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Influence of Chlormequatchloride on Morphogenesis and Productivity of Cruciferous Plants

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ABSTRACT

At present, there are burning issues concerning waste utilization of the agro-industrial complex, rational usage of nature and transition to the use of environmentally friendly and low-waste technologies. One of the most forward-looking areas is the application of the principles of the so-called "green chemistry", in particular, the development of technological processes using renewable raw materials and chemicals, the chemicals with a short half-life and low cost while ensuring maximum efficiency. The article presents the results of antigiberrelic preparation effect, which is characterized by low dosage and half-life in soil from 3 to 43 days on productivity, growth processes, anatomical, morphological features and functioning of the leaf apparatus of white mustard plants and rapeseed. The research shows that CCC-750 reduced the height of mustard and rapeseed plants. Inhibition of growth processes of white mustard plants at the beginning of the flowering phase with an anti-gibberellic preparation led to increased stem branching, which, in its turn, resulted in an increase in the number and area of leaves throughout the growing season. Moreover, the use of quaternary ammonium salt on winter rape plants has led to a reduction in the number of leaves and leaf area in comparison to the control samples. The application of the retardant caused an increase in the productivity of the studied cultures due to the increase in the number of pods, the increase in the mass of thousands of seeds and the number of seeds in the pod.

Keywords: productivity, winter rape, morphogenesis, plant growth regulators, chlormequat chloride, mustard white, mesostructure of leaves.

INTRODUCTION

High pollution of soils and groundwater with mineralized water and toxic substances demanded the search for efficient ways of diminishing this influence, while maintaining high soil productivity and achieving maximum productivity. The search for effective and safe technologies for growing agricultural products is one of the tasks in the domain of nature-bearing. Nowadays, it seems impossible to obtain high yields, ensuring the use of advanced agricultural technologies without the introduction of essential elements for plant nutrition and plant growth inhibitors (Tymchuk et al., 2020). Therefore, it is pivotal to ensure the following basic conditions for the successful use of chemical compounds as fertilizers or retardants:

- nutrients must be in a form accessible to plants;
- the content and ratio of elements must correspond to the physiology of plant nutrition.
- dosage of preparations should not exceed the normative values of chemicals in soil and food.
- environmental safety of preparations must be due to their non-toxicity, low half-life, lack of harmful effects on living organisms (Palamarchuk et al., 2022).

Generally, plant growth inhibitors, which provide all the aforementioned conditions, are highly specialized preparations that show efficacy in only one group of plants. The presented studies were conducted in order to expand the spectrum of action of antigiberrelic preparations in agriculture.

The investigation of the donor-acceptor system functioning so as to develop means of redistribution of assimilate flows to economically significant organs is an important issue in plant physiology to tackle burning issues of up-to-date agriculture. Growth is a single integrated process in which the growth of shoots and leaves is to some extent coordinated with each other, so exogenous growth inhibition will definitely cause some changes in the photosynthetic apparatus. The main donor of assimilates is, first and foremost, leaves. The leaf donor function is largely determined by the peculiarities of their morphology, anatomical structure and the ratio of the sizes of individual tissues. Retardants are known to act on subapical meristem cells, thereby slowing stem growth in length, but the effect of antihypertensive preparations on the apical meristem, which is responsible for leaf formation, remains largely unexplored. The application of retardants on many crops during the period of intensive growth and morphogenesis of leaves leads to an increase in their number, while reducing the area. However, treatment of poppy plants with antigiberrelic preparation – CCC – caused the growth of the leaf surface (Kuryata, Polyvanyi 2018).

Nevertheless, the effect of chlormequat chloride on morphological and mesostructural parameters of agricultural plants has not been studied to a full extent. There are controversial results on the effect of this antigiberrelic preparation on the morphogenesis of oilseeds (Koutroubas et al., 2016; Matysiak et al., 2013; Polyvanyi et al., 2020). Thus, the aim of this experiment was to find out the influence of CCC on morphometric indicators in plants of the cruciferous family.

MATERIAL AND METHODS

Oslava white mustard plants and *Galytskyi* rapeseed plants were treated during the budding period with a 0.5% chlormequat chloride solution using a Mastertool hydraulic sprayer, while the control plants were treated with water. The research plots were established in farms of the Podillia region in 2020-2021. The number of experimental plots of each of the experimental variants is 5, the area is 5 m^2

Chlormequat chloride (CCC) is an organic substance $[ClCH_2CH_2N(CH_3)_3]^+ Cl^-$, which is used as an inhibitor of the onium-type. It has got the 3rd class of toxicity (slightly hazardous to humans). The preparation does not accumulate, is not absorbed by the body and is excreted within 2 days. These features allow its vast use in agriculture.

The half-life in the soil, depending on the soil temperature and humidity, varies between 3 and 43 days. The optimal conditions for the decomposition of the preparation in the soil are the temperature of 25 C and soil moisture of about 60% (Vasilenko et al., 1990). In the soil, the preparation breaks down into choline chloride, choline and betaine, which are natural products of metabolism. Insects, as well as other animals, do not die in the treated areas, and the activity of microorganisms is not inhibited even at high doses (Kuryata, 2009).

Recent studies demonstrate the possibility of obtaining antigiberrelic preparations, namely, chlormequat chloride reagent decomposition of off-balance sheet pesticides (Petruk et al., 2002), that are stored for years in warehouses and non-specialized sites, which further increases the environmental attractiveness of these retardants.

The *Oslava* white mustard variety was created by the method of individual - family selection from the *Carolina* variety due to late spring sowing dates. It is has been added to the Register of Plant Varieties of Ukraine since 2011. The originator of the variety is the Institute of Feed and Agriculture of Podillya of NAAS. It is recommended to be used as fodder, industrial and green culture. It is characterized by increased fodder and seed productivity. The vegetation period to mowing maturity is 40–45 days, to seed gathering - 85–95 days. The dry matter yield is 6.0–6.5 t/ha, seeds – 2.8–3.0 t/ha. The dry matter content of crude protein is 18–19%, fiber – 21–22%. The seeds dry matter content is: oil – 26–28%, crude protein - 28–30%. The variety is adapted to mechanized harvesting.

The *Galytskyi* winter rape variety was bred at the Institute of Cruciferous Crops of the Ukrainian Academy of Sciences (Ivano-Frankivsk). It is added to the State Register of Plant Varieties of Ukraine (1997). The yield is 18–35 kg/ha. It is suitable for edible oil, the yield of which is 42–44%. The rapeseed is also grown to use its green mass for animal feed. Its yield is 440 c/ha. This variety is of early ripening, the growing season is 300–305 days. The plants are 140–145 cm tall. The bush is semi-closed. The stem is 0.5–2.5 mm in diameter with a slight anthocyanin color, pubescent. The leaves are smooth, lyre-shaped, pinnately dissected, pubescent. The inflorescence is a loose bouquet, with light yellow flowers. The seeds are spherical and dark brown. Each plant forms up to 420–450 pods and gives about 50 g of dark brown seeds. The pod contains up to 50 seeds. The weight of 1000 seeds is 4.5–5.0 g.

Morphometric parameters were measured every 10th day from the day of treatment. The area of leaf blades was determined by the method of felling. The study of the anatomical structure of the leaves of the experimental and control samples was performed on the canned material. The leaves to investigate their anatomical structure were stored in a solution that consisted of equal parts of C_2H_5OH, H_2O and $C_3H_8O_3$ and 1% CH_2O The study of cell size was conducted on a microscope XS-2610 MICROmed equipped with an ocular micrometer. Parenchyma cell sizes were detected in leaf tissues treated with a macerating solution of 5% ethanoic and two normal hydrochloric acids.

The residue amount of CCC in rapeseed and mustard seeds was established by a chromatographic method in a thin layer of cation exchange resin on plates "Silufol UV-254" The obtained results were statistically processed in accordance with the Student's test, the difference is significant at the level of $p = 0.05$.

RESULTS AND DISCUSSION

The use of synthetic growth regulators influences the anatomical parameters of different parts of the plant body, due to which there is a redistribution of assimilates to the fruit. Growth is one of the essential morphometric indicators of a plant. The scientific literature contains a considerable amount of information about the influence of retardants on the linear size of plants (Kuryata, 2009). One of the retardants, the most widely used of the quaternary ammonium compounds, chlormequat chloride, inhibited the growth of soybeans (Kuryata et al., 2019), poppies (Kuryata, Polyvanyi 2018), and flax (Kuryata, Khodanitska 2018).

The linear size is also a principal indicator of plant morphogenesis. The scientific literature presents information on the influence of the retardants on the height of the shoot of oilseeds, but they have conflicting results (Kuryata, Khodanitska 2018; Kuryata, Polyvanyi 2018). Therefore, it is urgent to study the effect of chlormequat chloride on the morphometric parameters of mustard and rapeseed plants.

Our research results testify to the fact that the antigiberrelic preparation – chlormequat chloride – caused differences in the ontogenesis. In particular, the retardant slowed down the growth of experimental crop plants, which is a typical reaction to the use of quaternary ammonium salts (Fig. 1).

The use of chlormequat chloride on mustard plants in the budding phase caused a decrease in growth in the phase of wax ripeness by 4.87%, and the use of retardant on winter rape plants

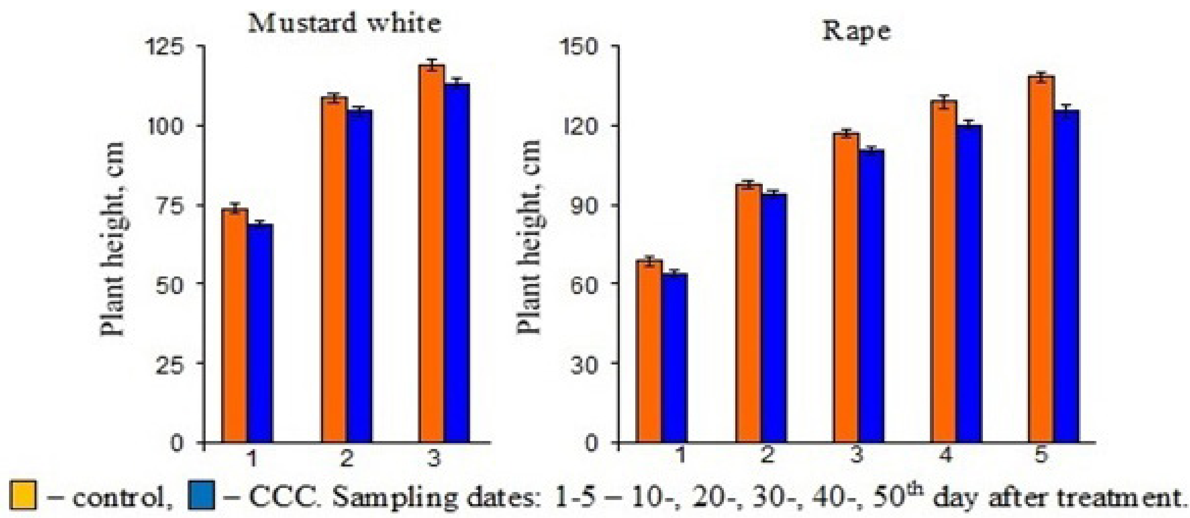

Figure 1. The effect of CCC on the linear dimensions of plants of the cruciferous family

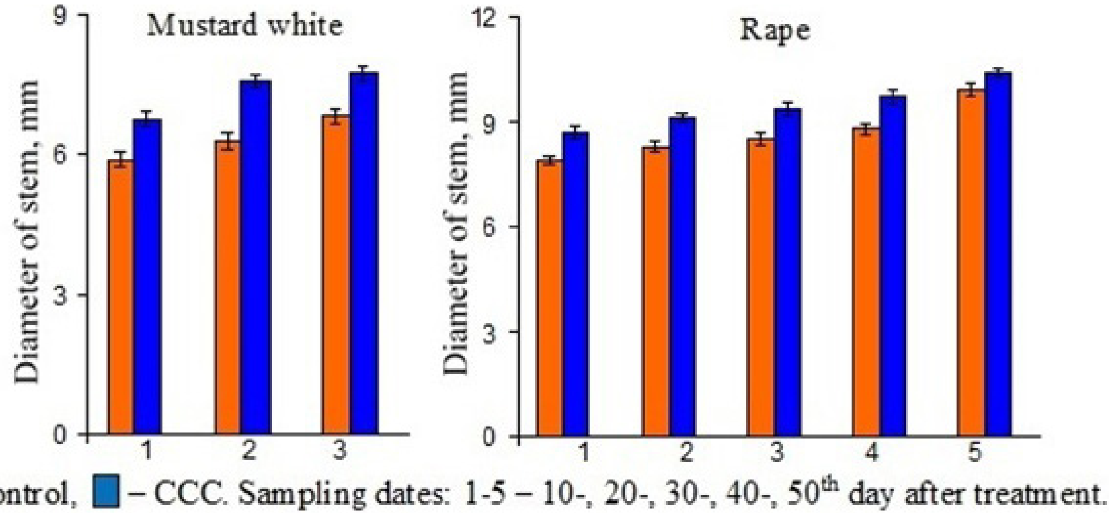

Figure 2. The effect of CCC on the diameter of the stem of cruciferous plants

caused a decrease in shoot length by 9.36% compared to the control sample.

Lying down is a serious problem for agricultural plants, especially for cereals. There is information in the scientific literature that plant growth regulators with antigiberrelic mechanism of action are used to tackle this problem. The issue of oilseeds lying is also essential for agriculture. This problem is also relevant for oilseeds, including rapeseed and mustard.

In the course of the research we found that the use of exogenous chlormequat chloride growth inhibitor caused the thickening of the shoot of experimental plants (Fig. 2).

The most considerable shoot thickness at the end of its development increased with the use of the retardant and the white mustard culture, an average of 13.63% compared to the control sample. Similar results in increasing the stem thickness were obtained on plants of flax oil, poppy oil in the study of the action of chlormequat chloride.

Therefore, the use of CCC enlarges the stem diameter, improving the resistance of the aforementioned plants to lying.

The photosynthetic apparatus plays a major role in fruit formation. It depends on the anatomical structure and number of leaves, as well as leaf area (Kuryata, 2009; Shadchyna et al., 2006). That is why it was necessary to analyse the peculiarities of the formation of the leaf surface of white mustard and winter oilseed rape under the action of a synthetic growth inhibitor.

We found that the treatment of mustard and rapeseed crops with chlormequat chloride leads to remarkable changes in the number of leaves, their area between experimental plants and the control

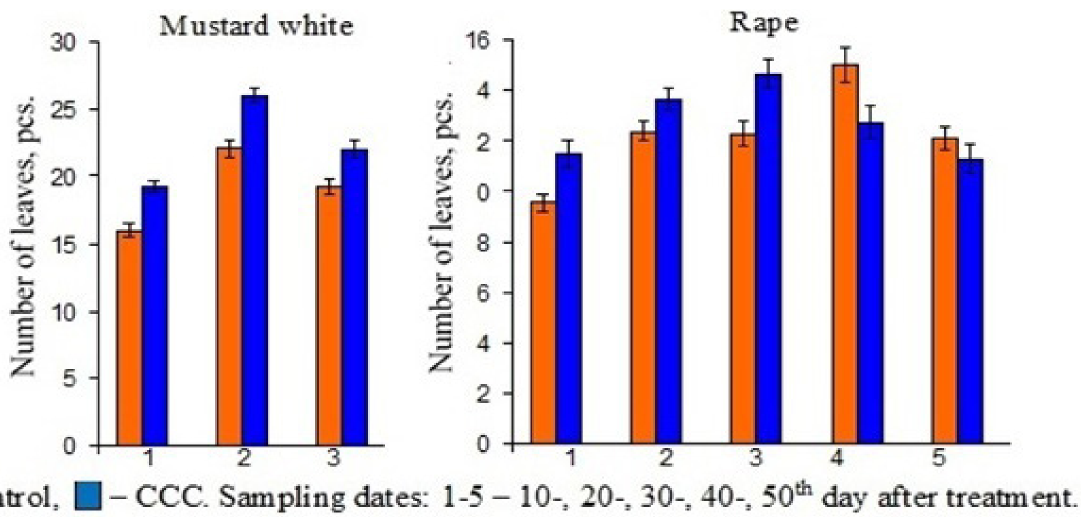

Figure 3. The effect of CCC on the number of leaves of rapeseed and mustard

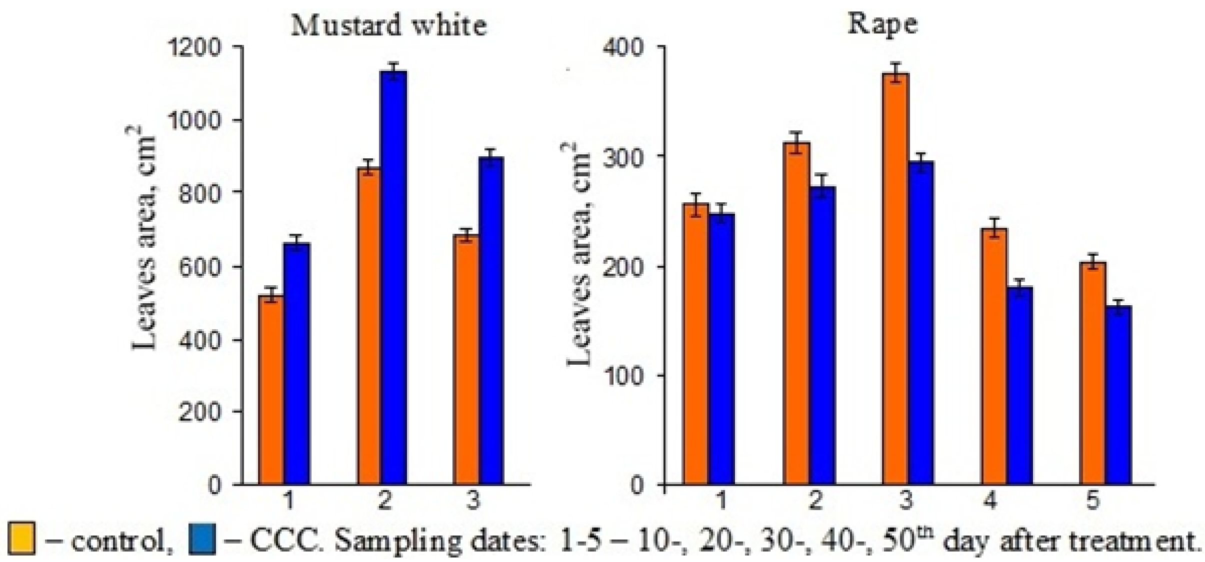

Figure 4. The effect of CCC on the leaf area of mustard and rapeseed plants

sample. The number of leaves in mustard plants under the action of the applied retardant increased during the ontogenesis (Fig. 3). A similar effect from the application of antigiberrelic preparations was observed in flaxseed plants. However, the use of CCC on winter rape plants was accompanied by a decrease in the number of leaves.

The increase in the number of leaves after treatment with CCC is due to an increase in the number of additional shoots of the first order and is 8.16 ± 0.30 as compared to the control sample with 7.00 ± 0.27. Enhancement of stem branching under the action of growth stimulants is a general reaction of plants to the action of antihypertensive preparations. Moreover, similar changes were observed in plants of oil flax and poppy oil (Kuryata, Khodanitska 2018; Kuryata, Polyvanyi 2018; Sousa Lima et al., 2016).

The leaf area plays a crucial role in fruit formation. The existing literature data show that retardants affect the leaf area, specifically, the use of chlorcholine chloride on sugar beet plants (Shevchuk et al., 2021) caused a decrease in leaf area.

The results indicate that the treatment with chlormequat chloride changed the leaf surface (Fig. 4). In particular, the measuring of the total leaf area on one mustard plant indicates its increase as compared to the control sample throughout the observation period. Thus, the increase in the number of leaves per plant under the action of the retardant provided an increase in the total area of leaves. At the same time, the use of quaternary ammonium salt on winter rape plants led to a decrease in leaf area in comparison to the sample sample.

The leaf physiological condition is determined by leaf anatomical and morphological features. In the field experiment it was found that in plants of white mustard and rape on the 10th day after treatment with chlormequat chloride solution there was a notable increase in leaf thickness on account of the growth of the assimilation parenchyma layer in both cases (Table 1).

The increase in the thickness of the parenchyma layer under the action of retardant is caused by a change in the size of its cells. Thus, in the

Table 1. The effect of CCC on the mesostructure of rapeseed and mustard leaves

Indicators	Thickness of the leaf blade, μm	Thickness of chlorenchyma, μm	Cell volume of the columnar parenchyma, μm^3	Cell width of t he spongy parenchyma, μm	Cell length of the spongy parenchyma, μm
Mustard white					
Control	205.46 ± 3.96	167.31 ± 3.89	2038.49 ± 101.92	16.48 ± 0.39	23.03±0.81
CCC	*286.59 ± 7.06	*245.44 ± 3.99	*2489.86 ± 120.66	*22.49 ± 0.48	*37.04±0.84
Rape					
Control	228.58 ± 2.35	185.69 ± 6.46	1355.72 ± 25.91	13.25 ± 0.70	18.69 ± 0.72
CCC	*283.76 ± 2.57	*230.63 ± 2.39	*1436.61 ± 27.26	*22.10 ± 1.04	21.55 ± 1.35

untreated mustard plants the cross section of the leaf is 205.46 ± 3.96 microns, whereas under the action of 5% CCC it is 286 ± 7.06 microns. In rapeseed plants treated with anti-giberrelic drug, the leaf thickness is 283.76 ± 2.57 microns as compared to the control sample of 228.58 ± 2.35 microns. Similar results were obtained as regards to potato, tomato and soybean crops (Kuryata et al., 2019; Rohach et al., 2020).

The plant organism is an open system whose metabolism is closely linked to the external environment. This connection is carried out by means of different formations in integumentary fabrics. One of these is the stomata, which play a dramatic role in the transpiration process.

It is found that growth inhibitors affect the number of stomata per abaxial leaf surface unit (Polyvanyi et al., 2020; Rohach, Kiriziy et al., 2020). Studies of the effect of the retardant on the respiratory tract of white mustard leaves reveal that the number and area of stomata increased in the treated plants (Table 2). However, our research shows that the area of stomata in rapeseed plants increased, but their number decreased per leaf area.

It is noteworthy that considerable changes occur in the leaf epidermis due to the retardants. Treatment of white mustard leaves with a growth inhibitor in the budding phase led to a decrease in the number of epidermal cells per unit of abaxial surface of the leaf compared with the control sample, which testifies to the increase in the cell size of the lower epidermis. It is also remarkable that the number of epidermal cells per unit of abaxial leaf surface in experimental rapeseed plants decreased compared to the control plants.

The use of physiologically active preparations in the technological complex of field cultivation contributes to crop productivity increase with a fairly high rate of economic and energy efficiency, without producing a toxic effect on the environment.

Inhibitors of a growth type are used to increase productivity. For this purpose, quaternary ammonium salts are also used in vegetable (Kuriata et al., 2016) and oil (Matysiak et al., 2013) crops. In particular, under the influence of CCC in flax plants there was an increase in seed productivity (Kuryata, Khodanitska 2018; Sang-Kuk et al., 2014).

We found that under the action of the retardant a more vigorous leaf apparatus of rapeseed and mustard plants was formed, which led to the formation of an abundance of assimilates used for fruit formation.

The use of antigiberrelic preparations on mustard and rapeseed plants has led to changes

Table 2. Change in the leaf respiratory system in rapeseed and mustard plants treated with CCC

Indicators	Number of stomata per 1 mm^2 of abaxial leaf surface, pcs.	Area of one stomata, μm^2	The number of epidermal cells per 1 mm^2 of abaxial surface of the leaf, pcs.
Mustard white			
Control	328.13 ± 13.89	120.76 ± 1.79	993.24 ± 18.02
CCC	*377.79 ± 10.78	*158.84 ± 2.72	*1065.98 ± 16.06
Rape			
Control	118.80 ± 5.36	228.99 ± 7.93	535.48 ± 24.87
CCC	*101.83 ± 5.57	*251.53 ± 6.08	*445.63 ± 10.90

Table 3. Influence of CCC on the productivity of mustard and rape plants

Indicators	Control	CCC 05%
Mustard white		
Number of pods per plant, pcs.	167.37 ± 6.79	*224.65 + 7.83
Number of seeds per pod, pcs.	3.75 ± 0.14	*4.25 ±0.13
Weight of 1000 seeds, g	6.38 ± 0.04	6.52 ± 0.05
Yield, c/ha	9.4 ± 0.30	*12.83 ± 0.37
Rape		
Number of pods per plant, pcs.	39.6 ± 1.11	42.5 ± 1.04
Number of seeds per pod, pcs.	26.3 ± 0.38	27.8 ± 0.07
Weight of 1000 seeds, g	4.3 ± 0.42	4.4 ± 0.11
Yield, c/ha	25.6 ± 0.73	*28.4 ± 0.74

in the crop structure: an increase in the amount of pods, an increase in the mass of thousands of seeds and the number of seeds in the pod. These changes increase the yield of the crops under investigation (Table 3).

Given the requirements of environmental safety when using growth regulators, it is necessary to determine the content of residual amounts of the preparations in the seeds.

The results of our studies demonstrate that in the experimental samples of white mustard seeds, the residual content of CCC was 0.023 mg/kg, and in winter rapeseed it was 0.05 mg/kg. According to the permissible doses of pesticides in agricultural raw materials (8.8.1.2.3.4.-000-2001), the residual amount of CCC should not exceed 0.1 mg/kg, i.e., the seeds of treated crops meet the existing sanitary standards.

CONCLUSION

The research, presented in this article, was carried out with the aim of expanding the spectrum of action of ecologically safe antigiberrelic preparations in agriculture. The application of chlormequat chloride to inhibit the growth of mustard and rapeseed plants is proposed.

Slowing of linear growth of the shoot at the beginning of the growing season under the action of CCC caused the stem thickening as regards both the plants under investigation, and some differences in the number and area of the leaf surface. The treatment of plants with a growth inhibitor led improving the anatomical structure of the leaves. Moreover, there was a thickening of the main assimilation tissue of the parenchyma due to the growth of its cells. The abovementioned application led to increased productivity of mustard, rapeseed.

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