate, then *n*-butanol. The chloroform, ethyl acetate, and *n*-butanol fractions were obtained after evaporation of the solvents under vacuum (Makhmudova et al., 2019).

As a result of extraction, 4.985 kg (24.92%) of dry weight was obtained. The obtained mass was extracted with organic solvents (benzine, chloroform, ethyl acetate, *n*-butanol) to isolate the sum of vitasteroids. As the results were obtained the following amount of products: a benzine fraction of 51.5 g (0.26%), a chloroform fraction of – 511.1 g (2.55%), an ethyl acetate fraction of – 303 g (1.52%), a *n*-butanol fraction 1.621 kg (8.11%).

The *n*-butanol fraction of *D. stramonium* L. (was named extract later) was analyzed by HPLC-mass spectrometry.

Mass spectra of substances were obtained by ESI mass spectrometry (electrospray) using a 6420 Triple Quad LC/MS mass spectrometer (Agilent Technologies, USA). Registration of mass spectra of the samples was carried out with positive and negative ionization. The parameters of the mass spectrometer were selected by SCAN followed by EIC (extracted ion monitoring) mode to determine individual substances: fragmenter tension 70.0 V, desiccant gas flow rate 6 l/min, gas temperature 300°C, gas pressure at the atomizer needle 20 psi, evaporator temperature 300°C, capillary voltage 4000V (Morena Pedraza et al., 2019).

Growing and receiving of pests

The laboratory population of common cereal aphids (*Schizaphis graminum*) was kept on seedlings of wheat of

the Saratovskaya-29 variety in a thermostatic room with a temperature of 22 ± 2°C and the duration of daylight, 16 hours.

Potato aphids were collected in the Kibray district of the Tashkent region, the laboratory population was kept on seedlings of potatoes in a thermostatically controlled room with a temperature of 26-27°C, air moisture of 70-80% atm and the duration of daylight, 14 hours.

Apple aphids were collected at the "Super garden" horticultural farm in the Kibray district of Tashkent region.

Screening for insecticidal activity

The extract of *D. stramonium* with the next concentrations 10 mg/mL; 5 mg/mL and 1 mg/mL were prepared for conducting insecticidal activity (Berestetskiy et al., 2018). The leaves of apple, wheat, and potatoes were lowered into the studied extract solutions, then they were placed in Petri dishes on wet filter paper to avoid early drying of leaf discs. 200 µl of the extract was applied to the filter paper, and then twenty adults of each test object were added. A variant of experiments with different doses and test objects were carried out four times and kept at 24°C, relative moisture 65%, and at a photoperiod of 16:8 (D:N) h. Mortality was evaluated under a binocular microscope following 24 hours after exposure. Aphids were considered dead if the movement was imperceptible. Water was used for control and a Karate insecticide as a reference.

Field experiment

Field experiments were carried out in the Kibray district of the Tashkent region. The effectiveness of the extract was studied against potato aphids (*Macrosiphum euphorbiae*) in a small experimental potato plant area about 100 m^2. The experimental area was allocated randomly. The experimental design involved spraying in terms of 0.12-0.23 kg/ha of extract. The Karate 5 EC (Syngenta Crop Protection AG, Switzerland) insecticide was used as a reference with normal application 0.2 l/ha. The experiment was carried out four times according to the generally accepted method (Dospehov, 1979). The processing was conducted using a 10-liter Avtomax knapsack sprayer in the evening. The counts were carried out on the 3rd and 7th day after processing. The biological effectiveness of the extract was calculated using the Abbot formula (Abbot, 1925):

Phytotoxicity screening

The method of chipped leaf discs was used for evaluate the phytotoxic effect of the extract (Berestetskiy et al., 2018). Well-developed leaves of wheat, cucumber, and tomato crops were used as test-plants.

Screening for antimicrobial activity

The antimicrobial activity of the extract was evaluated by the method of paper disks at a concentration of 60 µg/disk (Egorov, 2004). The bacteria *Bacillus subtilis, Escherichia coli* and the yeast *Candida tropicalis* were used as test microorganisms. The radius of the inhibition zones was measured after 24 hours of incubation at 30°C.

Biotest for cytotoxicity

The cytotoxic activity of the extract was studied against the cell line of maize deciduous scoops (*Spodoptera frugiperda*), supported in the microbiological defense laboratory of All-Russian Research Institute of Plant Protection. 100 µl of a 10 mg/mL extract diluted with acetone was added to the well plate and the solvent was evaporated. The dry residue was dissolved in 20 µl of dimethyl sulfoxide after that 900 µl culture medium of SF900II (Thermo Fisher Scientific, USA) and 100 µl of a suspension of cells at a concentration of 300 thousand cells / well were added to each well. Cell lines were incubated for 24 hours at 27°C, stained with trypan blue and the percentage of dead (stained) cells was determined in relation to the total number (at least 50) of several areas of view (Watts et al., 2003).

Express method of determining total toxicity

The evolution of the toxicity of the extract was also conducted against the ciliates (*Paramecium caudatum*). The culture was provided by D. O. Vinokhodov (St. Petersburg Technical University) and was cultivated in the medium of Lozin-Lozinsky. 0.5 ml suspension of cells ciliates (approximately 100 cells/ml) was added to a 24-well plate, the existence and activity of ciliates were determined visually using a microscope. 5 µl of a 10 mg/mL extract was added to the suspension of ciliates. It was repeated three times. Ciliates survival was analyzed 3, 30 min and 3 hours after the start of the experiment (Rao et al., 2007). Cell counting was performed in a Goryaev chamber. A culture medium with ciliates was used as a control.

Statistical analysis

The insecticidal activity was determined by the number of dead insects for 1 day, the percentage of the mortality was calculated according to the Abbots formula (Abbot, 1925). The extract was highly active, leading to the death of insects from 61 to 100% (I-II level) (Sukhoruchenko et al., 2006). Analyzes were performed in triplicate, and the results are expressed as mean ± SE.

The obtained results of the research were processed by the mathematical method of analysis of variance using the Original Program 6.

Results

Analysis of metabolite profiles of the extract

HPLC-MS analysis of the *n*-butanol fraction of *D. stramonium* L. extract showed high similarity by the qualitative composition of major metabolites (Figure 1). Analysis of chromatography revealed 7 major peaks in the extracts, which differed in retention time and charge of mass. The following seven total peaks were found in the extract: Agmatine (Figure 2), 4-Trimethylammoniobutanal (Figure 3), 6, 7-Epoxy-5, 12-dihydroxy-1-oxowitha-2, 24-dienolide; (5α, 6α, 7α, 12α, 22R)-form (Figure 4), 8-Methyl-8-azabicyclo [3.2.1] octane – 3,6-diol; (1R*,3S*,6S*,7R*)-form, 3-tigloyl, 6-propanoyl (Figure 5), 3-Phenylacetoxy-6-hydroxytropane (Figure 6), 6-Hydroxyhyoscyamine; (2′S,3S,6S) – form, stereoisomer (Figure 7), 8-Methyl-8-azabicyclo

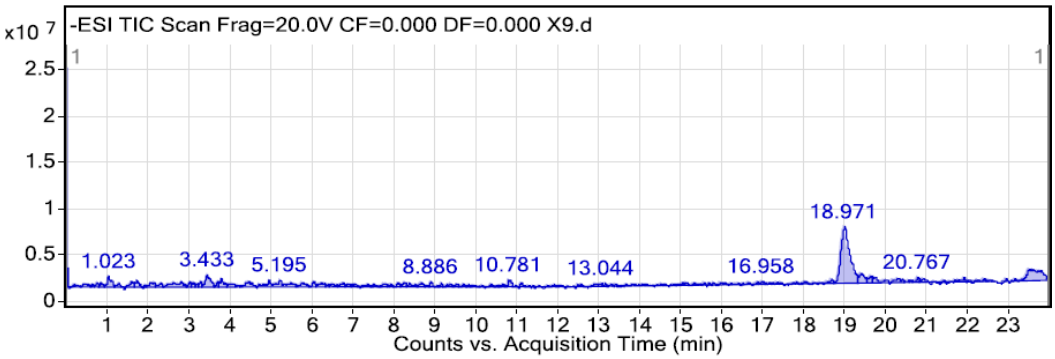
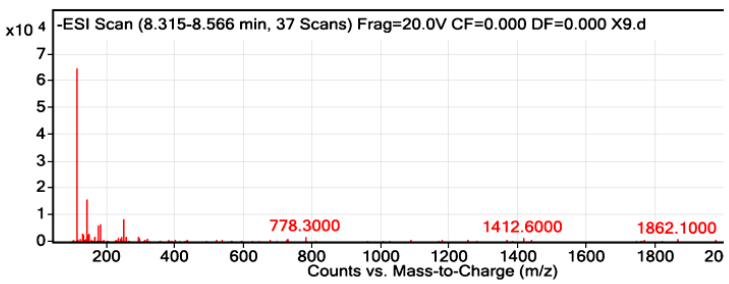
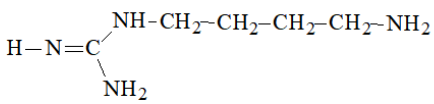


Fig. 1. Mojor phytochemicals from leaves of *Datura stramonium* L. by HPLC-MS

Peak List

m/z	Abund
112.8	64839.58
129.7	3258.5
130.7	2792.8

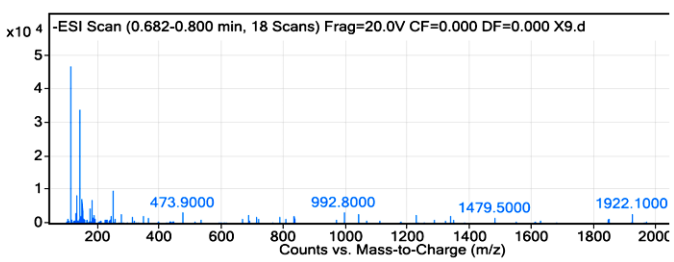


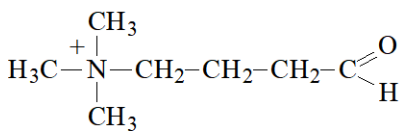


Agmatine (**1**), $C_4H_{14}N_4$, Мм. 130,12,

Fig. 2. HPLC chromatogram of Agmatine from *n*-butanol extract of *Datura stramonium*

m/z	Abund
112.8	46907.89
129.8	3170.5
130.9	8338.17

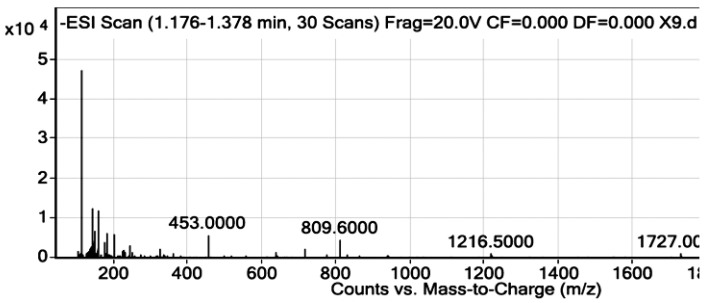




4-Trimethylammoniobutanal (**2**), $C_7H_{16}NO$, Мм. 130,21,

Fig. 3. HPLC chromatogram of 4-Trimethylammoniobutanal from *n*-butanol extract of *Datura stramonium*

m/z	Abund
112.8	47429.77
140.8	12504.77
146.9	6935.99
156.9	11780.66
174.8	4041.32
180.8	6380.06
200.9	5892.01
242.5	3073.26
453	5762.12
809.6	4541.46



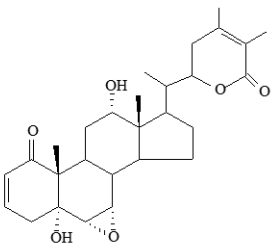
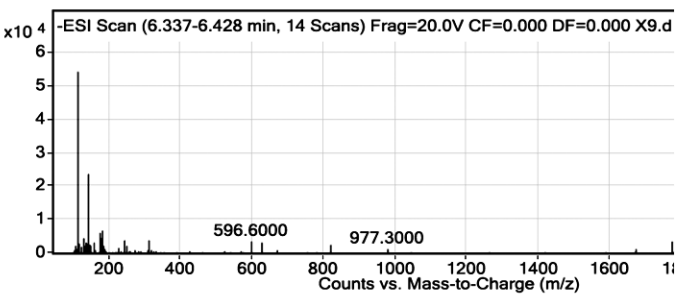


Fig. 4. HPLC chromatogram of 6, 7-Epoxy-5, 12-dihydroxy-1-oxowitha-2, 24-dienolide; (5α, 6α, 7α, 12α, 22R)-form *n*-butanol extract of *Datura stramonium*

6,7-Epoxy-5, 12-dihydroxy-1-oxowitha-2, 24-dienolide; (5α, 6α, 7α, 12α, 22R)-form (**3**), $C_{28}H_{38}O_6$, Mm 470.61

Peak List

m/z	Abund
112.9	54350.04
129.9	4556.47
140.9	23879.75
174.8	6301.86
178.7	4644.11
180.7	6974.9
242.5	3915.31
311	3789.23



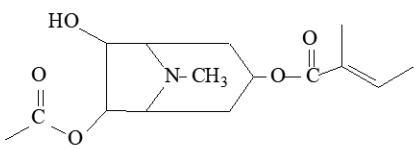


Fig. 5. HPLC chromatogram of 8-Methyl-8-azabicyclo [3.2.1] octane – 3,6-diol; (1R*,3S*,6S*,7R*)-form, 3-tigloyl, 6-propanoyl *n*-butanol extract of *Datura stramonium*

8-Methyl-8-azabicyclo[3.2.1]octane- 3,6-diol; ((1R*,3S*,6S*,7R*)-form, 3-tigloyl, 6-propanoyl (**4**), $C_{16}H_{25}NO_5$, M.m. 311.

[3.2.1] octane – 3,6-diol; (1R,3R,5R,6R)-form, 6-tigloyl (Figure 8).

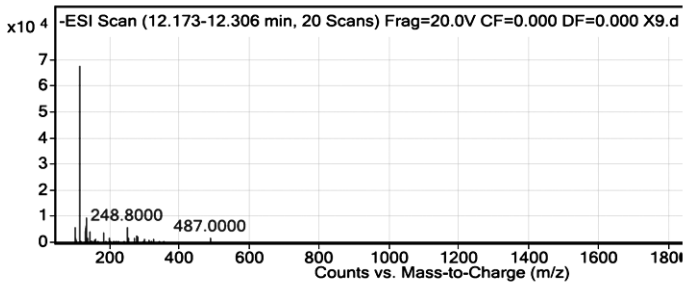
These compounds can be identified as Agmatine, 4-Trimethylammoniobutanal, 7-Epoxy-5, 12-dihydroxy-1-oxowitha-2, 24-dienolide; (5α, 6α, 7α, 12α, 22R)-form, 8-Methyl-8-azabicyclo [3.2.1] octane – 3,6-diol; ((1R*,3S*,6S*,7R*)-form, 3-tigloyl, 6-propanoyl, 3-Phenylacetoxy-6-hydroxytropane, 6-Hydroxyhyoscyamine; (2′S,3S,6S) – form, stereoisomer, 8-Methyl-8-azabicyclo [3.2.1] octane – 3,6-diol; (1R,3R,5R,6R)-form, 6-tigloyl.

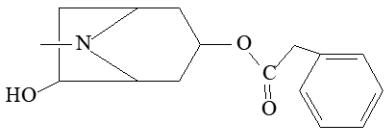
Laboratory testing of insecticidal activity

Laboratory testing for the insecticidal properties of the fraction of *D. stramonium* extract was carried out against three species of sucking pests of the genus *Aphididae*: *Schizaphis graminum, Aphis pomi* and *Macrosiphum euphorbiae.* Studies have shown that the lowest activity of all three studied concentrations of 1mg/mL, 5mg/mL and 10mg/mL of the extract was shown against *Schizaphis graminum* and the percentage of mortality was 15.1%, 19.5%, and 23.8%, respectively, while when using the insecticide Karate –

Peak List

m/z	Abund
100.8	6196.8
112.9	67914.85
129.9	2640.51
130.7	9680.5
140.9	4338.59
180.8	4053.27
197.6	2140.07
248.8	6291.77
276.6	3044.82



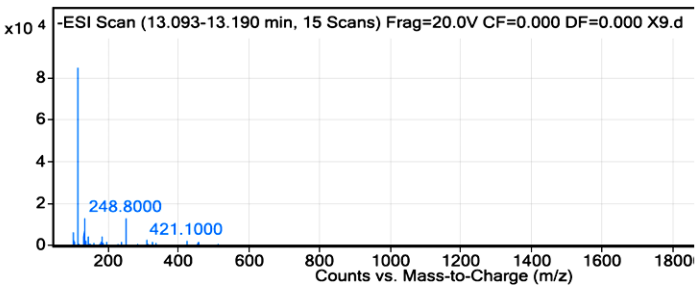


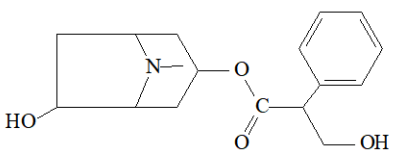
3-Phenylacetoxy-6-hydroxytropane (**5**), $C_{16}H_{21}NO_3$, M.m. 275.35

Fig. 6. HPLC chromatogram of 3-Phenylacetoxy-6-hydroxytropane *n*-butanol extract of *Datura stramonium*

Peak List

m/z	Abund
100.7	6602.54
112.9	85254.73
129.7	4608.18
130.7	13288.54
140.8	4522.5
180.9	4352.11
248.8	13408.21
250	6484.12
306.5	3075.03
421.1	2529.06



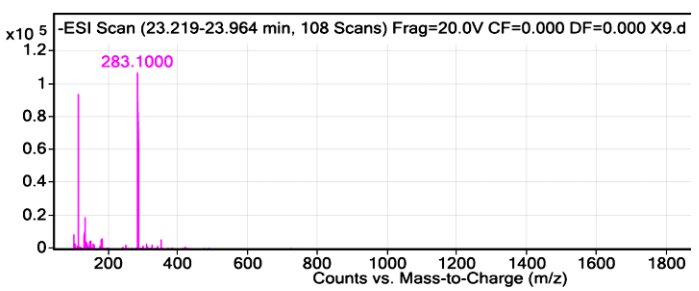


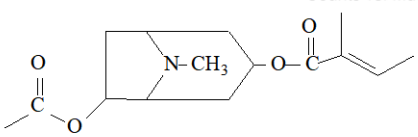
6-Hydroxyhyoscyamine; (2'S,3S,6S)- form, stereoisomer (**6**), $C_{17}H_{23}NO_4$, M.m. 305.37.

Fig. 7. HPLC chromatogram of 6-Hydroxyhyoscyamine; (2′S,3S,6S) – form, stereoisomer *n*-butanol extract of *Datura stramonium*

Peak List

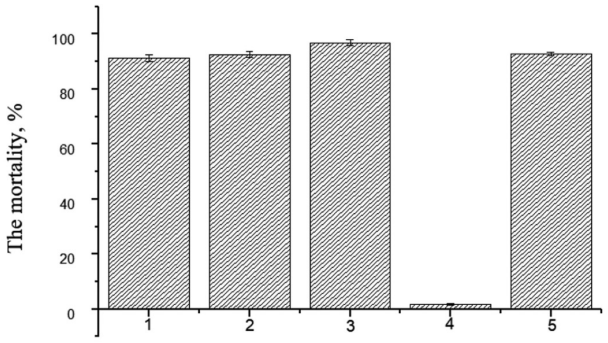
m/z	z	Abund
100.7		8848.83
112.9		93958.8
129.8		7785.82
130.8		19328.6
146.7		5167.69
178.7		5631.36
180.8		6260.62
283.1	1	106886.34
284.1	1	17421.38
351.1		5932.71





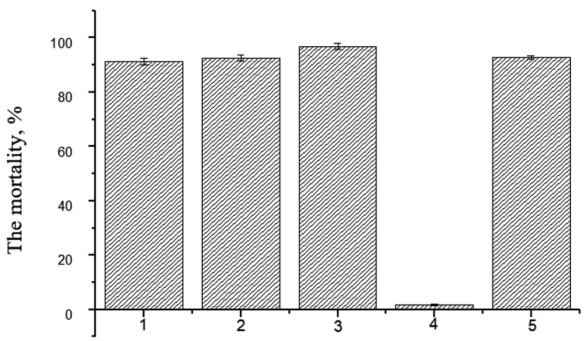
8-Methyl-8-azabicyclo[3.2.1]octane- 3,6-diol; (1R,3R,5R,6R)-form, 6-tigloyl (**7**), $C_{15}H_{23}NO_4$, M.m. 281.35.

Fig. 8. HPLC chromatogram of 8-Methyl-8-azabicyclo octane – 3,6-diol; (1R,3R,5R,6R)-form, 6-tigloyl *n*-butanol extract of *Datura stramonium*



1. Extract – 1mg/mL; 2. Extract – 5mg/mL; 3. Extract – 10 mg/mL; 4. Control; 5. Karate 5 mg/mL

Fig. 9. Mortality (M±SEM) of adults *Schizaphis graminum* in 24 hours after exposure to plant extract *Datura stramonium* L.



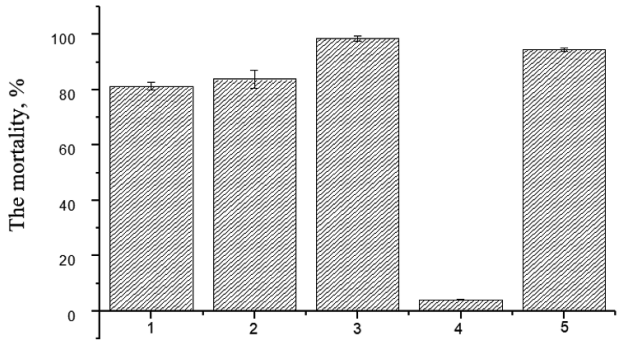
1. Extract – 1mg/mL; 2. Extract – 5mg/mL; 3. Extract – 10 mg/mL; 4. Control; 5. Karate 5 mg/mL

Fig. 9. Mortality (M±SEM) of adults *Aphis pomi* in 24 hours after exposure to plant extract *Datura stramonium* L.

Fig. 10. Mortality (M±SEM) of adults *Aphis pomi* in 24 hours after exposure to plant extract *Datura stramonium* L.

91.5% (Figure 9). The high activity was shown by the extract against *Aphis pomi* when exposed with 1mg/mL and 5 mg/mL dose, mortality of insects was 91.2% and 92.6%, accordingly. The first level activity was showed at 10mg/mL concentration, mortality was 96.8% and exceeded the value of the insecticide Karate (92.7%) (Figure 10).

The extract of the plant *D. stramonium* also shown high activity against *Macrosiphum euphorbiae.* The percentage of the mortality was 81.3% and 83.3% on the used concentrations 1 mg/mL and 5 mg/mL respectively (Figure 11). The high activity (98.5%) was shown by the extract at 10 mg/mL concentration so it was higher than the value (94.55) of the insecticide Karate.



1. Extract – 1mg/mL; 2. Extract – 5mg/mL; 3. Extract – 10 mg/mL; 4. Control; 5. Karate 5 mg/mL

Fig. 10. Mortality (M±SEM) of adults *Macrosiphum euphorbiae* in 24 hours after exposure to plant extract *Datura stramonium* L

The biological efficiency of the 1 mg/mL extract against *S. graminum, A. pomi* and *M. euphorbiae* was 12.8% – 91.0% – 80.5%, respectively. Moreover, the efficiency of the extract at a dose of 5mg/mL reached 17.4% – 92.3% – 82.6% accordingly. The highest efficiency (96.7% – 98.4%) was shown by the extract at 10 mg/mL concentration against *A. pomi* and *M. euphorbiae*. The least (21.7% effectiveness at a dose of 10 mg/mL) sensitivity to the effect of the extract was shown against *S. graminum* (Figure 9).

Field studies

As a result of the field experiment, it was revealed that the biological effectiveness of the extract in the 10 mg/mL dose was 100% after processing.

According to the results of field tests, the biological effectiveness of the extract at a consumption rate of 0.23 kg/ha after processing was 97.0% and 100% on 3 and 7 days accordingly which was practically at the level of experiments that were conducted using of Karate insecticide. The biological efficiency was 97.0% and 100% on 3 days and 7 days respectively. The biological efficiency when spraying potato leaves with an extract at a dose of 0.12 kg/ha at the time of the registrations was 88.7% and 92.5%, respectively (Table 1).

Phytotoxic activity

The phytotoxic activity of the extract of *D. stramonium* at the concentrations of 10 mg/mL, 5 mg/mL and 1 mg/mL was studied in biotests on segments of wheat, tomato, and cucumber leaves. According to the results of registration, only at 10 mg/mL concentration showed very weak activity on the leaves of cucumbers, leaf necrosis did not exceed 1.0 mm. The necrotic symptoms were not found in the remaining variants (Table 2).

Table 1. Biological efficiency of the extract of *Datura stramonium* L. against *Macrosiphum euphorbiae* when spraying potatoes varieties of Connect. (M±SEM)

Tretment	Consumption rate, kg/ha	The number of aphids, sample/leaf per plant			Biological effectiveness,%	
		before processing	3 days	7 days	3 days	7 days
Control		15	23	25	0.0	0.0
Karate	0.2	11	2	0	89.0	100.0
Extract	0.23	20	1	0	97.0	100.0
Extract	0.12	16	3	2	88.7	92.5

Table 2. Phytotoxic activity of the extract of *Datura stramonium* L.

Tretment, dose, mg/mL	Phytotoxic activity		
	wheat	tomato	cucumber
Control	i	i	i
Extract, 1.0	i	i	i
Extract, 5.0	i	i	i
Extract, 10.0	i	i	1.0±0.01

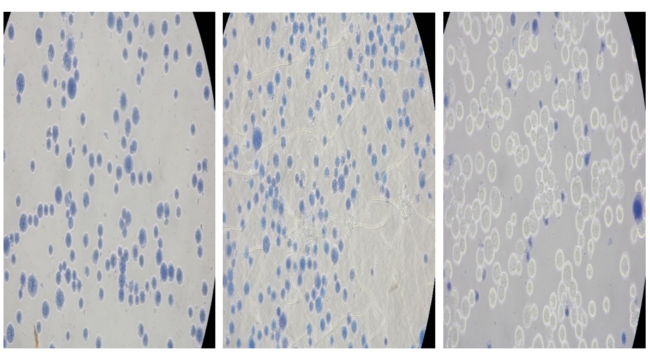
Note: i – inactive; the length of the necrotic spot on the segments of plant leaves, mm

Antimicrobial activity

According to the results of the antimicrobial effect of the extract of *D. stramonium*, only 10 mg/mL of the dose among the three studied concentrations showed activity against *Bacillus subtilis* and *Escherichia coli*, the inhibition zone was about 0.1 mm for both cultures, but the extract did not show activity against *Candida tropicalis* yeast (Table 3).

Table 3. Antimicrobial activity of the extract of *Datura stramonium* L.

№	Concentration, mg/mL	The inhibition zone of the test-culture, mm		
		Bacillus subtilis	*Escherichia coli*	*Candida tropicalis*
1	Extract, 1.0	–	–	–
2	Extract, 5.0	–	–	–
3	Extract, 10.0	0.15	0.10	–



Extract, 100 µl/mL Extract 500 µl/mL Control

Fig. 11. Cytotoxic activity of extract of *D. stramonium* against the *Spodoptera frugiperda* cell line

Toxic activity

Studies on the determination of acute toxicity on a culture of ciliates (*Paramecium caudatum*) showed that a 5 µl extract with 10 mg/mL concentration did not show toxicity after 3 hours of exposure which *Paramecium caudatum* was live in a well plate.

Cytotoxic activity

The extract showed a high level of cytotoxic activity against the *Sf9* cell line at concentrations of 100 µl/mL and 500 µl/mL (Figure 12).

Discussion

According to literary sources, it is known that secondary metabolites have sublethal consequences, such as a decrease in fertility, a decrease in the viability of insects. Compounds reduce the number of individuals in populations both directly (as a result of death) and primarily, indirectly. Secondary plant metabolites can disturb development, lead to malformations or malfunctions, extend the duration of developmental stages (Weissenberg et al., 1998; Buyukguzel et al., 2013; Buyukguzel et al., 2013; Friedman, 2002; Nenaah, 2011), or act as repellents (Chopa et al., 2016; Dinesh et al., 2014).

One of the plant taxons that produce highly toxic compounds is the *Solanaceae* family. In this family, in which some of the most poisonous plants can be found (Lee, 2007; Lee, 2006; Fowler, 2015), substances and extracts obtained from these plants are also widely used as pesticides (Kanteh, 2015).

Previous work indicates that chemical composition depends on the geographical area where the plant grows. In the aerial parts of the plant *D. stramonium* L., contains atropine from 0.037% to 0.07% and scopolamine from 0.09% to 0.02% (Bhakta, 2005).

All organs of this species contain alkaloids, mainly hyoscyamine, atropine, scopolamine: in leaves – 0.23-0.37%, stems – 0.06-0.24%, roots – 0.12-0.27%, flowers – 0.13-1.9%, seeds – 0.08-0.22%. In addition, *D. stramonium* leaves contain up to 0.04% of essential oil, up to 0.1% carotene and 1.7% tannins (Samylina, 1999).

The insecticidal activity of the compounds of the plant *D.*

stramonium is known. In a previous work (Abbasipour, 2011), extracts of powdered leaves, stems, and seeds were obtained on a rotary evaporator and tested in laboratory conditions for its ability to control some stored products from the attack of *C. maculatus.* There is a high increase in mortality after 12 hours. The data of Probit analysis demonstrated that the lethal concentration for the death of 50% of the population (LC_{50}) was estimated at 1680 and 16058 ppm for 24 and 48 hours, respectively. The authors found that the extract of *D. stramonium* can be of great importance when storing grain against *C. maculatus* (Bekuzarova et al., 2015) developed the composition of *D. stramonium* and Veratrum Lobelianum Bernh against the potato beetle. Hyoscyalin contained in *D. stramonium* lowers the tone of the pest, and when mixed with five steroins, alkaloids of Veratrum Lobelianum Bernh cause death in the Colorado potato beetle.

Conclusion

The study of the chemical composition of the n-butanol fraction of the extract of the aerial part of the plant *D. stramonium* collected in the Ferghana region of the Republic of Uzbekistan showed that its main components were 1,8-dihydroxy-naphlhalene (1), atropine (2), scopolamine (3), anisodamine (4), campesterol (5), withanolideglucosides (6).

The high efficiency of the n-butanol fraction was established against juice-sucking pests of the genus *Aphididae – A. pomi* and *M. euphorbiae* as a result of the studies. The *n*-butanol fraction of extract showed high insecticidal activity against *A. pomi* and mortality was 96.7%, 92.3%, and 91.0% at a dose of 10 mg/mL, 5.0 mg/mL and 1.0 mg/mL respectively.

The first level of activity also was shown at a 10 mg/mL dose of the fraction against *M. euphorbiae* with 98.4% mortality. The mortality was lower at 1.0 mg/mL and 5.0 mg/mL concentration, which was 82.6% and 80.5%, respectively.

The extract did not have a significant insecticidal effect on *S. graminum*, pest mortality was 12.8%, 17.4%, and 21.7% at the dose of 1.0 mg/mL, 5.0 mg/mL and 10.0 mg/mL accordingly.

As a result of the field experiment, it was revealed the biological efficiency of the fraction with a 10.0 mg/mL concentration against *M. euphorbiae* was 100% the next 7 days after the processing of plants.

Some biological activities of the extract of *D. stramonium* were studied. Studies have shown that the fraction at a concentration of 10.0 mg/mL did not show phytotoxicity on the leaves of wheat, tomato, and cucumbers. For the extract, moderate antimicrobial activity against *B. subtilis* was revealed (the inhibition zone was about 2 mm). The fraction with the concentrations of 500 µl / ml and 100 µl / ml showed 100% cytotoxicity against the cell lines of *S. frugiperda* under *in-vitro* conditions. The extract was not toxic to the ciliates (*Paramecium caudatum*) in 5 µl of 10 mg/mL extract.

Thus, it is potential to consider the possibility of using the n-butanol fraction of the extract of the aerial part of the plant *D. stramonium* as an insecticidal agent against *A. pomi* and *M. euphorbiae*.

Financial supporting of the work

This work was supported by the State Foundation for Fundamental Research of the Republic of Uzbekistan under the research grant PZ-20170927326 "Development of a domestic anti-inflammatory drug based on vitasteroids from plants of the genus *Datura* and *Physalis*"and as well as partical support from the El Yurt Umidi Foundation.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

The work was performed at the Institute of Chemistry of Plant substances of the Academy of Sciences of the Republic of Uzbekistan and the All-Russian Research Institute of Plant Protection.

References

Abbasipour, H., Rastegar, F., Mahmoudvand, M., Hosseinpour, M. H. (2011). Insecticidal activity of extract from *Datura stramonium* (F.) (*Solanaceae*) against *Callosobruchus maculatus*. *Integrated Protection of Stored Products IOBC/wprs Bulletin, 69,* 251-256 (Eng).

Abbott, W. S. (1925). A method of computing the efectiveness of an insecticide. *Journal Econ. Entomol., 18 (2),* 265–267.

Bekuzarova, S. A., Bekmurzov, A. D., Dzobelova, Z. T. & Gazzaev, G. T. (2016). Method of controlling the Colorado potato beetle. Patent RU 2 586 901 C1 (Ru).

Benelli, G. (2016). Natural products as effective weapons against mosquito-borne diseases. *Current Organic Chemistry, 20,* 2647.

Berenbaum, M. (1995). Phototoxicity of plant secondary metabolites: Insect and mammalian perspectives. *Archives Insect Biochemisty and Physiology, 29 (2),* 119-134.

Berestetsky, A. O., Grigoryeva, E. N., Petrova, M. O. & Stepanycheva, E. A. (2018). Insecticidal and phytotoxic properties of extracts from cultures of certain cereal pathogens. *Mycology and Phytopathology, 52 (6),* 408-419.

Bhakta, P. G. (2005). Monographs on *Datura stramonium* L. The School of Pharmaceutical and Biomedical Sciences Pokhara University, Dhungepatan-12, Lekhnath, Kaski, Nepal, 2008 29, 80 (Eng).

Buyukguzel, E., Buyukguzel, K., Erdem, M., Adamski, Z., Marciniak, P., Ziemnicki, K., Ventrella, E., Scrano, L. & Bufo, S.

A. (2013). The influence of dietary alpha-solanine on the waxmoth *Galleria mellonella* L. *Archives Insect Biochemisty and Physiology, 83,* 15–24.

Buyukguzel, E., Buyukguzel, K., Snela, M., Erdem, M., Radtke, K., Ziemnicki, K. & Adamski, Z. (2013). Effect of boric acid on antioxidant enzyme activity, lipid peroxidation, and ultrastructure of midgut and fat body of Galleria mellonella. *Cell Biology Toxicology, 29*, 117–129.

Chopa, C. S., Benzi, V., Alzogaray, R. & Ferrero, A. A. (2009). Repellent activity of hexanic and ethanolic extracts from fruits of *Solanum eleagnifolium* (*Solanaceae*) against *Blattella germanica* (Insecta, Dictyoptera, Blattellidae) adults. *Boletin Latinoamericano Caribe, 8,* 172–175.

Dinesh, D. S., Kumari, S., Kumar, V. & Das, P. (2014). The potentiality of botanicals and their products as an alternative to chemical insecticides to sandflies (Diptera: Psychodidae): *A review. Journal Vector Borne Diseases, 51,* 1–7.

Dospehov, B. A. (1985). Methodology of Field Experience. Moscow, *Kolos,* 351 (Ru).

Egorov, N. S. (2004). Fundamentals of the Doctrine of Antibiotics. M., *MSU,* 19, C. 528 (Ru).

Fowler, C. J. (2015). Plant sources of antimuscarinics. *BJU International, 115 (6),* 4–7.

Friedman, M. (2002). Tomato glycoalkaloids: Role in the plant and in the diet. *Journal Agricultural Food Chemistry, 50*, 5751–5780.

Kanteh, S. M. & Norman, J. E. (2015). Diversity of plants with pesticidal and medicinal properties in southern Sierra Leone. *Biological Agriculture Horticulture, 31*, 18–27.

Kurapov, P. B., Shain, S. S. & Dmitriyeva, V. L. (1999). Secondary metabolism and hormonal balance of peppermint under the influence of exogenous growth regulators. *Agricultural Biology, 3,* 73-78.

Lee, M. R. (2006). The *Solanaceae*: Foods and poisons. *Journal Royal College Physicians Edinburg, 36,* 162–169.

Lee, M. R. (2007). *Solanaceae* IV: *Atropa belladonna*, deadly nightshade. *Journal Royal College Physicians Edinburg, 37,* 77–84.

Makhmudova, M. M. & Babayev, I. D. (2018). Vitanolides of the plant *Datura stramonium. Collection of Abstracts of the International. Scientific Conf. "Medicines Based on Natural Compounds", 18 (20),* 185-186.

Makhmudova, M. M., Saidkhodjaeva, D. M., Babayev, I. D., Syrov, V. N. & Abdullaev, N. D. (2019). Anti-inflammatory activity of the sum of Vitasteroids of *Datura stramonium*. *Zhurn. Infection, Immunity and Pharmacology, 1,* 100-106.

Mohammadhosseini, M., Venditti, A., Frezza, C., Serafini, M., Bianco, A. & Mahdavi, B. (2021). The Genus *Haplophyllum* Juss.: Phytochemistry and Bioactivities. A Review. Molecules, *26,* P. 4664. DOI.org/10.3390/molecules26154664

Morena Pedraza A., Gabriel J., Treutler H., Winkler R., Vergara F. (2019). Effect of Water Avialability in the Soil on Tropane Alkaloid Production in Cultivated *Datura stramonium*. *Metabolites, 9,* 131. DOI;10/3390/metabo9070131.

Morkunas, I., Mai, V. C. & Garrys, B. (2011). Phytohormonal signaling in plant responses to aphid feeding. *Acta Physiol. Plant., 33(6),* 2057- 2073. DOI10.1007/11738-011 07517.

Nenaah, G. (2011). Toxic and antifeedant activities of potato glycoalkaloids against *Trogoderma granarium* (Coleoptera: Dermestidae). *Journal Stored Product Research, 47,* 185–190.

Ojebode, M. E., Olaiya, C. O., Adegbite, A. E., Karigidi, K. O. & Ale, T. O. (2016). Effcacy of some plant extracts as storage protectants against *Callosobruchus maculatus*. *Journal Biotechnology Biomaterials, 6,* 217. DOI:10.4172/2155952X.1000217.

Pascual, N. & Marco, M. (1990). Azadirachtin induced imaginal-moult deficiencies in *Tene-brio molitor* L. (Coleoptera: Tenebrionidae). *Journal Stored Product Research, 26,* 53-57.

Petrova, O. E. & Sauer, K. (2017). High Performance liquid chromatography (HPLC) based detection and quantitation detection- and quantitation of cellular c –di GMP. *Metods Journal Molecular Biology, 1657,* 33-43. DOI: 10.1007/978-1-4939-7240-1_4

Pushkina, G. P., Tropina, N. S., Osipov, V. I. & Melnikova, G. V. (2016). Tests of a new natural retardant Hardi on medicinal crops, Materials of Reports of the IX Scientific-Practical Conference "Prospects for the Use of Innovative Forms of Fertilizers, Protective Equipment and Plant Growth Regulators in Agricultural Technologies of Agricultural Crops. Moscow, Anapa. *VNIIA Publishing House,* 120-124.

Rao, J. V., Gunda, V. G., Srikanth, K. & Arepalli, S. K. (2007). Acute toxicity bioassay using *Paramecium caudatum*, a key member to study the effects of monocrotophos on swimming behaviour, morphology and reproduction. *Toxicology. Environmental Chemisty, 89,* 307–317.

Samylina, I. A. & Severtseva, V. A. (1999). Medicinal plants of the State Pharmacopoeia. *Pharmacognosy*. M., AMNI.1, 488.

Schmutterer, H. (1990). Properties and potential of natural pesticides from the neem tree, *Azadirachtaindica. Annual Review Entomology Palo Alto (Calif.), 35,* 271-297.

Sokolov, P. D. (1990). Plant resources of the USSR, Flowering plants, their chemical composition, use; Families Caprifoliaceae, Plantaginaceae, Leningrad, Akademia Nauk, 5, 328 (Ru).

Sukhoruchenko, G. I., Dolzhenko, V. I. & Novozhilov, K. V. (2006). Metods for evaluating the action of insecticides on arthotopest. *Plant Protection News, 3*, 3-12 (Ru).

Volociuc, L. (2008). Promoting technological strategies and agricultural and ecological research. *Agricultural Science, 2,* 26-29 (Ru).

Vvedenskiy, A. I. (1961). Flora of Uzbekistan. *AS of UzSSR, 5,* 668 (Ru).

Watts, P., Kittakoop, P., Veeranondha, S., Wanasith, S., Thongwichian, R., Saisaha, P., Intamas, S. & Hywel-Jones, N. L. (2003). Cytotoxicity against insect cells of entomopathogenic fungi of the genera Hypocrella (anamorph Aschersonia): possible agents for biological control. *Mycology Research, 107,* 581–586.

Weissenberg, M., Levy, A., Svoboda, J. A. & Ishaaya, I. (1998). The effect of some solanum steroidal alkaloids and glycoalkaloids on larvae of the red flour beetle, *Tribolium castaneum*, and the tobacco hornworm, *Manduca sexta. Phytochemistry, 47,* 203–209.

Received: **April, 16, 2022**; *Approved*: **May,27, 2022**; *Published:* **October, 2022**