Effect of metal nanoparticles on the accumulation and structure of rapeseed carbohydrates

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Abstract. The purpose was to conduct field tests of the most active metal nanoparticles of iron, cobalt and copper to realize the maximum accumulation of polysaccharides in plants; to develop a method for obtaining and studying the structure of polysaccharides obtained from plants treated before sowing with metal nanoparticles; to study the physical-chemical characteristics of polysaccharides and the monosaccharide composition under the influence of metal nanoparticles. Before sowing, seed treatment was carried out with suspensions of nanoparticles with a concentration of 0.5 g / ha (hectare seed sowing rate). Observed under the influence of copper nanoparticles, the yield increased by 15 % comparison to the control one. When determining the monosaccharide composition, in addition to changing the quantitative content of monosaccharides, a high concentration of galacturonic acid was observed, especially when treating seeds with copper nanoparticles before sowing.

1. Introduction

Carbohydrates and their derivatives perform various functions in plants and have a high biological value. The content of polysaccharides in plants varies, not only depending on the type of plants, but also on the conditions of their growth, on age, stages of vegetation, and the ecological state of the environment. For the same reasons, along with a change in the amount of carbohydrates, qualitative changes in these biopolymers also occur, therefore, during the analysis it is necessary that they are in the same vegetation stage. Raw materials for such studies should be collected and stored under the same conditions.

Depending on the monosaccharide composition, plant polysaccharides are distinguished into acidic, neutral and basic. A certain role in this division belongs to uronic acids, namely their content in the polysaccharide. Depending on the amount of uronic acids, polysaccharides belong to three groups and, by their chemical nature, are glycuronoglycans. The first group consists of polysaccharides, which contain only uronic acids. The second group includes carbohydrates, which include less than fifty percent of uronic acids, as the remaining components are a variety of neutral monosaccharides, as well as their derivatives. The main part of the polysaccharide composition is polygalacturonic acid.

Polygalacturonic acid mainly consists of D-galacturonic acid residues. However, glucose, arabinose, galactose, rhamnose, and mannose are also included in the bulk of the polysaccharide. Polysaccharides incorporate a branched polysaccharide chain. Branching points are monosaccharides of different chemical composition. The monosaccharide composition determines the functional
characteristics of the polysaccharide, its role in the growth and development of plants, biological activity and feed value.

The study of the influence of trace elements on the physical-chemical constants of polysaccharides, structure and monosaccharide composition is of particular interest. Currently used trace elements in the form of biologically active nanoparticles of biogenic metals are used as stimulants of plant growth and development. Their influence on the accumulation of polysaccharides and enhancement of their biological activity due to a quantitative change in the monosaccharide composition is assumed. There is no analysis of the influence of nanometals on the accumulation and structure of biologically active compounds in agricultural and medicinal plants in the scientific literature.

The effect of metal nanoparticles (NPs) on medicinal plants and polysaccharides isolated from them, which are used as natural medicinal products present great interest. The composition and structure of plant polysaccharides determine their properties, biological activity and nutritional value. The study of the biological activity of NPs and some physical-chemical properties of plant polysaccharides will expand their scope in agricultural production when developing new forms of biologically active substances.

2. Materials and methods
Field tests took place at the instructional farm of Ryazan State Agrotechnological University Named after P.A. Kostychev according to the work [1]. The soil of the test areas was gray forest. The land plots area was 100 m$^2$ and the replication was 3-fold.

The plants for determining the accumulation of polysaccharides in the herbage were selected in different growing periods. The samples of aboveground parts of spring rape of the same mass were taken from experimental and control plots at the same time for further research. It was brought to constant weight by drying the herbage in laboratory conditions, without direct sunlight at a constant temperature and periodical turning of the collected plants by hand. The dried mass was subsequently used for physical and physical-chemical analyzes.

3.1 Extraction of polysaccharides from plants
The variety of monosaccharides that make up natural polysaccharides makes these compounds unique in their properties, and the study of natural biopolymers requires certain schemes for their extraction from plant materials. The simplest method of extraction is water extraction, both at room temperature and at elevated temperature [2].

The separation of carbohydrates from dry plant materials was carried out according to the method [3], based on the practical determination of the highest release of polysaccharide under conditions of extraction with distilled water at least two times of the same raw material. The third water extraction gives insignificant amount of carbohydrate from the plant and it is more advisable to treat it a third time not with water, but with an electrolyte solution, then the third yield can reach 1 gram. The amount of extractives depended not only on the number of extracts, but also on the processing time of plant material. We studied the range of increasing temperatures from 40 °C to boiling water with a stepwise increase in temperature at 10 °C and the application time, which was 30, 60, 90 and 120 minutes. Water-electrolyte solutions containing polysaccharides were evaporated to a volume of 100-130 ml, cooled and ethanol was added, creating a concentration gradient from 0 to 60 %, achieving complete precipitation of polysaccharides insoluble in ethanol. The precipitates were separated by centrifugation, washed with 90 % alcohol, acetone and dried over calcium chloride. The quantitative content of polysaccharides by phenological phases was controlled by the gravimetric method. The separation into acidic and neutral fractions was carried out according to the procedure [3, 4]. The purification of the isolated polysaccharides was carried out with a 10 % solution of lead acetate.

3.2 Methods to study the physical-chemical characteristics and structure of polysaccharides
The following steps were used to study the structure of the isolated polysaccharides.
The first step is the purification of the isolated polysaccharides. For this, the method of dialysis and ion exchange chromatography on high molecular weight polymer resins was used. Then, ash was determined in the purified polysaccharides by burning.

Acidity, content of uronic acids and methoxy groups were determined by the Zeisel method and titrimetric analysis method [5, 6]. The hydrogen parameter of the medium was measured on a laboratory pH meter with a measurement range of 0.00-14.0, a resolution of 0.01, and an accuracy of ± 0.01. Molecular weights were calculated by spectrophotometric method with the help of spectrophotometer SF-2000-02. The optical density was determined by the AP-300 instrument - automatic polarimeter at 5-20 dm, wavelength 589 nm.

The value of optical activity \([\alpha]_{20}^\circ\) was calculated by the following formula:

\[
[\alpha]_{20}^\circ = \frac{\alpha}{c \cdot l} \cdot 100,
\]

where: \(\alpha\) - deflection angle; \(l\) - tube length, dm; \(c\) – concentration of the test substance, g / 100 ml.

When studying the composition of the polysaccharide, the precipitation method was used. A fairly wide range of reagents was used: barium hydroxide, sodium acetate, copper salts, calcium hydrochloride, cetavlol, Feling's solution and ethyl alcohol. Reagents were selected under certain conditions. The homogeneity of the extracted polysaccharides was confirmed using DEAE cellulose and the electrophoresis method. Electrophoresis was performed with the help of an EFK-4 device (voltage 400 V, current 20 \cdot 10^{-3} \text{ A}, time 60-150 minutes).

When determining the monosaccharide compositions of the isolated polysaccharides, all the types of hydrolysis were used: partial, complete acid and enzymatic. Sulfuric acid, oxalic acid and hydrochloric acid of various concentrations were used. The hydrolysis time depending on the polysaccharide varied from 2 to 40 hours.

Different chromatography methods with Shimadzu GC-2014 chromatograph were used [7]: if necessary, paper (PC), thin-layer (TLC), column, and gas-liquid in all cases. Columns having powdered cellulose and coal as a solid phase were used for column chromatography, when necessary. As a solvent, a solution of butanol-1 saturated with water was used.

Chromatography on paper was carried out in accordance with [8] on paper of brand FN-7, FN-11, according to the method previously washed with an aqueous solution of ethanol-water (9:1). The method for performing chromatography is top-down. Depending on the objects, chromatography was performed using various solvent systems:

1) butanol-1 – pyridine - water (6: 4: 3);
2) ethyl acetate - formic acid - water - acetic acid (18: 1: 4: 3);
3) ethyl acetate - pyridine - water - acetic acid (5: 5: 3: 1);

For the development of chromatograms, the following systems were used:
- a mixture of potassium permanganate - sodium periodate;
- aniline phthalate reagent;
- a mixture of silver nitrate and KOH solutions.

When conducting gas-liquid chromatography, the analysis was carried out on a device with a Shimadzu GC-2014 flame ionization detector. Stainless steel 1 m long columns with a diameter of 0.3 m were used. To separate monosaccharides, polyols and tartric acids N-AW-DMCS chromaton was selected with the addition of 5 % XE-60 silicone; grain diameter 0.16 - 0.20 mm. 3 % polyethylene glycol on chromaton N-AW-DMCS was also used.

Thus, using physical, chemical, and physical-chemical methods (hydrolysis with methylation and periodical oxidation, gas-liquid, thin-layer, paper chromatography, IR spectroscopy), we obtained results that allowed us determining the monosaccharide composition and structural elements of polysaccharides extracted from spring rape, as well as the nature of the change in these characteristics under the action of nanoparticles (NPs) of iron, cobalt, copper as a result of a single treatment of plant seeds before sowing.
3.3 Nanoparticles characteristics
Nanoparticles were obtained chemically: by precipitation of metal hydroxides from salt solutions, followed by their low-temperature reduction in a stream of hydrogen [9]. The physical-chemical characteristics of nanoparticles were determined by such parameters as specific surface area (m$^2$/g), phase composition (%), and size distribution of nanoparticles. The specific surface area of the studied nanoparticles was measured by low-temperature nitrogen adsorption by BET using the Quantachrome NOVA 1200e analyzer: Fe 45.7 m$^2$/g (18 - 44 nm); Co 52.1 m$^2$/g (23 - 46 nm); Cu 6.5 m$^2$/g (16 - 63 nm). The phase composition of nanoparticles was determined using x-ray phase analysis (XRA) according to the powder method on an XRD-7000 diffractometer (Shimadzu). The dispersion and morphology of the obtained metal nanoparticles were studied using a Tescan Vega 3 scanning electron microscope [10]. They had a shape close to spherical. The aggregation resistance was low.

To treat rapeseed, aqueous suspensions of nanoparticles were used.

4.1 The content of polysaccharides in spring rape after seed treatment before sowing with NPs of cobalt, copper and iron
During field tests, it was noted that the treatment of spring rape seeds with NPs of iron (Fe), cobalt (Co) and copper (Cu) at optimal concentration (0.5 g / g, hectare sowing rate of seeds) before sowing affected not only the growth and development, but also accumulation of polysaccharides in the plant (Table 1).

| Date      | Variant                  | Control | 0.5g Cobalt NP | 0.5g Iron NP | 0.5g Copper NP |
|-----------|--------------------------|---------|----------------|--------------|----------------|
| June 4    |                          | 2.44    | 2.67           | 2.54         | 2.61           |
| June 15   |                          | 2.57    | 2.74           | 2.67         | 2.79           |
| June 20   |                          | 2.60    | 2.80           | 2.72         | 2.80           |
| June 23   |                          | 2.65    | 2.73           | 2.79         | 2.82           |
| July 4    |                          | 3.08    | 3.53           | 3.54         | 3.55           |
| July 15   |                          | 3.77    | 4.07           | 4.17         | 4.21           |
| July 22   |                          | 4.10    | 4.99           | 4.98         | 5.06           |
| August 6  |                          | 5.91    | 6.75           | 6.26         | 6.77           |

The amount of polysaccharide accumulation in rapeseed reaches its maximum during the flowering phase, and shows a wave-like dependence. At the early stages of growth and development of rapeseed, like other plants, a leaf apparatus is being created and seedlings are growing intensively. Approximately the same amount of polysaccharides in the control and test samples is from 2 to 3 g per 100 g of spring rape herbage. The content of polysaccharides in the test samples, depending on NP, before flowering was higher (up to 15 %). However, during flowering, their number increased. With active photosynthesis, the processes of neoplasm are inhibited, which stimulates the accumulation of polysaccharides.

During the formation of generative organs (pods), a decrease in the number of polysaccharides is observed both in plants treated with metal nanoparticles and in control untreated variants.

However, at all stages of development, the content of water-soluble polysaccharides remained above the control up to 15 %, therefore, NPs of iron, cobalt, and copper intensively stimulate metabolic processes.

The completeness of hydrolysis of the studied polysaccharides was determined according to the results of 32-hour hydrolysis.

The results of studies on the intensity of hydrolysis show the influence of metal NPs on the number of polysaccharides, as well as, possibly, on their structure (Table 2).
Table 2. Effect of NP on the change in the intensity of hydrolysis of spring rape polysaccharides

| #  | Variant         | Hydrolysis time, hour |
|----|-----------------|-----------------------|
|    |                 | 4        | 8        | 12       | 16       | 20       | 24       | 28       | 32       |
| 1  | Control         | 0.40     | 0.32     | 0.22     | 0.19     | 0.10     | 0.02     | 0.01     | trace    |
|    |                 | 0.20     | 0.15     | 0.10     | 0.08     | 0.02     | 0.01     | -        | trace    |
| 2  | 0.5g Iron NP    | 0.47     | 0.39     | 0.29     | 0.26     | 0.17     | 0.13     | 0.09     | trace    |
|    |                 | 0.24     | 0.19     | 0.14     | 0.12     | 0.06     | 0.03     | -        | trace    |
| 3  | 0.5g Cobalt NP  | 0.45     | 0.37     | 0.27     | 0.24     | 0.15     | 0.11     | 0.07     | trace    |
|    |                 | 0.23     | 0.18     | 0.13     | 0.11     | 0.05     | 0.04     | -        | trace    |
| 4  | 0.5g Copper NP  | 0.46     | 0.38     | 0.27     | 0.23     | 0.12     | 0.06     | 0.04     | trace    |
|    |                 | 0.23     | 0.19     | 0.13     | 0.10     | 0.02     | 0.01     | -        | trace    |

* precipitate remaining after hydrolysis, g
**precipitate precipitated with alcohol, g

Unlike polysaccharides of control plants, polysaccharides of test samples underwent a lower hydrolysis rate, over the whole time, that is, for a shorter hydrolysis time, a greater amount of monosaccharides was released compared to monosaccharides from plant polysaccharides not treated with NPs. Apparently, polysaccharides after treatment of plant seeds with nanoparticles had a large molecular weight and more complex structure, which was proved in our further studies. In addition, an increase in the amount of non-hydrolyzed polysaccharide extracted from treated plants suggests a higher content of acid fractions, including galacturonic acid since according to some sources polygalacturonic acid is hydrolyzed within 30 hours, which is consistent with our results. At the same time, an increase in the amount of water soluble polysaccharides precipitated by alcohol suggests an increase in branch points.

4.2 Physical-chemical characteristics of the spring rape herbage polysaccharide after seed treatment with NPs of cobalt, copper and iron before sowing

As it was previously shown, over the entire period of plant growth and development, the level of water-soluble polysaccharides in the control variant was 5.0 - 15.0 % lower than in experimental variants (treated).

The nanoparticles of iron, cobalt and copper after a single treatment changed not only the amounts of water-soluble polysaccharides in the rape herbage, but their structure and monosaccharide composition. The hydrolysis time of polysaccharides (Table 3) indicates the presence of galacturonic acid in polysaccharides.

Table 3. Effect of metal NPs on chemical parameter, % titrimetric analysis and their content (%), 2019 (0.5 g / ha)

| #  | Chemical parameters | Control | Cu NP | Co NP | Fe NP |
|----|---------------------|---------|-------|-------|-------|
| 1  | Free OH groups, (Kc) | 1.2     | 3.31  | 2.4   | 1.4   |
| 2  | Esterified OH groups, (Kc) | 1.98 | 0.99  | 1.30  | 0.51  |
| 3  | -COOH groups        | 1.20    | 2.59  | 2.31  | 1.1   |
| 4  | CH₃O- groups        | 1.40    | 0.59  | 0.88  | 0.4   |
| 5  | pH                  | 6.9     | 5.8   | 6.0   | 6.3   |
| 6  | Galacturonic acid   | 20.1    | 27.8  | 25.2  | 24.9  |

The melting point of 155 – 157°C and [α]D20 = + 560 prove the presence of galacturonic acid in the hydrolysis products. Its derivatives give plants mechanical strength. According to Table 2, NPs of iron, copper, and cobalt do indeed increase the content of galacturonic acid in polysaccharides, which...
should make plants more resistant to lodging. The pH of the polysaccharide solutions also changes (Table 3), decreasing in relation to the control.

The highest content of galacturonic acid was in the variant with Cu NP. The excess to the control was 7.8 %, whereas in the variant with Fe NP and Co NP it was 6.2 % and 4.9 % respectively. As it was described above, under the influence of copper NPs, the highest accumulation of polysaccharides in rapeseed was noted.

4.3 Monosaccharide composition of polysaccharide of the spring rape herbage after seed treatment with NPs of cobalt, copper and iron before sowing

The monosaccharide composition of polysaccharides extracted from rapeseed was studied during their collection during mass flowering. This is the most favorable time to study the quantity and quality of polysaccharides [11]. At this time, the main biochemical changes in plants are realized. Carrying out the previously described methods of hydrolysis and chromatographic analysis made it possible to prove the following monosaccharides in the polysaccharides extracted from rapeseed plants after and without treatment with NPs of copper, iron and cobalt: D-ramnose, D-glucose, D-xylose, D-mannose, D-galactose and L-arabinose.

During the hydrolysis of rapeseed polysaccharide, the seeds of which were not treated with NPs after 24 hours, it was found: xylose, mannose, glucose in the amount of 64.2; 22.7; 10.4 %, and in experimental ones ramnose appeared additionally.

The amounts of galactose and arabinose in the polysaccharide extracted from rapeseed after treatment with iron nanoparticles were 50 % and 65 % lower than those in the polysaccharide from rapeseed without treatment. At the same time, galactose monosaccharide was 65 % higher in the polysaccharide extracted from rapeseed after treatment with copper nanoparticles.

After 4 hours of hydrolysis, the content of arabinose and galactose was 3 % lower in the experiment with Fe NP, and the galactose content in the experiment with Cu NP was 3 % higher than in the control.

Mannose monosaccharide in solution was detected after 8 hours of hydrolysis for all polysaccharides. Later its content was insignificant, but significantly decreased in polysaccharides of plants whose seeds were treated with metal NPs.

Xylose monosaccharide was detected in the solution during hydrolysis for 16 hours. Quantitatively, it was significantly less than in solutions after hydrolysis of rapeseed plant polysaccharides, the seeds of which had single treatment with iron, cobalt and copper nanoparticles before planting.

The xylose content in the hydrolysis products proves that nanoparticles alter the quantitative content of monosaccharides in the polysaccharide. If in the variant with Fe NP xylose was detected after 12 hours of temperature exposure, then in the polysaccharide of rapeseed, the seeds of which were treated with Co nanoparticles it was found after 16 hours. When hydrolysis of a polysaccharide extracted from rapeseed with the participation of copper nanoparticles, xylose in the solution was observed after 20 hours of reaction.

In the study of the conditions of ramnose hydrolysis in the control, its presence was observed after 24-28 hours of hydrolysis, and in the study of the influence of nano-iron, it appeared in the solution after 12 hours of hydrolysis and did not disappear anymore, and in the sample of Co NP and Cu NP, it was detected and present after 16 hours until the end of hydrolysis.

Thus, metal nanoparticles not only enhance the photosynthetic and growth processes of plants, but also affect the ratio of polysaccharide monosaccharides, changing the biological activity of the latter.

If we consider carbohydrates as a long chain, then it begins with the arabinose monosaccharide, which alternates with galactose, with more galactose. The main chain is glucose, in the middle there are apparently xylose and mannose, and the amount of mannose is gradually increasing.

The hydrolysis results indicate changes in the ratio of individual polysaccharide monosaccharides under the influence of metal NPs, and the changes are less, but depend on the chemical composition of the nanoparticles, as indicated by changes in plant growth and rapeseed productivity.
Thus, NPs activate the enzymatic dependence of the plant, which affects the change in metabolism and carbohydrate content.

5. Conclusion
Field tests of the most active metal nanoparticles for the maximum accumulation of polysaccharides in plants were carried out. Field studies were conducted with seeds of spring rape plants. Seed treatment with NPs increased the content of water-soluble polysaccharides in the rape herbage by 6.9 - 14.5 %, depending on the metal.

The physical-chemical characteristics of polysaccharides, the monosaccharide composition, and their change under the influence of metal nanoparticles were studied. Using chemical and physical-chemical methods of analysis, data were obtained that contribute to the determination of structural elements of plant polysaccharides when their seeds were treated with NPs before sowing. Seeds treatment with NPs in a case of constant qualitative composition changes the quantitative ratio of monosaccharides in the polysaccharide, increases the number of branch points of the carbohydrate chain, contributing to an increase in the molecular weight of individual fractions.

For polysaccharides of the rape herbage changes in the quality of the monosaccharide composition are directed towards improving parameters such as increasing plant resistance to adverse weather conditions (heat, drought). The increased content of galacturonic acid is considered as an opportunity to increase the resistance of rape plants to lodging, which is an important factor for crops.

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