Original Research Article

Evaluation of the effect of SiO₂ and Fe₃O₄ nanoparticles on *Pisum sativum* seeds in laboratory and field experiments

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ABSTRACT

The present study assessed the toxic effects and prospects of using nanoparticles of SiO₂ and Fe₃O₄ by studying the influence of pre-sowing priming of *Pisum sativum* L. seeds with a suspension of nanoparticles of SiO₂ and Fe₃O₄ in a concentration range of 10⁻² to 10⁻⁵ mg/l. The results demonstrated the stimulating effect of the SiO₂ suspension (10⁻³ mg/l and 10⁻⁴ mg/l) and the mix of Fe₃O₄+SiO₂ at the corresponding concentrations of 10⁻³ mg/l and 10⁻⁴ mg/l on the length of roots and seedlings, and the increase in the viability of plant cells under the influence of a stress factor (based on Evans blue staining). Field experience has shown the ambiguous effect of nano-printing of seeds on plant productivity.

Key Words - Growth indicators, Metal powder, Nanoparticles, Oxidative status, *Pisum sativum*, Seed germination

INTRODUCTION

Rapid and uniform germination is the key to agricultural production and can be achieved by seed "priming" methods (Gerna et al., 2018).

"Priming" is a well-known treatment for improving the quality of seeds. The seeds are primed showing an increase in germination, which leads to a high level of resistance to biotic / abiotic stress, yield and yield. All these characteristics, which increase the competitiveness of products, are directly related to seed energy, a complex of agronomic traits controlled by a variety of genetic and environmental factors (Paparella et al., 2015).

Priming of seeds with nanomaterials is an effective and development of equipment to increase strength of seedlings and the growth rates applied for certain types of plants. The small size and large surface area of nanoparticles allow them to demonstrate the unique physical,

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chemical and biological characteristics used in agriculture due to their high potential to improve seed germination and growth, plant protection, pathogen detection. The reaction of plants to nanoparticles depends on the plant species, its growth stage, and the nature of nanomaterials (Maroufi et al., 2011).

The most widely used NPs in agriculture are biogenic nanocrystalline compounds (Fe, Mo, Zn, Cu, Co, Se), due to their active participation in various redox processes and their presence in many enzymes and complex proteins (Quoc et al., 2014). In turn, Si is one of the most common macronutrients that have a positive effect on plant growth and development, but little information is available about its use for seed pre-treatment (Janmohammadi et al., 2015; Hoe et al., 2018; Hussain et al., 2019). Nano-iron has a high degree of bioavailability, which indicates its alternative use in living systems. Iron forms part of the catalytic centres of many redox enzymes, and also contributes to the formation of chloroplast proteins, which stimulate the development of the root and shoot systems (Kovalenko et al., 2006; Carvalho et al., 2018).

In the context of the above, the purpose of our research was to study the effect of colloidal solutions of nanoparticles of nutrients on the germination rate, oxidant status, yield and quality of products on the test plant *Pisum sativum*.

MATERIALS AND METHODS

Materials

The SiO₂ NPs with a size of 30.7±0.3 nm and a ζ-potential of 27±0.12 mV were acquired from the company "Plasmotherm" (Moscow, Russia, <http://plasmotherm.ru>). Nanoparticles of Fe₃O₄ (80–100 nm, z-potential of 20 ± 0.14 mV) were acquired from the company "Advanced Powder Technologies" (Tomsk, Russia, www.nanosized-powders.com) (Figure 1).

For the preparation of NP solutions, exact amounts of the preparations were placed in glass flasks with distilled tap water and intensively dispersed by ultrasound at a frequency of 35 kHz for 30 minutes. Fold-dilutions were prepared to reduce the amount of nanomaterials. For seed treatment, the following concentrations were used: for Fe₃O₄ 10⁻², 10⁻³ and 10⁻⁴ mg/l; for SiO₂ 10⁻², 10⁻³ and 10⁻⁴ mg/l; a mixed solution was obtained by mixing a suspension of Fe₃O₄ (10⁻³ mg/l) and SiO₂ (10⁻⁴ mg/l) at a 1:1 ratio. The control version of the experiment included the treatment of seeds in deionized water.

To assess the efficiency of using nanoforms of iron and silicon, a traditional product for pre-sowing seed treatment was used (MivalAgro, acquired from AgroSil company (<http://agrosil.ru>)). The active substance of MivalAgro is a mixture of 760 g/kg of triethanolammonium salt of orthrocrekoxyacetic acid and 190 g/kg of 1-chloromethylsilatrane.

The pea variety «Flagship 12» was used as a test object. Seeds were provided by the FSSI «Samara RIOA» (Russia, <http://samniish.ru>), complied with all the requirements of the guidelines

«The Order of biological assessment of effects of nanomaterials on plants using morphological characters» and were 1st class quality and not treated with disinfectant.

Experimental design

The first phase of the experiment began with the pre-treatment of seeds of *P. sativum* in solutions of various agents (NPs SiO₂, Fe₃O₄, MivalAgro) with subsequent drying in air. To determine the effective concentrations, the seeds were transferred to Petri dishes with 20 ml of deionized water.

Staining of plant root cells for loss of cell viability was carried out by staining freshly harvested roots from the control with Evans blue aquatic solution (0.25% v/v Evans blue, Sigma-Aldrich, Spain) for 10–15 min at room temperature followed by washing with CaCl₂ solution (100 µm; pH 5.6) three times and visualization under a light microscope (Vijayaraghavareddy et al., 2017).

The second stage of the experiment involved the germination of plants in opaque plastic containers with soil in a climatic chamber. The containers measured 15 cm long, 12 cm wide and 10 cm high, and were filled with 2 kg of soil.

The soil was collected from an experimental field near the village Nezhinka Orenburg region of Russia (51°46'4"N55°22'7"E). The soil mass was collected from the 0–20 cm layer, dried, and the inclusions were selected and thoroughly mixed. Each container was sown with 20 pea seeds with germination in a climatic chamber (Pol-eko-1200 KK TOP+; Pol-EKO-Aparatura, Poland) at a relative humidity of 30%, air temperature of 25±2°C, and substrate temperature of 23±2°C. In total, the experiment involved 30 containers. Plant samples were collected and analysed 15, 25 and 35 days after emergence. The scheme of the experiment is shown in figures 2 and 3.

Field experiment

The experiments were conducted at the experimental site at village of Nezhinka (Orenburg region, Russia). The total size of the plot is 990 m², which was further divided into 20 plots of 49.5 m² (each measuring 1.65x30 m). The soil at the site is a Calcic Pachic Chernozem (IUSS Working Group WRB 2014). The soil characteristics are presented in Table 1.

Before sowing a two-step cultivation was carried out in order to destroy weeds, loosen and level the soil, and create a dense seed bed. The creation of favourable conditions for seed germination was achieved by rolling the field after sowing.

Pea seeds treated with a working solution of 10 l of agent per 1 ton of seeds were sown in the seed bed to a depth of 8 cm. As the growing conditions were very dry, and the contamination of the plots by weeds was minimal, no further care of the plots was necessary. The peas were harvested by combining (TERRION-SAMPO SR 2010) 82 days after sowing.

Evaluation of vital, morphometric and biochemical parameters of test plants

There was a fixed germination percentage. The root and shoot lengths of seedlings were recorded using the standard centimetre scale. Measurements were carried out on roots after washing off the soil and drying on filter paper. The tolerance index (TI) of the plants was calculated (Wilkins, 1978).

The total activity of superoxide dismutase (SOD) was determined according to Giannopolitis and Rice (1972) (Bradford, 1976; Sibgatullina et al., 2011). Determination of catalase (CAT) was carried out using the method of Maehly and Chance (1955). The amount of lipid peroxidation products (LPO) in the soluble fraction of the homogenate and in the tissues was determined by the content of malondialdehyde (MDA), as described previously (Korotkova et al., 2017). Protein fragmentation was performed according to Chen and Bushuk (1970) and Bradford (1976).

Statistical analysis was performed using standard ANOVA techniques followed by the Tukey test (SPSS ver. 17.0). The Spearman method was used to determine the coefficient of correlation. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Laboratory experiment

The laboratory experiment on the germination of seeds treated with 10^{-3} mg/l Fe_3O_4 , 10^{-3} and 10^{-4} mg/l SiO_2 , and the mixture at a ratio of 1:1 (Figure 3) demonstrated reliable stimulation of germination with respect to the control by 10^{-3} mg/l and the mixture of Fe_3O_4 and SiO_2 , and demonstrated their effectiveness in relation to MivalAgro (Figure 4).

The study of the viability (V) of plant root cells was carried out using Evans blue dye, which is not able to penetrate into living cells but stains dead cells. The viability of pea root cells after exposure to the "stress factor" was determined only for those treatments having a stimulating effect on germination. Figure 5a shows a directly proportional increase in V after treatment of plants with 10^{-3} mg/l of the NPs SiO_2 (up to 56.8 and 63.4% relative to plants in the positive control, 100% dead cells) and $\text{Fe}_3\text{O}_4 + \text{SiO}_2$ (up to 55%) ($p \leq 0.05$).

At the same time, the most pronounced protective effect against the impact of the stress factor on the root systems of plants was shown by SiO_2 NPs at 10^{-4} mg/l. The data obtained were confirmed by microscopic assessment of the root elongation zone 7–14 days after germination (Figure 5B).

Climatic chamber experiment

The experiment examining the germination of plants in soil under climatic chamber conditions showed that the maximum germination relative to the control sample was achieved in seeds treated with Fe_3O_4 NPs at 10^{-3} mg/l and $\text{Fe}_3\text{O}_4 + \text{SiO}_2$ at 10^{-3} mg/l and 10^{-4} mg/l (Figure 6).

140 Note that the lowest level of germination was observed in seeds treated with MivalAgro (total
141 ~78% relative to control).

142 The stimulating effect of the NPs on *P. sativum* was most clearly manifested by the
143 increased growth rate after germination. Thus, the studied compounds had a specific effect on
144 the length of the seedlings, which significantly exceeded the control in the SiO₂ treatment at 10⁻³
145 and 10⁻⁴ mg/l at all stages of the study (from 15 to 35 days), and after 25 days in the 10⁻³ mg/l
146 Fe₃O₄ and Fe₃O₄+SiO₂ treatment (Figure 7A).

147 MivalAgro significantly stimulated root growth only after 15 days of the experiment, and
148 this stimulation was gradually offset in subsequent periods of growth. At the same time, it had no
149 significant effect on the length of the above-ground organs of peas. Similar results were obtained
150 regarding the length of pea roots, with maximum development in treatments with SiO₂ solutions
151 at different concentrations, 10⁻³ mg/l Fe₃O₄ and Fe₃O₄+SiO₂ (Figure 7B).

152 The integral metric characteristic of model plants in response to exogenous stimulators
153 (TI) is presented in Figure 8. A significant effect of seed treatment on the TI of plants at all
154 stages of the study was observed at both concentrations of SiO₂, and after 15–25 days for Fe₃O₄
155 combined with SiO₂. The obtained data are consistent with the literature (Nazaralian et al., 2017;
156 Yuan et al., 2018).

157 The beneficial effects of SiO₂ reported by Tahir and his colleagues (2010) were
158 associated with its hydrophilicity (Romero-Aranda et al., 2006), and in experiments with wheat
159 led to a significant increase in biomass and yield.

160 The results of the experiment showed that the enzymes SOD and CAT in peas react
161 differently to the presence of the studied compounds (Figure 9). Thus, catalase activity in
162 seedlings was 50–60% lower than that of the control in the first two weeks and on the 25th day
163 there was an increase in CAT to the level of control plants. In turn, a sharp increase in the index
164 was noted on the 35th day of exposure to the treatment agents, with the exception of MivalAgro,
165 which indicates the exhaustion of the existing enzyme pool. Thus, an increase in CAT was
166 recorded after SiO₂ treatment at both concentrations (by up to 83% and 146%), Fe₃O₄ (by up to
167 111%) and Fe₃O₄+SiO₂ (by up to 47%) (p<0.05).

168 CAT is the main antioxidant in plants, preventing their oxidative damage and acting as a
169 neutralizer of radicals. Our results show that SiO₂ NPs can cause oxidative stress only at
170 concentrations above 200 mg/l. Similar results have been published previously (Yang et al.,
171 2012; Wang, Zhang, 2014).

172 Differences in stress-dependent total SOD activity were less noticeable. A slight increase
173 in enzyme levels (over 30%) relative to the control was recorded in plants after 25 days of
174 exposure to MivalAgro and SiO₂ (p<0.05). At the same time, on the 35th day, the level of SOD

175 decreased to the control value, which indicates the effective neutralization of superoxide radicals
176 and intensification of CAT activity.

177 As a biological indicator of the development of oxidative stress in plants, the product of
178 lipid peroxidation (LPO) of cell membranes – malondialdehyde (MDA) – was used. Free
179 radicals are able to initiate LPO, as a result of which the membranes become permeable to ions
180 and organic acids. Analysis of the degree of LPO in the seeds showed that pretreatment with
181 nanoparticles had a multidirectional effect depending on the phase of growth. Thus, on the 25th
182 day of the experiment, there was a small increase in the MDA level compared to the control in
183 the case of treatment with 10^{-3} and 10^{-4} mg/l SiO_2 and a mixture of $\text{Fe}_3\text{O}_4+\text{SiO}_2$. However, this
184 indicator was much lower than the level recorded after treatment with MivalAgro (1.5–2 times
185 lower). Under experimental conditions, even within 35 days, the less strong accumulation of
186 MDA in SiO_2 and $\text{Fe}_3\text{O}_4+\text{SiO}_2$ treatments (not more than 40%) indicates the resistance of plants
187 to the nanoform of these elements.

188 In contrast, plants treated with Fe_3O_4 had a slightly higher content of MDA from 15 to 35
189 days, comparable to the drug MivalAgro i.e. up to 176% ($p<0.05$). These results show that
190 nanoparticles can also lead to the formation of free radicals and damage the integrity of the
191 membrane.

192 It has previously been reported that SiO_2 nanoparticles cause oxidative stress in plants
193 and lead to LPO (Slomberg et al., 2012). Our results are consistent with other studies indicating
194 an increase in LPO in different plant species after SiO_2 treatment (Yang et al., 2012; Wang,
195 Zhang, 2014).

196 Thus, the activity of SOD and CAT can be considered an important element of
197 antioxidant protection of plant cells from stress factors, including excess or lack of trace
198 elements. However, under experimental conditions, the activity of these enzymes with a small
199 increase in MDA indicates the stress of a redox imbalance due to mineral deficiency caused by
200 the lack of nutrients in the growing medium.

201 **Field experiment**

202 The weather conditions prevailing during the growing season of the *P. sativum* were
203 extremely unfavourable: extremely high air temperature (30–40°C) accompanied by a high daily
204 deficit of air humidity from 16 to 22 MB. The negative impact on the grain yield was caused by
205 the lack of productive soil moisture due to the low snow-cover during winter and deep freezing
206 of the soil. During the growing season only 38 mm of precipitation fell, which is 39% of the
207 mean annual values.

208 The moisture supply during the period from germination to full ripeness was only 28.4%
209 of the level of demand. Due to the negative influence of the above factors, the grain yield was
210 low (1.9–2.6 c/ha), while the difference between treatment groups was insignificant (Table 2).

211 A tendency for a decrease in productivity under the influence of the treatments was noted,
212 except for the low-concentration SiO₂ treatment, in which grain yield was higher than in the
213 control (without treatment) by 0.1 c/ha.

214 Analysis of the components of grain yield showed that the number of beans on the plant,
215 seeds in the bean, and grain weight were at or below the control level in the treatment groups.
216 The positive impact of the treatment of SiO₂, Fe₃O₄ and organic silicon (MivalAgro) manifested
217 itself in the form of an increased density of 5–7 plants per 1 m² compared to the control variant.

218 Analysis of the proteins in the plant after treatment with exogenous stimulators in the
219 field showed a marked increase in the albumin pool after exposure to a mixture of Fe₃O₄+SiO₂
220 88% (Figure 10). The content of globulins decreased to 9.8% relative to the control.

221 Treatment with SiO₂ led to a small increase in the amount of globulins relative to the
222 control (no more than 5%) while reducing the fraction of gluten. It should be noted that there
223 was a marked increase in the amount of gluten after exposure to MivalAgro (by 50%), which
224 was probably caused by specific activation of protein metabolism.

225 A decrease in prolamine and glutelin in rice grown in medium with nanoparticles of CeO₂
226 was previously reported (Rico et al., 2013). A study by Zhao and colleagues (2014) showed that
227 ZnO NPs did not affect protein fractions in cucumber, but CeO₂ at a dose of 400 mg/kg
228 increased the amount of globulins and reduced the amount of gluten. Thus, the effect of
229 treatment of seeds with nanoparticle suspensions on the composition of the protein complex has
230 no element and/or species-specific character.

231 CONCLUSION

232 The influence of colloidal solutions of nanoparticles of Fe₃O₄, SiO₂, and their mixture at
233 a ratio of 1:1 on the complex of physiological and biochemical parameters of the plant *Pisum*
234 *sativum* was studied. The study was based on laboratory and field experiments, from which the
235 following conclusions can be drawn:

236 1. The comparison of different compositions of nano compounds with the organic
237 analogue MivalAgro in the laboratory showed that reliable stimulation of seed germination and
238 growth of seedlings was achieved by the NPs Fe₃O₄ (10⁻³ mg/l), SiO₂ (10⁻³ and 10⁻⁴ mg/l) and
239 their mixture (Fe₃O₄+ SiO₂).

240 2. After more than 25 days, SiO₂ at 10⁻⁴ mg/l and Fe₃O₄ showed a stimulating effect
241 on plant growth relative to MivalAgro.

242 3. The results of the field experiment did not demonstrate an increase in plant
243 resistance to environmental stress factors, which indicates the need for further research on the
244 introduction of nanotechnological solutions in crop production.

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255 <http://www.ckp-rf.ru/ckp/77384>).

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a) Fe_3O_4 NPs

b) SiO_2 NPs

Figure 1 - Electron microscopy of nanoparticles: a) transmission electron microscopy Fe_3O_4 NPs, b) scanning electron microscopy SiO_2 NPs

Figure 2 – Experiment design (step 1, 2, 3)

Step 4. Execution of a model experiment.

Step 5. Implementation of the experiment in the field.

Figure 3 – Experiment design (step 4,5)

Figure 4 – Pea seeds in germination dough

Figure 5 – Viability of *P. sativum* roots after treatment of seeds with solutions of agents: A) diagram, % of control; C) percentage of living cells unstained with Evans dye after.... days of treatment: * variant significantly different from the control (value $P \leq 0.05$); C) cells with a damaged membrane in the elongation zone.

Figure 6 –*P. sativum* germination. Data points with some / no symbols (*) represent no statistical significance at $p \leq 0.05$.

Figure 7 – Growth parameters of *P. sativum* plants after exposure to various compounds: A - leaf length, B - root length. Bars are mean $\pm$ SEM (standard error of the mean). Data points with some/no symbols (*) represent no statistical significance at $p \leq 0.05$.

Figure 8 – Tolerance index of *P. sativum* plants after exposure to various compounds. Bars are mean $\pm$ SEM (standard error of the mean). Data points with some/no symbols (*) represent no statistical significance at $p \leq 0.05$.

Figure 9 – The activity of antioxidant enzymes (CAT, SOD) and the degree of lipid peroxidation (according to the MDA level) in *P. sativum* after exposure to various compounds: 1- $\text{Fe}_3\text{O}_4 + \text{SiO}_2$; 2 - Fe_3O_4 10^{-3} mg/l; 3 - SiO_2 10^{-3} mg/l; 4 - SiO_2 10^{-4} mg/l; 5 – MivalAgro. Bars are mean $\pm$ SEM (standard error of the mean). Data points with some/no symbols (*) represent no statistical significance at $p \leq 0.05$.

Figure 10 – Protein content of *P. sativum* seeds. Data points with some/no symbols (*) represent no statistical significance at $p \leq 0.05$.