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ORIGINAL ARTICLE

Effect of treptolem on morphogenesis and productivity of linseed plants

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It has been established the effect of growth stimulator with auxin, gibberellin, cytokinin compounds complex on the features of growth processes, anatomical organization of vegetative organs, productivity of linseed oil plants (*Linum usitatissimum* L.) and energy efficiency under application of growth regulator. Application of treptolem during the budding period leads to increase in the productivity of linseed oil by the increasing of morphogenesis process of vegetative organs with simultaneous restructuring of anatomical structure of shoots and leaves. The increase in stem diameter due to better development of bark, xylem, thickening of bast fibres enhances the resistance of linseed oil plants to lodging. Stimulator induces enhanced development of the photosynthetic apparatus: formation of a larger number of leaves, prolongation of their active functioning, increasing of chlorenchyma cells size and improving of chloroplastogenesis. The enhancement of photosynthetic productivity of linseed oil plants leads to an intensification of carpogenesis, an increase in yield and an improvement in the crop structure. Treptolem treatment increased the oil content in flax seeds and its unsaturation. The content of residual amount of morphoregulators in seeds is significantly lower than the permissible concentration. Improving of linseed productivity under treptolem as a stimulator of plant development is accompanied by changes in the amount of obtained crop energy, the structure of energy intensity and an increase in the energy efficiency ratio.
Keywords: *Linum usitatissimum* L.; growth stimulators; morphogenesis; productivity

Introduction

Phytohormonal regulation of plant morphogenesis is a perspective direction in the development of agricultural technologies (Fang et al., 2019; Macedo et al., 2017; Mao et al., 2018). Physiological activity in a plant organism is determined not by the content of particular groups of phytohormones, but mainly determined by the balance of biologically active substances (Hedden and Thomas, 2016; Kuryata et al., 2017; Mohammad and Mohammad, 2013). Exogenous treatment of compounds with analogues of phytohormones, their antagonists or regulators of activity, intensively affect on the metabolic processes and lead to changes in growth processes, allows to improve the seed productivity of plants (Kendall and Storer, 2017; Khan and Mohammad, 2013; Sang-Kuk et al., 2018). It is noted that application of growth-regulating compounds increase in simultaneity and seed germination energy, accelerate the shoot and root system growth, which leads to improve the absorption of nutrients from the soil, the resistance of plant organism to the action of extreme environmental factors (Kuryata and Khodanitska, 2012; Mo et al., 2016; Upreti and Sharma, 2016).

The mechanism of action of new-generation drugs is associated with the activation of cellular respiration reactions, enzyme systems, an increase in the rate of membrane transport and mineral nutrition processes that lead to enhance the biological effectiveness of growing plants methods (Giannakoula et al., 2012; Sang-Kuk and Hak-Yoon, 2014; Yu et al., 2015). Phytohormones and their synthetic derivatives compounds stimulate to increase in the proliferation and accelerate the processes of cell differentiation, that caused formation of a more powerful root system and changes of leaf mesostructural organization (McKenzie and Deyholos, 2011; Wani et al., 2016). The enhanced development of assimilative apparatus leads to the intensification of organic substances synthesis, which are used for the needs of formation and generative organs loading, that is an important key to improving crop yields (Shevchuk et al., 2019).

Modern plant morphoregulators are notable for their considerable efficiency and ecological safety, which is determined by their complex compositions (Kuryata et al., 2019; Pérez-Jiménez et al., 2015). Thus, the composite preparations that contain native compounds, synthetic phytohormones, a number of amino acids and organic acids, essential microelements are distinguished among the plant growth stimulators. Structural similarity of growth stimulators in the structure with natural phytohormones results in a similar physiological effect (Cai et al., 2014; Renuka et al., 2018). However, the molecular organization of stimulators and their native correspondences is not identical, that determines the greater stability of exogenous compounds and its prolonged action in the plant (Ciura and Kruk, 2018).

In modern agrobiology, composite cytokinin preparations with 2,6-dimethylpyridine are often used that include phytohormonal compounds of natural origin with N-oxide derivative pyridine (Rogach and Rogach, 2015). Thus, the growth stimulator treptolem, which contains a balanced mixture of 2,6-dimethylpyridine-1-oxide, as well as a succinic acid, substances of auxin, gibberellin, cytokinin structure, particular elements and amino acids, has a high efficiency in regulating of morphogenesis and productivity of linseed (Kuryata and Polyvanyi, 2018). Exogenous interaction of biologically active substances in the practice of crop production influences on the processes of ontogenesis, as well as create effective technologies for growing of staple food and industrial crops based on a detailed study of physiological and biochemical mechanisms of action (Shevchuk et al., 2018).

The advantages of growing linseed oil are economic feasibility and profitability, resistance to water deficiency, a significant oil content in the seeds, possibility of using it in crop rotation as a precursor for grain crops (Baud and Lepiniec, 2010). There are a number of problems in the cultivation of linseed crops, despite the widespread cultivation of flax products in various areas of production that cannot be solved by general agrotechnical methods and measures (Khodanitska and Kuryata, 2018). Thus, the development of flax cultivation technologies is aimed at improving the resistance to lodging, increasing the oil content within the normal range of the variety’s reaction, stable seed yield under unfavorable agrometeorological conditions, biologization of growing methods (Kuryata and Khodanitska, 2018). Accordingly, it remains relevant and has practical interest the possibility of regulation of seed yield magnitude and morphogenesis of linseed oil plants by growth stimulators treatment. In this regard, the objective of this study was to establish the features of growth processes, formation of leaf apparatus and crop of oil flax seeds under the action of complex growth-stimulator treptolem.

Materials and methods of research

Field studies were conducted on crops of Ukraine Institute of Feed Research and Agriculture of Podilly of NAAS to determine the effect of treptolem on linseed plants. The experiment followed a randomized block design (10 m^2) with five replication. Linseed oil cv. Debut was grown using agrotechnical methods and measures according to the standard scheme and technological map of the crop. A single treatment was applied with aqueous solution of treptolem in a concentration of 0.03 ml/l to complete wetting of leaves. The flow rate of active solution was 300 l/ha.

The height and diameter of stem, number and area of leaves, fresh and dry weight matter, as well as the photosynthesis productivity of linseed plants were studied every 10 days after preparation spraying. Anatomical structure of linseed vegetative organs was determined on the middle-layer leaves and shoot fragments on the same stage of plant growth development. The size of individual tissues and cells were determined by ocular micrometer MOB 1-15x and by a digital camera ScienceLab DCM 250 of microscope. According to necessity of the leaf mesophyll premaceration, palisade and spongy parenchyma cells were measured in a solution of 2 mol/l etanic acid in hydrogen chloride.

The total amount of oil content in linseed was determined by the extraction with petroleum ether as a solvent using the Soxhlet apparatus (Horwitz et al., 1970). The amount of residual treptolom content in the final product were determined by gas-liquid chromatography on a chromatograph «Crystal 2000M» (produced by SKB «Khromatek»).

The steel columns dimensions of chromatograph were 100 mm, as a sorbent used 5% SE-30, nitrogen and hydrogen were determined as a carrier gas; substance flow rate was 60 ml/min. The temperature parameters of columns were set at 240°C, for the evaporator -260 °C, and the indicators of flame-ionization detector were 300 °C (AOAC, 2010; Donato et al., 2017).

Statistical analysis of experimental data was performed by computer program «STATISTICA-6» (StatSoft Inc.). The reliability of obtained results between control and experiment varient was assessed with the use of Student's t-test. Tables and figures show average research data.

Results and discussion

Application of synthetic phytohormones, their compositions and analogues induces changes in the processes of histo- and morphogenesis, that leads to an intensification of metabolism and acceleration of growth processes of individual organs. Consequently, the reactions of synthesis and accumulation of assimilates, first of all products of photosynthesis are intensified, that actively used for processes of fruits laying and seeds formation.

According to the results of research, using of plant development stimulator treptolem on oil flax crops increased the growth of stem and formation of leaf apparatus of treated plants. Growth stimulator interaction accelerated the linear growth and increase in the height of linseed plants stem by 14.7% over the years of research. The average shoot height of drug-treated plants was 62.4 ± 1.7* cm, while the height of control plants was 54.4 ± 1.9 cm. The transverse dimensions of linseed stems were 3.2 ± 0.11 mm under morporegulator compared to 2.8 ± 0.12 mm in control, which is 6.9 %.

Anatomical studies show that the stem thickening of linseed drug-treated plants primarily occurred due to the more intensive formation of cortex and xylem (Table 1; Figure 1).

Table 1. Anatomical structure of stem under treptolem treatment on linseed.

Indicator / Variant	Epidermal thickness (μm)	Bark thickness (μm)	Number of xylem vessels in a layer (pcs.)	Xylem thickness (μm)	Bast fibre diameter (μm)	Number of bast fibres in a group (pcs.)	Cell wall thickness of bast fibre (μm)

Control	18.52 ± 0.41	241.40 ± 8.30	23.20 ± 0.50	541.70 ±10.50	29.10 ± 0.50	32.20 ± 2.10	11.32 ± 0.52
Treptolem	19.85 ±0.40*	298.75±12.12*	31.80 ± 0.71*	769.53 ±13.11*	36.75 ± 0.45*	33.60 ± 3.20	14.78 ± 0.42*

** - difference is significant at P<0.05.

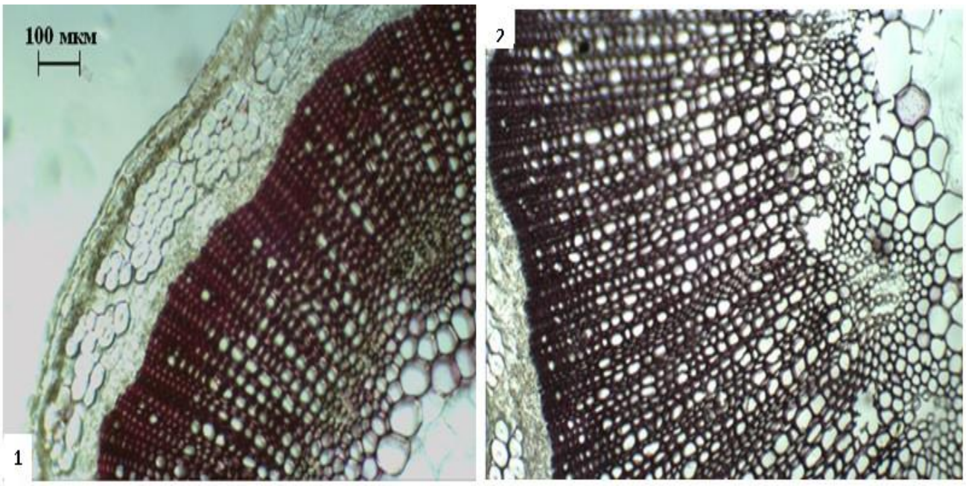

Figure 1. Anatomical organization of linseed stem. 1 – control; 2 – treptolem.

It was noted an increase in the xylem layer thickness under the influence of development stimulator in 1.4 times compared to control. Such thickening occurred due to the formation of a larger number of conducting elements – vessels, the number of xylem in a row increases by 37%.

Linseed culture is characterized by a significant development of bast fibres in the stem. According to the obtained results, treptolem treatment on linseed plants not affected on the number of bast fibres, however, the number of heavy type elements with developed cell walls increased. Thus, the cell wall thickness of bast fiber increased by 30.5% under influence of growth regulator. Such changes in the anatomical organization of linseed stems improved plant resistance to lodging and created technological advantages in harvesting.

The results of our research indicate that changes in the intensity of growth processes due to the action of growth regulator were accompanied by an increase in the dry matter accumulation of linseed plant (Figure 2). The largest weight gain of dry matter was observed for the stem of drug treated plants and amounted to 0.19-0.39 g more as compared to control.

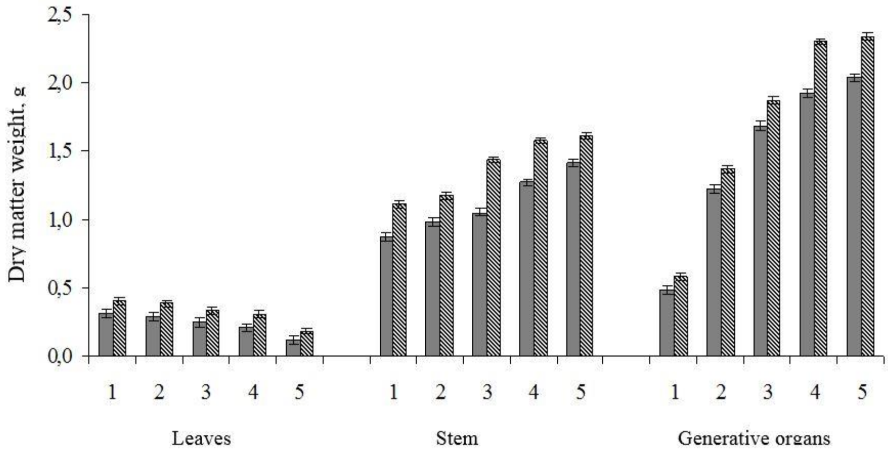

Figure 2. Accumulation of dry matter weight by linseed organs. 1, 2, 3, 4, 5 – accordingly 10-th, 20-th, 30-th, 40-th, 50-th day after treptolem treatment. ▭-control; ▨-treptolem.

Photosynthetic productivity performs a significant role in the formation of crop production and determined by the growth rate of leaf surface of the plants and the anatomical characteristics of leaf mesostructure. The activity of photosynthetic surface formation significantly depends on the balance of gibberellins, auxins and cytokinins in the plant organism.

According to the results of our work, a greater number of leaves on the plant were formed under treptolem application that contains the main classes of phytohormones and also total leaf surface of treated plants significantly increased (table 2). In addition to the formation of a large leaf surface area, the duration of photosynthetic apparatus functioning is not less important for the production process. The results indicate that the life of leaves is prolonged under the influence of growth regulator.

An increase in the number and leaf area of linseed plants under treptolem contributes to the formation of a powerful assimilative surface that leads to enhance photosynthetic productivity and more active accumulation of dry matter weight

(Figure 2, Table 2). Thus, the total dry matter weight of linseed plants under growth stimulator treatment increases to 4.07 ± 0.18 g, compared to control this indicator is 3.77 ± 0.22 g.

The intensity of photosynthetic processes is determined not only by the area of leaf apparatus, but also by the anatomical and morphological features of leave. It was found that an increase in leaf area under the influence of development stimulator was accompanied by its thickening. So, the thickness of lamina under treptolem action was 170.3 ± 2.1* μm compared to control 144.7 ± 1.5 μm. The lamina size of drug treated plant increased due to the proliferation of leaf mesophyll (Table 3.). In particular, the cell sizes of palisade assimilative tissue increased significantly, while the cell volume grew in 16 times. The cell sizes of spongy parenchyma not significantly changed and were close to control.

Table 2. Characteristics of leaf apparatus of linseed plants under treptolem action.

Indicator	**Variant**	**Vegetation period**		
		Flowering period	**Ripening period (green ripeness)**	**Ripening period (yellow ripeness)**
The total area of laminas per plant (m^2)	Control	0.013 ± 0.0005	0.012 ± 0.0006	0.010 ± 0.0011
	Treptolem	0.020 ±0.0006*	0.019 ± 0.0010*	0.015 ± 0.0017
Number of laminas per plant (pcs.)	Control	82.4 ± 2.2	73.7 ± 3.1	59.4 ± 4.4
	Treptolem	108.6 ± 3.5*	88.5 ± 2.8*	70.2 ± 3.1
Net photosynthetic productivity, g/(m^2 · day)	Control	0.18 ± 0.008	0.32 ± 0.011	0.26 ± 0.005
	Treptolem	0.38 ± 0.007*	0.63 ± 0.014*	0.46 ± 0.010*

* - difference is significant at P<0.05

The effect of treptolem on the photosynthetic apparatus of linseed was also realized through the processes of chloroplastogenesis (Table 3). Formation of a larger amount of chloroplasts and an increase in their volume was noted under drug application. In particular, the sizes of chloroplasts in the cells of palisade tissue increased by 29.5% relative to control, and respectively by 23.8% in the cells of spongy tissue.

Table 3. Leaf mesostructure of linseed under treptolem application.

Variant	**Length of cell (μm)**	**Width of cell, (μm)**	**Volume of cell, ($μm^3$)**	**Number of chloroplasts in cell (pcs.)**	**Volume of chloroplast, ($μm^3$)**
Palisade tissue of leave					
Control	34.1 ± 2.3	13.2 ± 0.8	3475 ± 171	12.7 ± 0.5	33.9 ± 1.7
Treptolem	40.8 ± 1.9	15.9 ± 0.7	5727 ± 215*	14.7 ± 0.7	43.9 ± 2.0*
Spongy tissue of leave					
Control	18.9 ± 0.8	16.1 ± 0.9	-	6.8 ± 0.4	31.4 ± 1.2
Treptolem	18.4 ± 0.9	14.8 ± 0.7	-	10.4 ± 0.4*	38.9 ± 1.6*

* - difference is significant at P<0.05

Mesostructural analysis of leaf apparatus indicators suggests that treptolem interaction result in formation of a more powerful photosynthetic apparatus: the leaf surface area and the number of leaves increased, the size of palisade parenchyma cells increased, chlorenchyma contained more chloroplasts compared to control, which is a necessary condition for enhancement of plant photosynthetic productivity and intensification of oil flax seed formation processes.

In modern agrobiology, exogenous introduction of physiologically active compounds is considered as a possible way to regulate the flow of individual developmental phases to mobilize plant resources and increase adaptive capacity under stressful conditions, which ultimately makes it possible to improve crop productivity. It is known that the application of phytohormonal drugs is accompanied by an increase in yield for oilseeds.

The analysis of treptolem effect on the productivity of linseed indicate that the application of growth stimulator led to increase in yield (table 4). Treptolem is included in the physiological processes in plant and affects on the stimulation of growth due to the hormones content of cytokinin and auxins nature. Intensification of metabolic processes and active development of photosynthetic apparatus by morporegulators action reinforce the processes of fruits laying, synthesis of plastic substances and seeds loading. Thus, interaction of drug on the linseed plant influenced on pods number and formed by 16 % more compared to control. The formation of a large number of generative organs and seeds on the plant, an increase in the seeds weight by drug treatement led to an increase in the number of seeds collected from a single plant. In general, the yield of linseed under treptolem influence increased by 4 %. Despite a small increase in yield, the results of the production process optimization of linseed under treptolem are valuable in a practical sense. So, it is possible to obtain cottonized, cotton-like fibers from short straw of linseed fiber for the production of mixed cotton-lined fabrics, medical cotton wool. Growth of linseed plants increases, stem length increases under the influence of treptolem. Thus, application of drug leads to a double positive effect - an increase in yield with simultaneous improvement in fiber quality.

Table 4. Structure of harvest under treptolem action on linseed plants.

Variant	Number of fruit per plant (pcs.)	Number of seed per pod (pcs.)	Seed weight per plant (g)	Weight of 1000 seeds (g)	Ratio of seed weight to plant weight	Yield (c/ha)
Control	25.0 ± 0.72	8.2 ± 0.17	1.5 ± 0.11	7.6 ± 0.04	442	18.2 ± 0.31
Treptolem	29.1 ± 1.02*	8.4 ± 0.21	1.8 ± 0.10	7.8 ± 0.02*	471	18.9 ± 0.27

Oil is valuable in high content of unsaturated fatty acids and accumulates as a reserve substance during forming and pouring of flax seeds. The analysis of the data suggest that application of treptolem led to a significant increase in yield of flax seeds that was accompanied by an increase in the oil content in it. The oil content in seeds of control plants was 34.2 ± 0.5%, whereas in the growth stimulator treated variant was 36.1 ± 0.6*%. The oil yield was 6.8 centners per hectare under the growth regulator influence despite the increase in seeds yield and its oil content compared to 6.2 centners per hectare in control.
Linseed oil is a very biologically valuable product. It is characterized by a high content of mono- and polyunsaturated fatty acids, in particular linoleic and linolenic acids, which are indispensable for humans. Polyunsaturated essential fatty acids are precursors of long-chain fatty acids and are part of cell membrane. α-linolenic acid has a particular importance, the content of which can reach 50% in individual linseed varieties. Consequently the fatty acids content and their ratio among themselves largely determine the oil quality. According to the results of gas-liquid chromatography, it was identified the main fatty acids of flaxseed oil. Among saturated acids are palmitic C 16, stearic C18, among unsaturated are palmitoleic C 16:1, oleic C 18:1, linoleic C 18:2, α-linolenic C 18:3, gondoic C 20:1 (Table 5).
The concentration of saturated fatty acids in the oil of treptolem treated linseed plants was decreased, but the total content of unsaturated fatty acids increased. The ratio of concentrations of unsaturated to saturated acids increased in the growth regulator variant as compared to control that indicates about improvement in the qualitative characteristics of linseed oil.
Toxicological control and research of residual amount of biochemical preparations in crop production is a prerequisite for environmental safety and developing technologies of growing crops under developing regulators application. The allocation of residual amounts of treptolem from flax seeds was carried out according to interstate standards (GOST) 13496.20-87. The results of chromatographic studies indicated that the residual amount of growth stimulator in linseed oil was 0.0073 mg/kg, that is, significantly lower than the permissible concentrations (0.03 mg/kg), which are regulated by State Sanitary Rules and Regulations (8.8.1.2.3.4.-000-2001) in Ukraine.

Table 5. Content of fatty acids in linseed oil (% per dry matter).

Fatty acids	Control	Treptolem
Saturated fatty acids:		
C 16 (palmitic acid)	5.11 ± 0.024	4.96 ± 0.031*
C 18 (stearic acid)	3.54 ± 0.052	3.40 ± 0.061
Content of saturated fatty acids	8.65	8.36
Monounsaturated fatty acids:		
C 16:1 (palmitoleic acid)	0.06 ± 0.010	0.06 ± 0.009
C 18:1 (oleic acid)	18.95 ± 0.104	19.11 ± 0.095
C 20:1 (gondoic acid)	0.24 ± 0.012	0.16 ± 0.010*
Polyunsaturated fatty acids:		
C 18:2 (linoleic acid)	13.95 ± 0.052	13.89 ± 0.041
C 18:3 (α- linolenoic acid)	58.16 ± 0.178	58.52 ± 0.144
Content of unsaturated fatty acids	91.36	91.74
Ratio of unsaturated/saturated acids	10.56	10.97

* - difference is significant at P<0.05.

Improving of the technological level of agricultural production and application of economically viable methods of management are one of the main tasks of the agricultural sector. At the present stage of plant physiology and agriculture development, one of the most important criteria for evaluating of growing crops technology is the assessment of economic and bioenergy efficiency of crop production. However, research and calculation of the economic efficiency of growing technologies is complicated by price instability in the means of production and final crop production. Energy assessment allows to level price policy fluctuations and get a more objective characteristic of technological processes. All types of labor, production inputs and material and technical means can be expressed in energy units (equivalents), which allows to bring all factors to a single indicator. The energy equivalent is determined as a cost of non-renewable energy to obtain mass unit of

production and labor inputs. The main advantage of this analysis is the ability to give an accurate assessment of the effectiveness of a separate agricultural method, identify the causes of inefficient production, identify ways to reduce energy resource costs, and program the energy intensity of growing technologies.
The energy efficiency ratio was determined to establish the energy efficiency of linseed growing. This indicator is a ratio of exchangeable energy amount contained in the grown products to the energy consumed amount for yield formation. It was used energy equivalents from the reference literature for calculation. The results of the study indicate that application of treptolem in the cultivation of linseed leads to increase in energy obtained by the yield enhancement (Table 6). Thus, treptolem treated linseed plants led to increase in the amount of accumulated energy by 3912 MJ/ha. The studied factors – application of plant growth regulators, have a rather high energy equivalent, therefore, the energy expenditure increase under drugs action.

Table 6. Energy efficiency of linseed cultivation under treptolem action (per 1 ha).

Growth regulators treatment	Energy recovery, MJ	Energy expenditure, MJ	EER
Control	62492.0	25557.2	2.45
Treptolem	66404.0	25757.2	2.58

The energy analysis of crop production technologies involves the establishment of energy price of yield - the energy efficiency ratio. It was found that the improvement of the linseed crop production under treptolem action as a stimulator of plant development is accompanied by changes in the energy amount obtained with the yied, the structure of energy intensity and an increase in the energy efficiency ratio. Application of growth regulator treptolem in process of linseed cultivation increases the bioenergy efficiency of flax production.
Analysis of energy intensity technology for linseed cultivation makes it possible to identify the most energy-intensive components (Table 7). The data of the structure of total energy expenditure in flax production indicates that more energy-intensive items are fertilizers and fuel.

Table 7. Structure of energy intensity in linseed cultivation under treptolem treatment (per 1 ha).

Expense item	Control		Treptolem	
	Energy intensity, MJ	Energy intensity, %	Energy intensity, MJ	Energy intensity, %
Mechanisms	1124.6	4.4	1169.9	4.5
Petrol, oil and lubricant	4273.7	16.7	4426.3	17.2
Electricity	204	0.8	204	0.8
Fertilizers	15450	60.5	15450	60
Herbicides	727.4	2.8	727.4	2.8
Plant growth regulators	-	-	2.1	0.01
Seeds	1280	5	1280	5
Other expenses	2497.5	9.8	2497.5	9.7
Total	25557.2	100	25757.2	100

Conclusion

Application of complex phytohormonal growth stimulator treptolem during the budding phase leads to improve the productivity of linseed plants due to changes in the processes of morphogenesis. Linear growth of vegetative organs is intensified with simultaneous restructuring of anatomical structure of shoot and leaves under the actions of developing stimulator. The increase in stem diameter due to better development of bark, xylem, thickening of bast fibres increases the resistance of linseed plants to lodging. The drug induces enhanced development of the photosynthetic apparatus: formation of a larger number of leaves, prolonging their active functioning, increasing the size of chlorenchyma cells and improving chloroplastogenesis. Enhancement of photosynthetic productivity intensifies the processes of laying and formation of generative organs, improves the structural indicators of crop-the number of fruits per plant, the number of seeds in pods, the seed weight increase that contribute to the growth of crop productivity. The increase in the oil content of flax seeds was accompanied by an increase in the concentration of unsaturated fatty acids. The bioenergetic efficiency of linseed cultivation technology was increased under the influence of treptolem.

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