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ORIGINAL ARTICLE

Effect of 2-chloroethylphosphonic acid on rate of fruit ripening and quality of tomato products

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We were studied the influence of 2-chloroethylphosphonic acid (2-CEPA) on the rate of fruit ripening of tomatoes Solerosso hybrid under production conditions. The treatment of 0.3% 2-chloroethylphosphonic acid at the stage of 25% fruit ripeness is highly effective method to accelerate the ripening of tomatoes. Such application increased in the friendliness of ripening and obtaining of the early yield production, reduced the number of tomato harvests and provided significant economic advantages. There was a significant restructuring of the cell wall of the fruit tissues due to intensive hydrolysis of hemicellulose and pentosans under the action of the drug. Interaction of ethylene-releasing compound increased in the total content of carboxyl groups, free and esterified carboxyl groups in the pectin fraction during the period of active fruit ripening. These resuts indicate a more intense hydrolysis of the non-galacturonic fraction of pectin substances. The hardness of the fruit tissue decreased and their consumer quality improved due to the hydrolysis of the structural polysaccharides of the cell walls. Pre-harvest treatment of 0.3% 2-chloroethylphosphonic acid led to increase in total acidity, decrease in the content of sugars and ascorbic acid in the fruits of treated plants compared to the mature fruits of untreated tomatoes. At the same time, these changes are within the range of fluctuations to typical product quality values for this crop.

Keywords: Tomatoes, ethylene-releasing compounds, polysaccharides, ripening, product quality.

Introduction

The growth regulators has become an important component of agrarian method that reduce the cost of manual labor and provide mechanization of care and harvest of crop products, a stable yield and its high quality. Analysis of trends of the gobal crop production chemicalization indicates that the application of plant bioregulators at this stage of development is effective and economically beneficial. The regulation of physiological processes by drugs of this group is highly specific and cannot be achieved by other methods (Panyapruek, et al., 2016; Kuryata and Khodanitska, 2018). The creation of national programs for plant growth regulators, the restructuring of agricultural research in many countries of the world has reached of this direction to a qualitatively new level, which is marked by the creation of new highly effective and environmentally sustainable growth regulators. The retardants (synthetic inhibitors of plant growth) has the greatest value in agricultural production among the known growth regulators. The application of these drugs in many cases leads to a significant increase in yields, which determined their introduction into complexes of effective methods for the cultivation of various crops: cereals (Wang et al., 2016), vegetables (Rogach et al., 2016), oilseeds (Koutroubas and Damalas, 2016; Kuryata and Polyvanyi, 2018) and technical crops (Matysiak and Kaczmarek 2013; Khodanitska et al., 2019; Shevchuk et al., 2019). A significant part of labor costs falls on harvesting in vegetable growing, therefore, the profitability of a crop and the prospects for its cultivation are determined by the mechanization of harvesting. Synthetic growth regulators affect the formation of the crown, decrease in the strength of attachment of fruits and the acceleration of their ripening. Such regulation can play a significant role in increasing the efficiency of existing and new harvesting machines and lead to an increase in the crop production. It is well known that the ethylene-releasing retadants regulate the growth rate and rearrangement of the donor-acceptor system of the plant (Kuryata and Poprotska, 2019; Kuryata et al., 2019), but also can accelerate the ripening of fruits and berries (Protsenko et al., 2013).

Ethylene-releasing compounds facilitate the separation of fruits and berries from the mother plant, therefore, pre-harvest processing with these preparations is recognized as a necessary condition for the effective application of fruit pickers. At the same time, one of the results of pre-harvest spraying is the acceleration of fruits and berries ripening, improvement of the decoration (Yang et al., 2021; Cui et al., 2021). The drugs can be used to reduce the number of harvests, post-harvest processing of fruits to accelerate their ripening. The synthesis of compounds that decompose with the release of ethylene in a plant opens up broad prospects for their application in plant growing practice for the mechanized harvesting, to avoid unfavorable weather conditions, early frosts. It has been established that the fruit and berry ripening is accompanied by a deep structural and functional rearrangement of the cell wall, an increase in the activity of enzymes that destroy polysaccharides (Yashoda et al., 2006; Majumder and Mazumdar, 2002), and significant structural changes in hemicellulose (Dzhan et al., 2012), the ratio of protopectin to soluble pectin and depolymerization of pectins (Kausik and Bibhas, 2005; Ding et al., 2017), increases resistance to infections (Dong et al., 2020). However, the development of industrial technologies for the ethylene-releasing compounds is largely limited. The physiology of their action on vegetable crops, in particular on tomatoes, has not been sufficiently studied.

It is promising to establish the physiological role of ethylene and ethylene releasing compounds in the regulation of carpogenesis, fruit ripening and senescence. In this regard, the aim of the study was to establish the features of 2-chloroethylphosphonic acid (2-CEPA) interaction on the structural polysaccharides of the cell walls of the tomato tissues in connection with their softening during the fruit ripening.

Materials and Methods

Field-based micro-trial experiments were established on tomato plants at a specialized farm "Solsky" (2016-2017) in Vinnitskiye khutory, Vinnytsia region, Ukraine. The Solerosso hybrid is the high-yield ultraprecocious deterministic hybrid of dutch selection «Nunhems» (Bayer CropScience, Holland). The growing season is 90-95 days. The plant height is up to 50 cm. The bushe of a plant is determinant and moderately sprawling. The fruit is roundish, the weight is 50-60 g, medium density, forms up to 6 seed chambers and is characterized by a good taste. The hybrid has a very high yield (about 100 t/ha), despite the small size of fruit. Tomatoes are characterized by amicable ripening (brushes of 5-6 pieces). It is used for whole-canning, processing for tomato products, as well as for the sale of early products on the market. The hybrid is tolerant to fusarium, verticidosis, cladosporiosis and is resistant to stressful growing conditions.

Tomato seeds were sown for seedlings in greenhouses on March 2, 2015, March 5, 2016 and March 12, 2017. Seedlings were planted on May 18, 2015, May 22, 2016 and May 27, 2017 by a tape method with the planting formula 50+50+50 × 50. It was used a mineral fertilizers $N_{50}P_{40}K_{30}$. The experiment followed a randomized block design (33 m^2) with five replication.

The treatment was applied via foliar spraying OP-2 with an aqueous solution of 0.3% 2-chloroethylphosphonic acid (2-CEPA, $C_2H_6ClO_3P$). The drug compound is esphon ("Agrosintez", Russia). Esphon belongs to ethylene releasing compound according to the mechanism of action. The active substance quickly penetrates into the plant and decomposes in its tissues with the formation of ethylene. The effect of ethylene producer significantly depends on the air temperature. The temperature range should be from +12 to +30°C. The maximum dose treatment is recommended at a temperature below 16°C. The resistance to rinsing is acquired after 4-5 hours of the treatment. 2-chloroethylphosphonic acid (2-CEPA) is a solid white waxy substance with a molecular weight of 144,5 Da. The melting point is 74-75°C. 2-CEPA is a hygroscopic, readily soluble in water, ethyl and isopropyl alcohols, acetone, propylene glycol solution. It hydrolyzes to ethylene, hydrochloric and phosphonic acid in water at pH above 4,1-4,5. It has a low toxicity for warm-blooded and belongs to the 3d class of toxicity. LD_{50} for rats is 4220 mg/kg at first injection (Kuryata and Poprotska, 2019).

Pectin preparations were isolated by extraction from dry material, extracted with 0.03 N HCl for 1 hour at a temperature of 80°C. The resulting extract was filtered and the residue was washed with 0.03 NHCl. The resulting extract was precipitated with ethyl alcohol (three times of the total volume –1:3). After decantation, the precipitate was centrifuged, dissolved in water, resepareted with alcohol (three times of the volume), centrifuged, washed with acetone until negative reaction with chlorine ions and dried. In the obtained preparations, it was determined the content of total, free and esterified carboxyl groups (Lukin et al., 2005). The hemicellulose content was determined by the iodometric method, the quantitative content of pentosans was determined colorimetrically at 610-660 nm wavelength by a qualitative reaction with an orcine reagent. The content of hemicellulose was determined by the iodometric method, the quantitative content of pentosans was determined colorimetrically at a wavelength of 610-660 nm by a qualitative reaction with an orcinic reagent. In mature fruits, it was determined the product quality indicators - the content of sugars, ascorbic acid and total acidity (AOAC, 2010). Statistical analysis of experimental data was performed by Statistica v.6.0. software. The reliability of obtained results between control and experiment variant was assessed with the Student's t-test. tables and figures show average values for the years of research and their standard errors.

Results and Discussion

The phytohormone ethylene regulates physiological processes at different stages of plant ontogenesis. This hormone controls the rate of fruit ripening and the stickiness of plants to unfavourable growth conditions. As a result, the physiological changes in the

plant under the actions of ethylene producer are important for fundamental research and for solving practical problems. (Zemlyanskaya et al, 2016). The treatment of ethylene releasing compound at the stage of 25% fruit ripeness significantly accelerated the rate of their ripening and reduced the number of harvests from three to two which created economic advantages in the early yield production (Fig. 1,2.).

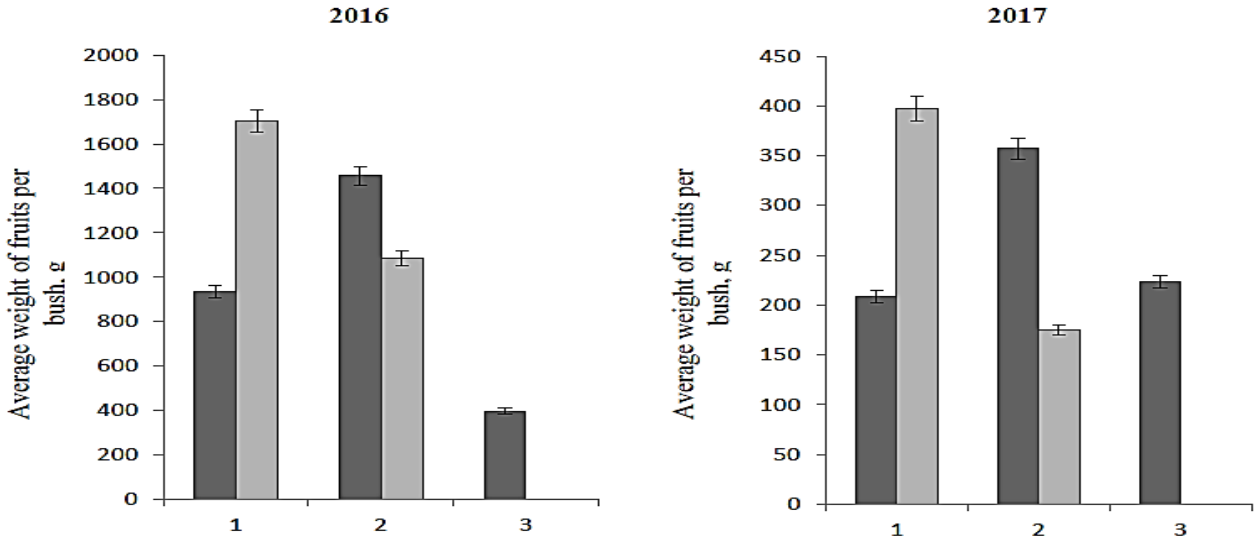

Fig. 1. Influence of 0.3% 2-chloroethylphosphonic acid on ripening rate of tomato fruits Solerosso hybrid. Treatment time: 2016–28th of july, 2017–3d of August. Sampling time: 1–after 3 days, 2–after 6 days, 3–after 9 days.

– control; – 0.3% 2-CEPA

Fig. 2. Ripening of tomato fruits Solerosso hybrid under 0.3% 2-chloroethylphosphonic acid treatment in field condition.

However, it was found that the application of ethylene inhibitor 1-methylcyclopropene (1-MCP) reduces the sensitivity of fruits to ethylene, thus controlling the rate of ripening and loss of density. 1-MCP application contributes to reduce the internal decay, retaine the content of sugars and organic acids and increase in the product stability during the storage and sale of pears. The 1-MCP effect depends on the degree of harvest maturity and storage duration (Melnyk and Drozd, 2019).

The degradation of cell wall structures during the fruits and berries ripening is accompanied by the enzymatic degradation of polyuronic polymers, a change in the ratio of protopectin to soluble pectin and depolymerization of pectins (Ding et al., 2017). The determination of molecular weight of berry pectins showed that the polyuronid complex undergoes significant changes within a week under 0.1% camposan M treated raspberry. The average molecular weight of pectins decreased upon ripening of berries, and

the process was faster in the treated trial. It was revealed that the mass fraction of high molecular weight fractions of pectins decreased in the experimental trial compared to control. The author concluded that one of the reasons for transition of protopectin to soluble pectin is the predominant cleavage of polygalacturonase of high molecular weight fractions of pectins upon maturation (Kuryata, 1991).

The study of the content and structural features of non-cellulose polysaccharides of tomato fruits at the maturation stage indicate the restructuring of polysaccharide complex of the cell walls during this period. The obtained analysis of the structural elements of pectins extracted at the different stages of ripening indicate significant structural changes in these polysaccharides throughout the entire ripening period both in the control and in the variant of ethylene producer treated plants (Table 1).

It was noted an increase in the free carboxyl groups content in the pectin fraction during the period of active fruit ripening. It can be explained by the processes of deetherification, enzymatic cleavage of methoxyl and acetyl groups. At the same time, it was found that the content of total and esterified carboxyl groups of pectin increased during this period, which does not allow us to draw the following conclusion. In our opinion, the growth of total carboxyl groups in pectin is determined by the more intensive hydrolysis of the non-galacturonic fraction of pectin substances.

Indicators	**Control**	**0.3% 2-EPA**	**Control**	**0.3% 2-EPA**	**Control**	**0.3% 2-EPA**
	01.08.2016		04.08. 2016		07.08.2016	
Free carboxyl groups, мg·Eq/g	0,06 ± 0,001	*0,14 ± 0,004	0,19 ± 0,004	*0,24 ± 0,006	0,33 ± 0,001	*0,29 ± 0,001
Esterified carboxyl groups, мg·Eq/g	1,55 ± 0,021	*3,01 ± 0,072	2,89 ± 0,091	3,00 ± 0,081	3,23 ± 0,122	3,41 ± 0,091
Total carboxyl groups, мg·Eq/g	1,61 ± 0,022	*3,15 ± 0,081	3,08 ± 0,052	3,24 ± 0,092	3,56 ± 0,112	3,70 ± 0,111
	07.08.17		10.08.17		13.08.17	
Free carboxyl groups, мg·Eq/g	0,101 ± 0,002	*0,15 ± 0,003	0,151 ± 0,003	0,161 ± 0,004	0,211 ± 0,001	0,191 ± 0,006
Esterified carboxyl groups, мg·Eq/g	2,55 ± 0,081	*3,83 ± 0,132	3,59 ± 0,112	*4,42 ± 0,141	4,27 ± 0,131	4,75 ± 0,151
Total carboxyl groups, мg·Eq/g	2,65 ± 0,071	*3,98 ± 0,111	3,75 ± 0,122	*4,58 ± 0,152	4,48 ± 0,141	4,94 ± 0,163

Note: Treatment time: 2016–28th of July, 2017–3d of August. *difference is significant at p<0,05.

Table 1. The content of free, esterified and total carboxyl groups of pectins under 0.3% 2-chloroethylphosphonic acid treatment on tomato fruit during ripening.

The ripening of unabi fruits is accompanied by a decrease in the content of hemicellulose with a simultaneous accumulation of sugars (Dzhan et al., 2012). It was established that the process of accelerated ripening of mango fruits due to the action of ethylene is accompanied by an increase in the activity of three glycosidases: β-D-galactosidase, α-D-mannosidase and β-D-glucosidase. The intensification of enzyme activity resulted in an accelerated accumulation of reducing and restoring sugars in fruit

tissues (Chidley et al, 2017). Accordingly, the effect of ethylene on the content of hemicellulose and their main fraction, pentosans during fruit ripening is not adequately explored, which necessitates a more detailed study of this issue. The analysis of the hemicellulose and pentosan content at the different stages of fruit maturity indicates an intensive hydrolysis of these polysaccharides, while these processes occur more intensively of 2-CEPA- treated trial (Fig. 3).

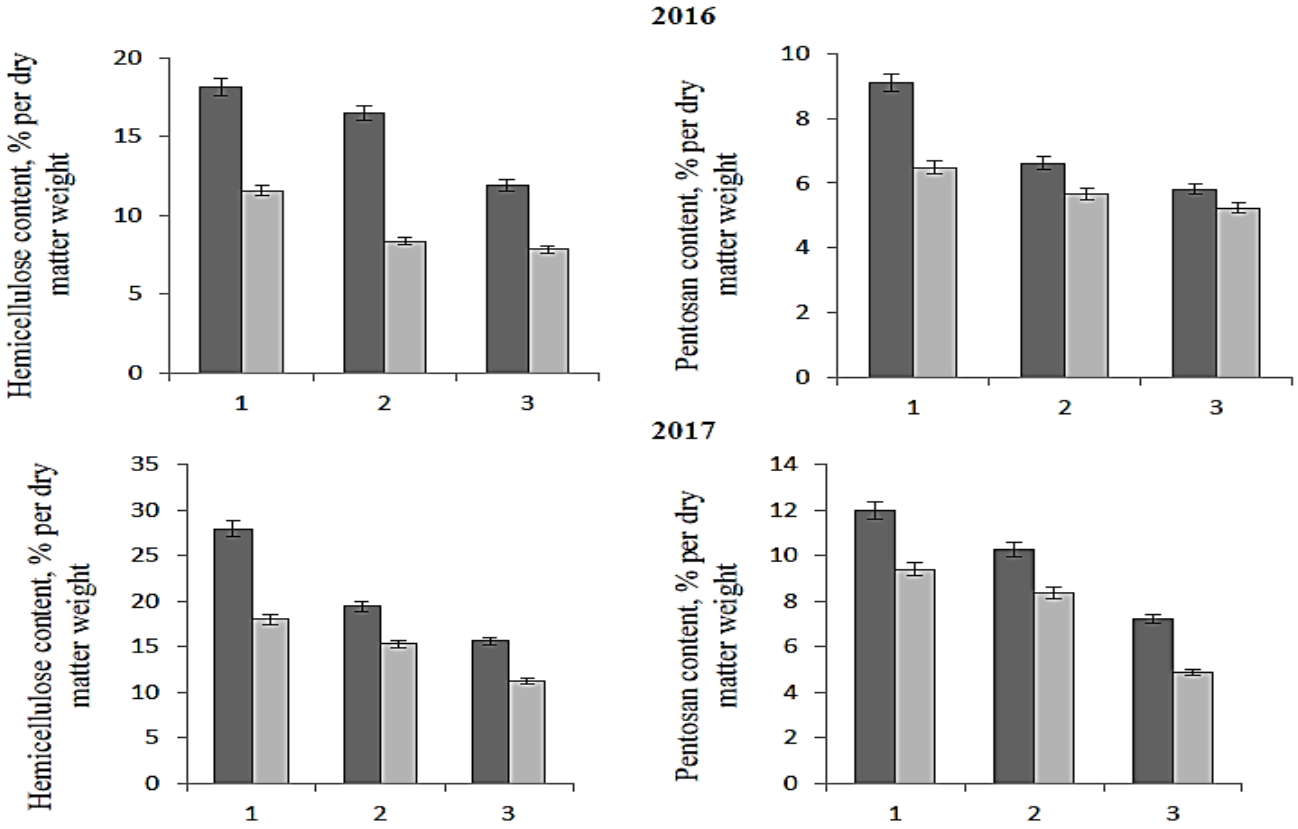

Fig. 3. The hemicellulose and pentosan content in tomato fruits under ethylene releasing compound application. Treatment time: 2016 – 28th of july, 2017 – 3d of August. Sampling time: 1–after 3 days, 2–after 6 days, 3–after 9 days.

–Control; –0.3% 2-CEPA.

Thus, the ripening process is largely determined by the intense maceration of fruit tissues, which is based on the hydrolysis of polysaccharide components of the cell wall. Moreover, the decrease in the content of hemicellulose and pectin occurs more intensively under 2-CEPA application compared to control. The yielding of sufficiently high quality characteristics products is an important condition for the synthetic growth regulators application. Literature data indicate certain differences in the quality of fruits under natural ripening conditions and processed to accelerate ripening with ethylene. It was noted that ethylene-treated fruits of banana, pineapple and tomato crops had a shorter shelf life than untreated samples. It was also established an insignificant decrease in the content of ascorbic acid and carotene in the treated fruits as compared to control (Hakim et al, 2012). Other studies have noted that the application of ethylene can accelerate the ripening process and improve the quality of chili peppers. Ehe maximum residue compound of ethephone in chili peppers after treatment was 21,18 mg/kg^{-1}, which is lower than the permissible residue level of 50 mg/kg^{-1} for chili peppers (Yang et al., 2021).

It was found that forced ripening of tomato fruits is accompanied by changes in the quality of crop production. 0,3% 2-CEPA pre-harvest treatment led to an increase in the total acidity, decrease in the content of carbohydrates, sucrose and ascorbic acid in the fruits of treated plants compared to mature fruits of tomatoes that ripened in vivo without the application of ethylene producers (Table 2). At the same time, these changes are within the range of fluctuations to typical product quality values for this crop.

Indicators	Control	0,3% 2-CEPA
Content of ascorbic acid, mg/100 g	26,38±0,82	26,02±0,69
Titrated acidity, g /100 g	0,52±0,02	*0,62±0,02

Reducing sugar, % per fresh matter	1,15±0,04	1,19±0,02
Sucrose, % per fresh matter	0,82±0,01	*0,62±0,01
Total sugars, % per fresh matter	2,07±0,03	1,83±0,03

Note: * difference is significant at $p<0.05$.

Table 2. Influence of 2-chloroethylphosphonic acid on product quality of tomato hybrid Solerosso (average values for 2016-2017).

Thus, the application of 0.3% 2-CEPA at the stage of 25% fruit ripeness significantly accelerated their ripening due to intensive maceration of fruit tissues, which is based on the hydrolysis of polysaccharide components of the primary cell wall–hemicellulose and pectins. The number of harvests was reduced and the share of early production in the crop structure was increased by drug treatment.

Conclusion

The treatment of 0.3% 2-chloroethylphosphonic acid at the stage of 25% fruit ripeness is highly effective method to accelerate the ripening of tomatoes. Such application increased in the friendliness of ripening and obtaining of the early yield production, reduced the number of tomato harvests and provided significant economic advantages. There was a significant restructuring of the cell wall of the fruit tissues due to intensive hydrolysis of hemicellulose and pentosans under the action of the drug. Interaction of ethylene-releasing compound increased in the total content of carboxyl groups, free and esterified carboxyl groups in the pectin fraction during the period of active fruit ripening. These results indicate a more intense hydrolysis of the non-galacturonic fraction of pectin substances. The hardness of the fruit tissue decreased and their consumer quality improved due to the hydrolysis of the structural polysaccharides of the cell walls. Pre-harvest treatment of 0.3% 2-chloroethylphosphonic acid led to increase in total acidity, decrease in the content of sugars and ascorbic acid in the fruits of treated plants compared to the mature fruits of untreated tomatoes. At the same time, these changes are within the range of fluctuations to typical product quality values for this crop.

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