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Analysis of the growth regulating activity of 1.1-dichlorofenilyclopropane

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ABSTRACT

This article deals with the issues of the effect of the synthesized compound (1.1-dichlorofenilcyclopropane) that has a specific morpho-regulatory specific function on the changes in the growth and development indicators of vegetable crops. In the course of the experiment, we assessed and compared the morpho- functional indicators of plants such as seed germination, growth rate, leaf area and the total leaf area of the whole plant, degree of pigmentation, timing of flowering and budding. We determined the sizes of the stem base, root system volume and dry weight of the whole matter. The obtained empirical data allowed designating a set of indicators, the changes in which were modulated with the preparation effect, including: decreased seed germination and plant growth, an increase in leaf area and the degree of its pigmentation, the earlier timing of flowering, a large degree of budding, an increase in the root system volume, thickness of the stem base and dry weight of the plant. Based on the pilot study, the authors proved that the synthesized compound refers to the class of phyto-hormones and shows pronounced retardant properties.

Keywords: retardant, temperature mode, seed vigour, budding, the number of sprouts.

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INTRODUCTION

In modern conditions, the development of efficient methods for synthesizing biologically active substances is regarded as one of the priorities in the development of basic technologies of the chemical and pharmaceutical industry. It is believed that the problem of creating modern domestic chemical and pharmaceutical industry should be included into the number of the most important elements of national safety.

The method of directed synthesis of reactive compounds is widely used to produce biologically active substances, which differ in their selective effect on individual objects, functional systems, tissues or organs. The role of heterocyclic compounds is exclusive in virtually all biological processes and their high modification ability determines the prospects of synthesizing these compounds in terms of practical use in various industries, including agriculture, synthesis of new medicinal compounds, medicine, industry (Kartsev, 2014; Pozharsky, 1985; Nametkin, 1981).

In the course of the research and assessment of the biological activity of synthesized preparations, we obtained a compound with the specific morpho-regulatory function. Therefore the task of assessing the nature of the synthesized preparation targeting effect and the manifestation degree of its biological activity was set. For this purpose, we determined the growth regulating function of 1.1-dichlorobenzocyclo-propane.

Scientific novelty and practical significance of the work lies in the fact that it has been shown experimentally that the synthesized compound (1.1-dichlorofenilcyclopropane) belongs to a class of phyto-hormones and demostarted pronounced retardant properties.

METHODOLOGY

To evaluate the growth-regulating role of the synthesized preparation, we used seeds of different vegetable crops: cucumber (cultivar of Elegans), tomato (cultivar of Bely Naliv) and beets (Bordeaux cultivar). As a control, we used distilled water and the biological preparation – Epin-extra that was extensively tested in practice; it is a stress adaptogen with the high growth-promoting activity. Concentration of the biological preparation and the synthetic preparation amounted to 0.002%.

Sowing and seed treatment was performed in accordance with the methods described in the literature (The method of evaluating the physiological condition of sprouts (Patent RU 2111639). Before sowing, the seeds were treated on a phased basis. Before that, if there was a need, we selected destroyed seeds, the rest of the seeds were weighed and counted. The sieved seeds were treated with 96% alcohol and washed in a stream of running tap water to remove mucor. Seeds were washed till all the mucor disappears from effluents under a microscope. After an hour of washing with regular water, the seeds were washed twice, and then soaked in distilled water for seed imbibition for 3 hours at a temperature of 22 °C.

Seeds were sown at the rate of 30 seeds per plate using the cluster method. Depending on the experiment, 20 ml of distilled water (temperature of 22 °C), 20 ml of 1.1-dichlorobenzocyclo-propane and 20 ml of the Epin Extra bioproduct were introduced into the cup. We covered the seeds with filter paper, in which 8 apertures for aerating the seeds were made beforehand. At certain time intervals from the start of treatment – soaking of seeds, i.e. we evaluated seed germination every 24 hours for five days.

In the research, we have used widely known assessment methods of seed vigour (Privalov, F.I., Burak, O.A), leaf area (Potapov, V.A., Bobrovich, L.V), root system volume, stem diameter (Efremov S., Golubev. V. N.), dry ashing method. In the course of the experiment, we registered the emergence of sprouts, beginning of budding and mass flowering. The experimental results were subjected to statistical processing on a personal computer using the programme called Primer of Biostatistics (Version 4.03 by Stanton A. Glantz, 1998). We calculated the arithmetic mean (M), standard error of the mean (m). Reliability of data (p) was determined by the Student t-test (Plohinsky, 1970).

MAIN PART

The conducted research allowed identifying several groups of facts. Differences were detected in the targeting and manifestation degree of the synthesized preparation effect depending on a temperature mode. The comparative analysis of seed vigour, soaked at a temperature of 23-25 °C and 15-17 °C, are shown in Tables 1 and 2.

Table 1. Comparative analysis of seed vigour; seeds were soaked at a temperature 23-25 °C

Vegetable culture	Statistical indicators	C-1	C-2	Preparation
Cucumbers	n M ± m p	6 29.33±0.19	6 30±0	6 30±0
Tomatoes	n M ± m p	6 6.16±0.15	6 7.6±0.30	6 6.16±0.15
Beet	n M ± m p	6 23±0.19	6 24±0.33	6 25.6±0.33

Note: Control-1 – water; Control-2 – biological preparation; n – number of experimental series.

Table 2. Comparative analysis of seed vigour with the seeds soaked at a temperature range of 15-17 °C

Vegetable crop	Statistical indicators	C-1	C-2	Preparation
Cucumbers	n M ± m p	6 15.3±0.58 ≥ 0.001	6 16±0.83 ≥ 0.001	6 12.3±0.88
Tomatoes	n M ± m p	6 6.6±0.57 ≥ 0.01	6 7.8±0.28 ≥ 0.001	6 4.8±0.28
Beet	n M ± m p	6 5.6±0.57 ≤ 0.01	6 6±0.47 ≤ 0.001	6 3.5±0.31

Note: Control-1 – water; Control-2 – biological preparation; n – number of experimental series.

Activity of the preparation different from the control groups was detected at a lower ambient temperature (14-15 °C). Assessment of the starting growth in these conditions showed a decrease in seed vigour and appearance of the first sprouts of all the vegetable seeds treated with the preparation (Table 1, 2). Differences in all cases were reliable.

Germinated and not germinated vegetable seeds were sown in the special trays with enriched soil. We recorded the timing of sprouts emergence, their number, the beginning of budding and mass flowering (Tables 3-5).

Table 3. Phenological observations of plants (cucumber)

Variant	Date				
	Emergence of sprouts	Formation of leaves	Appearance of buds	Efflorescenc e	Mass flowering
C1 (water)	24.03	31±03	01.05	03.05	25.05
Preparation	26.03	02.04	20.04	25.04	08.05
C2 (biological preparation)	21.03	28.03	26.04	30±04	18±05

Table 4. Phenological observations of plants (tomatoes)

Variant	Date				
	Emergence of sprouts	Formation of leaves	Appearance of buds	Efflorescence	Mass flowering
C1 (water)	28.03	05.04	-	-	-
Preparation	30.03	01.04	-	-	-
C2 (biological preparation)	26.03	03±04	-	-	-

Table 5. Phenological observations of plants (beet)

Variant	Date			
	Emergence of sprouts	Formation of leaves	Closure of rows	Opening of rows
C1 (water)	02.04	15.04	14.05	-
Preparation	02.04	10.04	08.05	-
C2 (biological preparation)	30.03	12.04	08.05	-

Throughout the entire period of observation, sprouts of the test plants lagged behind the control ones (C-1, C-2), had less pronounced axial organs. The treatment of cucumber plants with the biological preparation accelerated the emergence of sprouts and the formation of a first real leaf by 2 days in comparison with the control 1 and by 5 days in comparison with the synthesized preparation. The use of 1.1-dichlorofenilcyclopropane *accelerated the appearance of flower buds and blooming by 10-12 days as compared to the control 1, and by 4-5 days as compared to the control 2* (Table 3). The treatment of tomato plants with the 1.1-dichlorofenilyclopropane accelerated the emergence of sprouts and the formation of a first real leaf by 2 days in comparison with the control 2 and by 4 days in comparison with the control 1. For beet, the preparation accelerated the formation of a first leaf by 2 days as compared to the biological preparation and by 5 days as compared to the control. Variants of treatment with the biological preparation and 1.1-dichlorofenilcyclopropane shortened the closing of rows by 2 days as compared to the control 1.

Quantitative analysis of morpho-physiological indicators of vegetable sprouts is shown in Table 6. The table shows that the area of a cucumber leaf blade, treated with the biological preparation is larger in comparison with the plants in the control group 1. The largest area of a leaf blade was found in the plants, the seeds of which were soaked in 1.1-dichlorofenilcyclopropane, although the differences by this parameter were not statistically significant. However, in terms of the total leaf area of cucumbers, the differences were of a statistically significant nature between the plants of the two control groups (C1 and C2) and the test group. Under the same conditions of the experiment, a large leaf surface area of tomatoes and beet was typical for the plants, the seeds of which were treated with the biological preparation. Indicators with the test group of plants were comparable with the results of the control 1. Thus, in terms of leaf surface indicators, the highest indicators were found in cucumber plants treated with the synthetic preparation and in tomato and beet plants, the seeds of which were soaked in the biological preparation.

Table 6. Quantitative analysis of the morpho-physiological parameters of vegetable cultures sprouts

Plants	Number of plants			Area of the leaf blade, cm^2			
	K-1	K-2	Experiment	Stat. indicators	C-1	C-2	Experiment
1	150	155	67	n M ± m p	340 8.3±0.5 ≤ 0.5	382 9.04±1.3 ≤ 0.5	316 11.72±2.22

2	127	148	44	n M ± m p	820 1.7±0.7	946 2.9±0.2	423 1.2±0.6
3	50	123	39	n M ± m p	98 1.1±0.3	352 10.3±0.5	70 0.7±0.1

Note: C-1 – water; C-2 – biological preparation; experiment – preparation; n – the total number of leaves; plants: 1 – cucumbers, 2 – tomatoes, 3 – beet.

Thus, with the reduced seed vigour and seedling germination, the total leaf surface of cucumber plants was significantly higher in the plants treated with the experimental preparation. We observed large leaf sizes and the pigmentation degree of these plants. Change in the leaf blade area is a sufficient basis proving the improvement of the functional properties of the plant organism because it is the leaf that is the main assimilation organ of plants that provides the entire organism with organic substances and vigour during photosynthesis.

Processes related to the generative sphere of plants should be considered from the same position. Obtained phenological data indicate a significant reduction in the interphase periods of the beginning of flowering and mass flowering by 10-12 days compared to the control 1 (seeds, soaked in water) and 4-5 days in relation to the control 2 (seeds soaked in the biological preparation); early stages of budding and intensity of buds formation on one plant. Such data suggest a decrease in the interphase periods of plants post-emergence development and the overall positive effect of the studied substance on the development of the generative sphere of plants.

Factual differences between the experimental and control groups of plants are found by measuring their root system (Table 7).

Table 7. The results of the analysis of the root system volume, cm³

Plants	C2 biological preparation		C1 water		Preparation	
	Average value of the volume	Overall volume	Average value of the volume	Overall volume	Average value of the volume	Overall volume
Cucumbers	168.40	9,290.00	16.40	820.00	137.40	3,720.00
Tomatoes	50.06	2,403.00	13.40	611.00	169.00	2,370.00
Beet	45.52	1,912.00	24.33	365.00	104.00	940.00

In all cases, plants in the test group had a more developed root system, compared with the plants of the first control group. Thus, the root system volume of tomatoes was three times greater than of the plants in the test group relative to the control 2 (biological preparation) and an order of magnitude greater relative to the root system of plants in the control group 1. Considering the functional relevance of the given vegetative organ of higher plants, it can be concluded that the plants treated with 1.1-dicholorofenilcyclopropane are capable of more effective absorption, delivery and storage of organic and mineral substances, as well as of the formation of biologically active substances synthesized in the root.

The aspect that we considered next referred to the study of the sprout axial part, namely the stem.

Table 8. The analysis results of the diameter indicators of plants stem, cm

Plants	Stat. data	Biological preparation	Water	Preparation
Cucumbers	n M ± m p	155 0.25±0.01 ≥ 0.01	127 0.2±0.03 ≥ 0.001	50 0.33±0.01

Tomatoes	n M ± m p	148 0.25±0.09	127 0.24±0.02 ≥ 0.05	44 0.3±0.02
Beet	n M ± m p	123 0.23±0.08	50 0.17±0.01 ≥ 0.001	39 0.2±0.01

Note: n – total number of plants.

Data from Table 8 shows that diameter of the stem base of cucumbers treated with the preparation is substantially greater in relation to both control groups of plants. In case of tomatoes and beets, a reliable significant increase in the stem diameter in the test series was observed only in comparison with the seeds soaked in water. Such changes may be due to the vigorous activity of the formation tissue (cambium) at the account of division and differentiation of its cells. These changes are accompanied by an increase in stem strength, improvement of the mechanical function of its conductive and support base for leaves, buds, and flowers.

Significant differences in the experimental and control groups were identified by us in the course of the research on the dry weight of the studies objects. Dry weight of tomatoes and beets was greater if plants were treated with the biological preparation; of cucumbers – with the synthesized preparation. At the same time, for all three types of plants, the weight of dry residue in the test group exceeded the C-1 (water).
The ash content of the plants was calculated in grams by the total number of used plants.

Table 9. The results of the analysis of ash content of plants, g

Plants	Stat. data	Biological preparation	Water	Preparation
Cucumbers	M ± m p	7,74± 1.1 ≤ 0,01	5,42± 1.2 ≤ 0,001	9,95 ± 1.4
Tomatoes	M ± m p	8,88± 0,09	2,29± 0,02 ≤ 0,05	2,37± 0,02
Beet	M ± m p	6,43± 0,08	1,94± 0,01 ≤ 0,001	2,13± 0,01

Considering the quantitative variability of cucumber, tomato, beet plants for all three options (C-1, C-2, experiment) and a significant predominance of the first two over the experimental group plants, we can conclude that the actual dry residue weight of plants treated with 1.1-dichlorofenilcyclopropane exceeded the control groups. Mass fraction of plants ash is a main aspect of the plants chemism, it reflects the ability of synthetic and plastic processes and the accumulation of skeletal structures (structural polysaccharides) of a plant.

Thus, preparation-induced morphological and physiological changes in germination and development of a plant organism provide its greater resistance to adverse environmental conditions. High strength of mechanical and support systems that contributes to the prevention of plants lodging is of great practical and agricultural importance. Profitability from using such preparations is determined by the fact that with the lesser volumes of sprouts, and as a result, a smaller area of the land for planting, the plants provide greater efficiency.

CONCLUSIONS

Growth and development of plant organisms, as well as all life on the Earth, is determined by the combined interaction of external and internal parameters of the environment. Unlike external factors (light, daylength, temperature, humidity, etc.), internal factors, which are chemical substances, can be controlled and used to optimize plant growth processes. These factors include a wide variety of biologically active compounds, including the substances of hormonal or enzymatic nature. The central role of phyto-hormones in the regulation of plant growth is well known. However, due to the dual nature of its effect (as stimulators and inhibitors) hormones have come to be defined as chemical morpho-regulators of plants (Privalov *et al.*, 2012).

Such morphological regulators also refer to retardants – synthetic organic substances that have a specific impact on the morphological and physiological characteristics of a plant organism. It is believed that retardants include organic compounds, the functional activity of which decreases with increasing temperature, and which cause a reduction in seed germination and reduction in the plant growth rate. The stem base becomes thicker, skeletal structures (structural polysaccharides) accumulate, and consequently the dry stem weight increases. Axial organs may shorten (due to a significant inhibition of cell division in the subapical meristem of the stem), so formed plants with lower and thicker stems, interstices internodes, better-developed mechanical tissue and conducting system (Kefeli, 1984). An increase in the photosynthetic activity of leaves is indicated (their pigmentation increases) (Kuryata *et al.*, 1995), as well earlier periods of flowering and budding (Adams *et al.*, 1998), an increase in the number of plant sprouts (Zarenkova, 2014).

In the course of the experiment, we had the opportunity to assess and compare the morpho-functional indicators of plants such as seed germination, growth rate, leaf area and the total leaf area of the whole plant, degree of pigmentation, timing of flowering and budding. We determined the sizes of the stem base, root system volume and dry weight of the whole matter. The obtained empirical data allowed designating a set of indicators, the changes in which were modulated by the preparation effect, including: decreased seed germination and plant growth, an increase in leaf area and the degree of its pigmentation, the earlier timing of flowering, a large degree of budding, an increase in the root system volume, thickness of the stem base and dry weight of the plant.

Examining the obtained results in the light of the notions existing in literature concerning functional properties of retardants, it should be recognized that the examined preparation (1.1-dichlorofenilcyclopropane) shows all the properties that allow us to characterize it as a biologically active substance with the growth inhibiting effect.

The corresponding literature does not contain a lot of material concerning the mechanism of retardants effect. However, two directions of the effect of the substances of this nature are considered recognized: some of them are associated with the cell aging process, causing destruction of plant tissue cells, and others are the inhibitors of gibberellin and inhibit the stretching of cell in length during the active growth period and enhance their transverse division processes. As a result, the elongation of plants slows down, the stem thickens, which leads to increased plant resistance to lodging, its strength.

Originally, synthetic retardants were used as substances that prevent drowning. Later, inhibitory properties of retardants were used not only for other agricultural crops, but also for various plants, vegetables and fruit (Zarenkova, 2014; Baklanova, 1983; Murvanidze, 1985; Gorbachenkov, 2005; Pavlova, 2003).

It is necessary to emphasize one more aspect; it refers to the nature of biological activity of the synthesized substance. The analysis of obtained empirical data evidences in favour of their hormonal nature. It is known that phyto-hormones have a high specificity of effect, providing consistency and functional integrity of the plant organism (Abou-Dahab and Nahed, 2006; Wilkins, 1989).

The studies were carried out in the framework of the comprehensive targeting assessment and a manifestation degree of the biological activity of synthesized heterocyclic compounds. By their chemical nature, the synthesized preparation is a precursor that may be converted into five- and six-membered heterocycles.

The practical significance of the conducted work lies in the fact that it has been shown experimentally that the synthesized compound (1.1-dichlorofenilcyclopropane) belongs to a class of phyto-hormones and shows pronounced retardant properties.

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