Investigation of the effect of water treated with low-temperature plasma on the formation of seedlings and sprouts of vegetables

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Abstract. The article presents a study of the effect of plasma-activated water (PAW) on the development of seedlings from carrot and radish seeds, as well as forcing of garlic with its subsequent growth during its vegetative reproduction. The results of the experiment were obtained by a comparative analysis of the parameters of the development of crops and plantings, watering of which was carried out with treated, as well as not treated with low-temperature plasma water. During the germination of carrot and radish seeds, as well as forcing of garlic bulbs from the cloves, the positive effect of plasma-treated water was established. The germination numbers of carrot and radish seeds increased by an average of 18-20%, and the germination of garlic cloves by 20 ± 5%. Based on the results obtained, it was concluded that the PAW treatment of the planting material of root and bulbous vegetable crops contributes to the acceleration of certain physiological processes (removal from the latent stage of seeds and the beginning of bulb vegetation), as well as, possibly, to the acceleration of mitotic processes of the apical meristems of the studied plants. This work can form the basis of a fundamentally new eco-friendly, as well as cost-effective approach in the cultivation of food, medicinal and other industrially significant plants.

1 Introduction

In the global production of crop production, vegetable crops are in second place in importance after cereals and legumes. As you know, a common problem in plant cultivation is to increase germination and improve the development of seedlings. The first is solved by presenting general standardized requirements for the quality of seeds (for example, GOST R 52325-2005 "Seeds of agricultural plants. Varietal and sowing characteristics. General specifications"), and the second is the selection of optimal agrotechnical and agrochemical methods of cultivation known for a particular crop. Currently, chemical treatment of seed and soil has become widespread (mainly pesticides, growth stimulants and fertilizers) [1].

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Many of the substances used are capable of accumulating in plant organs, as well as forming metabolites toxic to humans, thereby reducing the marketable quality of agricultural products.

In recent domestic and foreign literature, it is proposed to use the method of plasma modification (treatment) of both the seed surface and the use of plasma-activated water before sowing to solve this problem. The authors obtained positive results on some cereals, legumes, oilseeds, melons, tubers, as well as on three forest woody plants from the genera: *Pinus* L., *Betula* L. and *Acer* L. [2-8].

Thus, such an electrochemical effect on seed and/or planting material, soil, and water is an actual object of a complex of biological studies. It is also worth noting the research aimed at disinfection treatment [9].

In order to logically continue the vector of scientific research described above, in our opinion, it is necessary to study the effect of water treated with low-temperature plasma on the formation of seedlings and other economically valuable plant species.

The purpose of this work was to study the effect of water treated with low-temperature plasma on the development of seedlings from carrot and radish seeds, as well as forcing of garlic with its subsequent growth during its vegetative reproduction.

The material for our study was carrot seeds of the Parisian Carotele variety, Aelita (Russia) and radish of the Superstar F1 variety, Siberian Garden (Russia), as well as garlic bulbs of the Gulliver variety (Russia).

Carrot (*Daucus carota* L. ssp. *sativus* Hoffm.) belongs to the parsley family (Umbelliferae Juss. (Apiaceae Lindl.)), is a biennial herbaceous plant. The root system is of the tap-root system. The leaves are macropodous, repeatedly pinnatisected to form a rosette, the stem of the peduncle is ribbed, the inflorescence is an umbel bearing flowers with small calyx serratures and white, reddish or yellowish petals. The fruits are diachenium elliptical in shape, 3 - 5 mm long.

Carrot seeds begin to germinate at low temperatures. High temperatures during the germination period can cause secondary dormancy of seeds. Light promotes germination. Carrot seedlings are sensitive to drought, increased salt concentration and lack of oxygen. All this, combined with a long germination period, causes unstable germination.

Two or three pinnatisected leaves with a wide leaf sheath form a rosette. A taproot is formed from the embryo root, which penetrates into the soil to a depth of 1 m. Only then, through the secondary growth of parts of the root and hypocotyl, the formation and staining of the root crop begin. The upper part of the hypocotyl is drawn into the soil by the main and lateral roots. Varieties that do not fully possess this property, for example, fodder carrots, form green heads.

The root crop consists of a core (heart), which is surrounded by cambium and bark.

Carrots can move to generative development only after the completion of the juvenile phase, the duration of which depends on the variety.

Depending on the genotype and environmental conditions, a more or less high (up to 1.5 m) and highly branched peduncle is formed.

Carrot flowering begins in June, pollination is mainly carried out by insects. The seeds ripen in August – September.

Wild forms of carrots are widely distributed in Asia and Europe.

Carrots are grown in all parts of the world on an area of 584,000 hectares. It is one of the most important vegetable crops. The largest sown areas are concentrated in Asia (28%), especially in China and Japan, and Europe (134,000 hectares) (mainly in Poland, France and the UK).

Carrots are a cold-resistant plant. It tolerates low temperatures and weak freezes well and suffers from heat and drought. A high content of carotene can be obtained under optimal growth conditions, as well as with increased temperature and low water availability.

The soil should be loose, permeable, free of stones, provided with nutrients, and when cultivating varieties with long root crops – with a sufficiently powerful arable horizon, have a high moisture capacity and do not form lumps.

Carrots are responsive to high doses of organic fertilizers.

For sowing in early spring, the main tillage is carried out to a depth of 30 cm with additional deep loosening in autumn. At the same time, phosphorus fertilizers can be applied, and potash fertilizers can also be applied on cohesive soils. Potassium removal by a crop is high and is 210 – 360 K_2O kg/ha. Increased doses of potash fertilizers have a positive effect on the sugar content, keeping capacity, taste and yield. The soil at the depth of seed embedding should be loose and structural, the seedbed should be sufficiently firm. During spring and summer plowing, a soil packer is used.

The need for nitrogen fertilizers in carrots depends on the yield and the size of the root crop and ranges from 80 (early carrots) to 150 kg N/ha, which should be in a soil layer with a thickness of 60 cm.

Since carrots, especially in drought, with high salt concentrations, damage to seedlings and slow growth are observed, the initial dose of nitrogen fertilizers (within 80 kg N/ha) should be applied either immediately before sowing, or when the first true leaf appears.

For middle-early and late varieties, along with the main dose of fertilizers, 70 kg N/ha is additionally added. The introduction of too high doses of nitrogen fertilizers leads to a slowdown in staining, excessive leaf growth, germination of root crops during storage, as well as to the coarsening of their tissues.

Before sowing, carrot seeds are released from the spikes and etched. With good seed quality and modern sowing techniques, seed pelleting is not necessary.

The timing of sowing depends on the variety and the purpose of production.

Sowing in spring is more reliable. The duration of cultivation is 90 -105 days. The degree of density of carrots should be 150 – 300 plants per 1 m^2. With a row spacing width of 20-25 cm, the seeding rate is 3 - 6 kg/ha. With a density of 800 plants per 1 m^2, the seeding rate is 15 kg/ha. With early harvesting, the degree of density can be 500 plants per 1 m^2 (12.5 kg/ha).

Seeds are embedded to a depth of 1.5 - 2.0 cm, single-seed planting is possible. Since the seeds do not swell well, with relatively dry soil, soil packers are used. Depending on the temperature of the soil, seedlings appear in 1.5 - 4 weeks.

Late varieties intended for storage and processing are sown from mid-April to mid-May (sowing is also possible in July), harvesting is carried out in October. In the early stages of sowing, high yields are obtained, but the keeping capacity of root crops is low.

Thickened crops are treated with a mesh harrow until the emergence of seedlings, which allows for simultaneous surface loosening of the soil and the destruction of weeds. In addition to harrowing, one or more inter-row cultivations (loosening) are carried out; rippers are often equipped with spherical plant-shielding wheels.

Weeding or herbicides are used to control weeds. The treatment is carried out both before and (or) after the emergence of seedlings, and preference is given to post-emergence treatments, in which the use of herbicides of selective action against cereal weeds is allowed.

Post-emergence treatment is carried out in phase of 3 - 4 of true leaves. After the application of herbicides, carrot yields, as well as the carotene content in it, may decrease, especially on soils with a low humus content. With late weed control, which can be carried out both mechanically at the same time as loosening, and chemically, varieties prone to the formation of green shoulders are hilled up.

Carrots are responsive to an even supply of water. Plants of early ripening varieties are irrigated from June, of late varieties, with slower growth in the early stages of development – from July. With early watering, soil compaction is possible, as well as inhibition of growth and development of root crops due to cooling. Abundant watering during the drought period leads to cracking of root crops.

When carrying out plant protection measures, starting from the middle of May, it is necessary to pay attention to the possibility of the appearance of carrot flies, aphids and jumping plant lice. Measures to protect plants from alternariosis (*Alternaria*) are also needed, especially with a high degree of density (cultivation of carotels). Various species of nematodes (*Meloidogyne hapla*, *Heterodera carotae*, *Ditylenchus dipsaci*, *Pratylenchus penetrans*) can be displaced primarily due to crop rotation.

The physiological importance of carrots in human nutrition is due to the high content of carotenes in it (especially (3- and a-carotenes), which, along with xanthophyll and lycopene, give the root crop an orange-red color. The carotene content varies depending on the variety and weather conditions from 50 to 300 mg/kg of fresh weight and averages 150 - 200 mg/kg. In addition, carrots contain vitamin C (30 - 90 mg/kg of fresh weight), pectins, sugars (4-6%), potassium and essential oils that give it a peculiar taste, especially terpenes. Pectins have a positive effect on the work of the digestive tract, have a therapeutic effect in gastric diseases. Essential oils contribute to the overall strengthening of the body, but, unfortunately, they are a solvent for fat-soluble pesticides. Carrots also have antibacterial properties, as well as diuretic and glycogenic effects, so they have long been used in folk medicine as a medicinal plant. The possible accumulation of nitrates in it is not significant.

Radish (*Raphanus raphanistrum* var. *sativus* (L.) Schmalh.) belongs to the cabbage or cruciferous family (*Brassicaceae* Burnett or *Cruciferae* Juss.) and is an annual plant.

The root system is of the tap-root system. The leaves are macropodous, pinnatisected to form a rosette, the stem of the peduncle is smooth, the inflorescence is a brush bearing four-membered flowers with light pink or purple petals. Fruits are pods with 6 - 10 rounded-oval seeds.

As a result of the growth of the hypocotyl in width, a root crop of round or oval shape is formed. There are also varieties with a pointed root crop, in which the root is involved in its formation. Varieties with a red color of the root crop predominate, but there are also white, yellow-cinnamon, pink and purple colors, as well as red ones with a white tip.

With a decrease in illumination, an increase in the degree of density and an increase in temperature, there is a tendency to reduce the formation of root crops.

Radish has an extremely short growing season and does not impose special requirements on the soil and climate. Soils with medium and light granulometric composition in combination with irrigation are favorable for this crop. The high content of clay fraction in the soil makes it difficult to wash the products afterwards. In Germany, radish production is developed in all areas of intensive vegetable growing.

In many crop rotations, fertilizers for radishes are not required, since it has enough nutrients contained in the harvesting residues of the previous crop. The nitrogen requirement is 40 kg/ha. It is recommended to use fertilizers with a low chlorine content.

In some areas, sowing in open ground in the presence of film coating begins in February or early March. The last sowing period is the beginning of September. Currently, radishes are in demand and are supplied to the market throughout the year. When sowing from May to August, the growing season is only 20-25 days.

Sowing is carried out with precision seeding drills. When using a well-calibrated material, it is possible to get rid of the pelleting. The seeding scheme, depending on the variety, is 10 – 15 × 3 cm, which corresponds to the seeding rate of 3 g/m^2. The sealing depth is 1 - 1.5 cm. It prefers sowing on ridges, the width of which corresponds to the track of the tractor.

To control weeds in the open ground, pre-emergence treatment with herbicides is used. On lightly polluted soils, you can refuse this event. On light soils, it is necessary to pay attention to maintaining sufficient moisture, especially during the germination of seeds and before harvesting. When cultivating radishes, there are no special problems with protecting plants from diseases and pests. Occasionally, measures are carried out to protect against downy mildew *(Peronospora),* black rot *(Aphanomyces)* and crater rot *(Rhizoctonia).*

Garlic (*Allium sativum* L.) is a perennial herbaceous plant of the Amaryllis family (*Amaryllidaceae* St. Hil.) originates, most likely, from Central Asia, from where it spread to the Mediterranean and East Asia. It belongs to the oldest vegetable and medicinal plants. Currently, it is cultivated mainly in southern latitudes, especially in the subtropics, as a winter crop.

Garlic forms long, relatively thin false stems with narrow grooved leaves. The peduncle is cylindrical, filled, the flower buds most often fall off, only a few form seeds, instead of them, so-called bulbils are often formed in inflorescences. *In the axils of the scales, under the influence of low temperatures, adventive buds, the so-called cloves, are formed.* Their growth, like that of the onion, is stimulated in conditions of a long day and high temperature.

For the cultivation of garlic, the most suitable are average temperatures (13 - 24 °C) and medium and heavy soils in terms of soil texture, well permeable (especially for autumn planting), with a deep arable horizon. Garlic is frost-resistant and tolerates light frosts well.

Nitrogen is introduced in a dose of 100 - 120 kg/ha, potassium – in the form of sulfate. In the crop rotation, garlic, like other species of *Allium* L., can be returned to its former place in at least 5 years.

Garlic is propagated in a vegetative way, with cloves or bulbils. Cold-resistant varieties are used for autumn planting, cloves weighing 3 - 9 g are planted from the middle to the end of October to a depth of 5 – 6 cm according to the scheme 30 × 8 cm (42 plants per 1 m^2). Plants begin to grow in February – March, depending on the weather, harvesting starts in July.

Cloves for spring planting are stored at a temperature of 10 °C. Storage at high temperature and late planting lead to the formation of weak cloves or they do not form at all. This is due to the insufficient duration of exposure to reduced temperatures. After a very long exposure to low temperatures, cloves are formed in the axils outside the lower leaves, and the cloves and bulbs in this case are immature. With early planting, yields are low, but thanks to late harvesting, garlic is stored better. Garlic is ready for harvesting when the wrapper around the bulbs becomes dense, and the outlines of the cloves are even. By this time, the leaves, as a rule, turn yellow and wither by $^1/_3$. The yield is 50-100 c/ha. Before storing the bulbs, they are dried well. Garlic is stored at a temperature of 0 °C and relative humidity of 70%. Storage duration is 6 - 7 months.

Cloves contain many valuable substances, among them alliin and garlic oil, which contribute to lowering blood pressure and have an antibiotic and anti-sclerotic effect, so garlic is not only a vegetable, but also a valuable medicinal plant. In some countries, green parts of plants are also used for food [10].

A series of experiments on the production of plasma-activated water (PAW) in a continuous stream was carried out at a laboratory installation for plasma chemical treatment of liquid, the scheme of which is shown in Figure 1. Tap water was used to obtain PAW. Untreated water was fed with a peristaltic pump at a constant volumetric rate of 82 ml/min through a tube (1) into an open discharge cell (2). With the help of another peristaltic pump, PAW was pumped out of the discharge cell through the tube (4). The volume of water in the discharge cell remained constant and amounted to 30 ml.

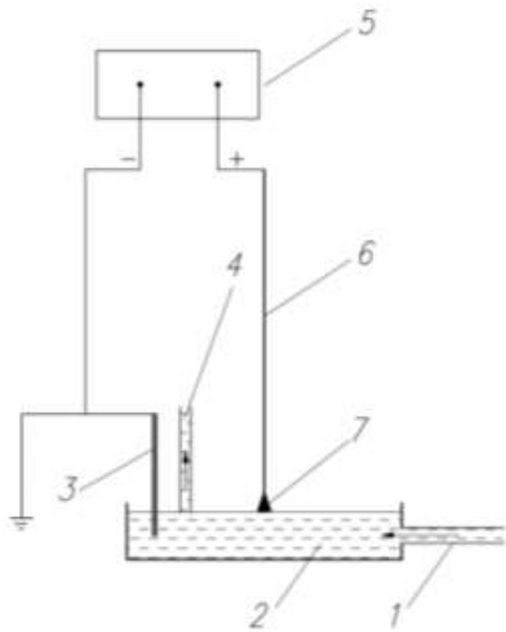

Fig.1. Scheme of experimental installation of plasma chemical treatment of liquid. 1 – liquid supply tube, 2 – discharge cell, 3 – cathode, 4 – liquid pumping tube, 5 – power supply, 6 – anode, 7 – discharge.

Water treatment was carried out by glow discharge at atmospheric pressure in the air. The discharge was created between a rod metal electrode (6), acting as an anode, and the surface of the solution, which is the cathode. The distance between the electrode and the solution surface was 6 mm. The discharge current was set by an adjustable DC source (5) and maintained at 80 mA. The voltage drop on the discharge cell was 1850 V.

The electrochemical parameters of the solution were measured at the outlet of the discharge cell. The conductivity of the solution was measured using a conductometer "Expert-002", the pH of the solution – liquid analyzer "Expert-001-3.0.4". After treatment with a DC discharge, the hydrogen index (pH) of the PAW was 7.4, and the specific conductivity was 266 µsm/cm

Water, which was used as a standard, was also passed through the installation, but without treatment with a DC discharge. Its hydrogen index (pH) was 7.4, and its specific electrical conductivity was 220 µsm/cm.

The study of the effect of water treated with low-temperature plasma on seed germination and forcing of cloves was carried out by the soil-culture technique. When setting up vegetative experiments with soil culture, the following basic operations were performed: purchase and preparation of soil with fertilizers, filling of pots, sowing (planting), care of plants and crop accounting.

The universal nutrient soil of the trademark Partterra® (TU 08.92.10-001-23539486-2017) was used, brand Nov Agro (Russia) with the content of nutrients: nitrogen ($NH_4 + NO_3$) – not less than 120 mg/l, phosphorus (P_2O_5) – not less than 150 mg/l, potassium (K_2O) – not less than 250 mg/l, the mass fraction of moisture – not more than 65%, acidity (Ph_{KCl}) – 5.2 – 6.5. Additional dressing and fertilizers were not introduced during the study.

Pots (60 pieces) were selected with a height of 20 and a diameter of 16 cm, containing about 5 kg of absolutely dry soil. As drainage, fine clay pellets, of the brand "Organic

Botanic" (Russia), fractions – 5-10 mm, were introduced to the bottom of the perforated containers.

The amount of soil in the pot was established by a trial packing with its complete uniformity in the entire volume.

Soil moisture in the experiment in all pots was the same, the same amount of water was introduced into the soil.

Seeds were sown in wells, which were evenly distributed on the soil surface in a pot. Sowing and planting was carried out according to standardized agrotechnical recommendations for each crop. The soil surface in the pot was leveled and slightly compacted after seeding, then watering was carried out. Garlic cloves were placed in containers of the same volume, their forcing was carried out by the method of hydroponic culture.

The plants were watered with the same amount of water (by volume) so that the soil moisture in all pots was at the same level of average moisture capacity.

During the growing season of plants, pots were periodically rearranged to provide them with the same lighting conditions and temperature. The rearrangement of pots was carried out during watering. Aeration was carried out manually, as the soil compacted in the vessels.

When conducting the vegetation experiment by the method of soil culture, the methods and conditions of cultivation described above in nursery conditions of the studied plants were taken into account.

During the excavation of plants, using a ruler and a caliper, the length/width of the aboveground and underground parts of the plant in each pot was measured, then mathematical processing of the data obtained was carried out [11].

Control photofixation was carried out at the beginning of cultivation. The equipment used was a Canon camera (Japan), model - EOS 6D Mark II Body. The results of the photo fixation are given below.

Fig. 2. Carrots, watered with control water, on the 10th day after planting.

Fig. 3. Carrots, watered with control water, on the 40th day after planting.

Fig. 4. Carrots, watered with PAW, on the 10th day after planting.

Fig. 5. Carrots, watered with PAW, on the 40th day after planting.

Fig. 6. Radish, watered with control water, on the 7th day after planting.

Fig. 7. Radish, watered with control water, on the 40th day after planting.

Fig. 8. Radish leaves, watered with control water, on the 40th day after planting.

Fig. 9. Radish, watered with PAW, on the 7th day after planting.

Fig. 10. Radish, watered with PAW, on the 40th day after planting.

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Fig. 11. Radish leaves, watered with PAW, on the 40th day after planting.

Fig. 12. Garlic, watered with control water, on the 7th day after planting.

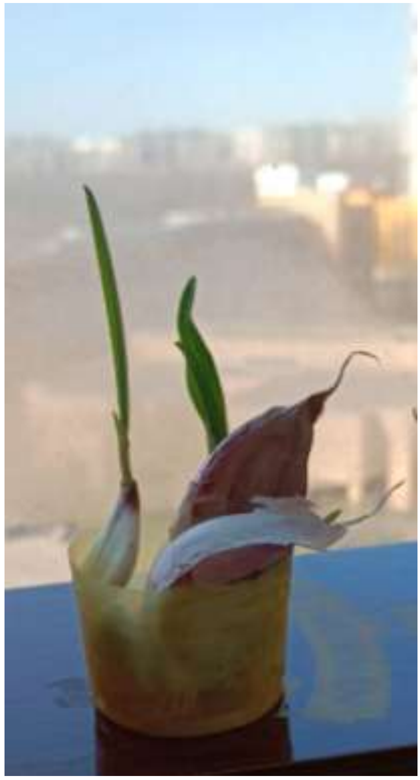

Fig. 13. Garlic, watered with PAW, on the 7th day after planting.

Fig. 14. Comparison of two samples on the 20th day after planting: on the left – garlic, watered with PAW, on the right – garlic, watered with control water.

The results of studying the germination of carrot seeds, radishes, as well as forcing and formation of leaves from garlic cloves are presented in Table 1

Table 1. The germination of carrot and radish seeds, as well as forcing of garlic cloves (in%).

Vegetable crops	Sowing/planting (n = 10)	
	plasma-activated water (PAW)	monitoring
Carrot	86±2	67±3
Radish	98±2	80±2
Garlic	95±1	75±4

As can be seen from this table, when soaking seeds and cloves, followed by regular watering with plasma-activated water (PAW), on average, the germination rates of carrot and radish seeds increased by 18-20%, and by 20 ± 5% – the germination of garlic cloves.

The data obtained in the study of the effect of plasma-activated water (PAW) on the growth and development of aboveground, as well as underground parts of the studied species are shown in Table 2.

Table 2. The effect of plasma-activated water (PAW) on the growth and development of vegetable crops (in %) (n = 10).

Vegetable crops	Number of plants in the container, pcs.	Shoot length, cm	Area of leaf blades, cm2	Area of the underground part, cm2
plasma-activated water (PAW)				
Carrot	12±1	13±1	7±3	10±4
Radish	6±1	12±1	25±2	20±3
Garlic	3±1	15±3	27±4	25±2
Monitoring				
Carrot	10±2	14±2	9±2	7±2
Radish	5±2	15±3	30±4	16±2
Garlic	2±1	13±2	22±3	21±1

According to the results given in the table above, it is possible to observe a significant excess of quantitative and morphometric characteristics of the studied vegetable crops during their treatment with plasma-activated water (PAW) compared with the control.

Thus, based on the results obtained, it can be concluded that pre-sowing / pre-planting treatment with plasma-activated water (PAW) of the planting material of root and bulbous vegetable crops contributes to the acceleration of some physiological processes (removal from the latent stage of seeds and the beginning of bulb vegetation), as well as, possibly, to the acceleration of mitotic processes of apical meristems of the studied plants.

This work is a logical continuation of the vector of complex applied research aimed at studying the electrochemical effects of low-temperature plasma both on living organisms themselves and on their environment (air, soil, water and nutrients and physiologically active substances soluble in it).

This work can form the basis of a fundamentally new eco-friendly, as well as cost-effective approach in the cultivation of food, medicinal and other industrially significant plants.

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