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EFFECT OF ZEOLITE ON THE MICRO-MORPHOLOGICAL AND BIOCHEMICAL FEATURES OF THE SPRING RAPESEED (*Brassica napus* L.)

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SUMMARY

The study aimed to assess the effect of a natural mineral fertilizer – zeolite on the micro-morphological and biochemical characteristics of the spring rapeseed *Brassica napus* L. cv. “Rif”). Experiments were held at the Federal Research Center for Breeding, Agro-technics and Nursery Horticulture, Moscow, and Agro-industrial Institute, Bunin Yelets State University, Yelets, Russia from 2018 to 2020. The study scheme comprises four treatments as follows: a. control (no fertilizer), b. NPK @ 60-60-60 kg ha^{-1}, c. Zeolite 5 t ha^{-1}, and d. NPK @ 60-60-60 kg ha^{-1} + Zeolite 5 t ha^{-1}. Results revealed that the maximum number of stomata was observed in the rapeseed crop treated with natural and mineral fertilizer – zeolite. On average, there were 537 pcs/mm^2 on the adaxial surface, and 480 pcs/mm^2 on the abaxial leaf surfaces. The mineral fertilizer application only, and in combination with a natural ameliorant, contributed an increase in photosynthetic pigments, which was about 22% more relative to the control. To determine the content of trace elements (Mn, Fe, Zn, and Ni) according to the phases of spring rapeseed development, the trace elements were decreased by the harvesting phase. The only exception was Co, where content was increased by the time of rapeseed ripening. The accumulation of the studied trace elements in spring rapeseed plants at maturity can be represented by the following decreasing series, Fe>Mn>Zn>Cu>Ni>Co. The correlation coefficients also revealed that there was a strong relationship among all the studied elements. A high correlation (r = 0.95) was found between the seed yield and the magnitude of the photosynthetic pigments.

Keywords: Spring rapeseed, natural mineral fertilizer (zeolite), micromorphology, mineral composition, photosynthetic pigments, seed yield

Key findings: The use of natural mineral fertilizer (zeolite) only, and in combination with other mineral fertilizers, influenced the micro-morphological features of the leaf surfaces, the number of stomata, the composition of microelements in plants, and the seed yield in spring rapeseed (*Brassica napus* L.).

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INTRODUCTION

Rapeseed (*Brassica napus* L.) is one of the most widely cultivated oilseed crops in the world. Breeders developed the non-erucic rapeseed cultivars with low glucosinolates that significantly contributed to the crop's potential in world agriculture and production (Vafina *et al*., 2016). Rapeseed is now an indispensable component of crop rotation in the agro-industrial sector of large growing regions such as Australia, Western Canada, Central China, and many countries of the European Union (Carre and Pouzet, 2014; Choudhary *et al*., 2015). Rapeseed is used as a raw material for the extraction of vegetable oil and meal (Gulidova *et al*., 2017). Rapeseed oil is also characterized by a high content of oleic acid (about 60%) and polyunsaturated linolenic acid (about 10%) (Zubkova *et al*., 2020). Rapeseed oil is also used as biofuel in automobiles, especially in Germany and other European countries (Singh and Singh, 2018; Vinogradov *et al*., 2019).

The generation of funding for seed development requires constant improvement in the growing technology of the crop under the specific soil and climatic conditions, considering characteristics of the cultivars and their response to various technological elements (Koh and Ghazoul, 2008; Rondanini *et al*., 2012). Fertilizers are predictable factors used in increasing soil fertility and crop yield, regardless of the nature of the cultivation sequence and environmental conditions (Ilieva and Vasileva, 2013).

Zeolite is a natural mineral fertilizer containing rocks, generally used in rapeseed crop production, that has become a very relevant agricultural practice today(Loboda, 2000; Leśny *et al*., 2021). Experimental data obtained from the non-black earth zone of Russia revealed a high efficiency of sewage sludge mixed with zeolite on the yield of crops (Khabarova *et al*., 2018). The zeolite fertilizer can be used in various types of soils as it is environmentally friendly that saturates the soil with microelements, and makes it breathable and water-absorbing in *Brassica campestris* L. (Singh *et al*., 2014; Vasileva and Lupova, 2021; Zakharova *et al*., 2021). The natural zeolite characteristics include an improvement in nitrogen nutrition and a decline in the contents of heavy metals and radionuclides (Mustafayev and Mazhaysky, 2018). Introducing zeolites in low-cost crop growing technologies can get a high return in net profit (Bakulina *et al*., 2020). Zeolites can also be used both as a carrier and a medium for nutrient releasing activities (Nurhasanah and Ecke, 2016; Sangeetha and Baskar, 2016). However, despite all the significant progress, further investigations are still required for the effective use of zeolites in crops.

Photosynthesis is an important and a key factor that has positive effects on the yield of crops (Katashov and Khryanin, 2014; Kosakovskaya *et al*., 2014). One of the indicators that contribute to an increase in the photosynthetic potential of plants is the presence of more stomata on leaf blades (Woodward, 1998). The more stomata per unit area are, the higher the intensity of stomatal transpiration, which can force water to rise inside the plant, making the upper leaves receive the required amount of moisture and nutrients creating photosynthates through photosynthesis (Mushinskaya *et al*., 2007).

Adequate water supply and nutrients provide ideal conditions for photosynthesis in crop plants (Woodward, 1998). Zeolite use further boost photosynthesis with its main property to absorb water and nutrients, then gradually releasing the required amount, with the right temperature. The use of zeolite increases photosynthetic activity, and consequently the biological productivity of crops (Krutilina *et al*., 2000).

Currently, much issues focus on high-grade plant nutrition, namely microelements. A balanced microelement composition can help in plants' physiological processes, and their deficiency can incite disease and contamination of plants. Vital trace elements used by rapeseed plants include manganese, zinc, copper, and iron. (Martin *et al*., 2012; Pandey, 2018). It has been proven that a balanced composition of trace elements in a plant ensures higher and constant yields, and also improves the quality of the harvested products (Choudhary *et al*., 2015; Tiwari *et al*., 2020).

In crops, particularly in spring rapeseed (*Brassica napus* L.), the higher yields are directly dependent upon the organo-mineral complexes made through trace elements in plants. Consequently, the determination of quantitative parameters of microelements accumulation in rapeseed (*Brassica napus* L.) plants and its yield with the introduction of a natural zeolite containing rock and mineral fertilizers is also very relevant. The present study aims to determine the effects of zeolite on the micro-morphological and biochemical traits of spring rapeseed under field conditions.

MATERIALS AND METHODS

The field experiments on spring rapeseed (*Brassica napus* L.) for determining the efficiency of mineral fertilizers and natural zeolite were carried out from 2018 to 2020 at the Yelets State University Named after I.A. Bunin, in Russia. The field experiments were based and laid out as per the methodology of Dospekhov (2014).

Field experiments on *Brassica napus* L. were carried out at the different sites located in District Yeletsk, Lipetsk region, Russia. The study area climate was moderately continental, and situated at 166 meters above the sea level, with coordinates at 52° 62′ north latitude and 38° 50′ east longitude. In general, and during the growing season of rapeseed, the weather conditions were most favorable in 2019 and 2020 for the formation of a good yield (Figure 1). In 2018, 2019, and 2020, from May to August, the average daily air temperature was 19.4 °C, 18.5 °C, and 17.9 °C; the precipitation was 137.0 mm, 214.8 mm, and 227.0 mm; the HTC was 0.58, 0.94, and 1.28 with an average long-term HTC equal to 1.21 (according to Selyaninov), respectively (Figure 1). The soil of the experimental field was leached chernozem (black earth). The humus content varies between 5.70 to 5.80%, the total nitrogen content ranging from 0.285 to 0.292%, phosphorus (196.2 to 198.3 mg kg^{-1}), and potassium (114.7 to 115.0 mg kg^{-1}).

The rapeseed experiments received the following four treatments i.e., a) control, b) NPK @ 60-60-60 kg ha^{-1}, c) Zeolite 5 t ha^{-1}, d) NPK @ 60-60-60 kg ha^{-1} + Zeolite 5 t ha^{-1}. To assess the effects of all the treatments, the spring rapeseed cultivar, Rif (obtained by crossing of the genotypes Rubezh and Magnum) was used. Rif is a high-yielding and disease-resistant rapeseed cultivar, characterized by no erucic acid in seeds. Its average plant height reaches 120 cm and 1000-seed weight is around 2.8 to 3.5 g. Fertilizers and natural zeolite were applied in the spring before cultivation. The area of the experimental plots was 50 m^2, and the

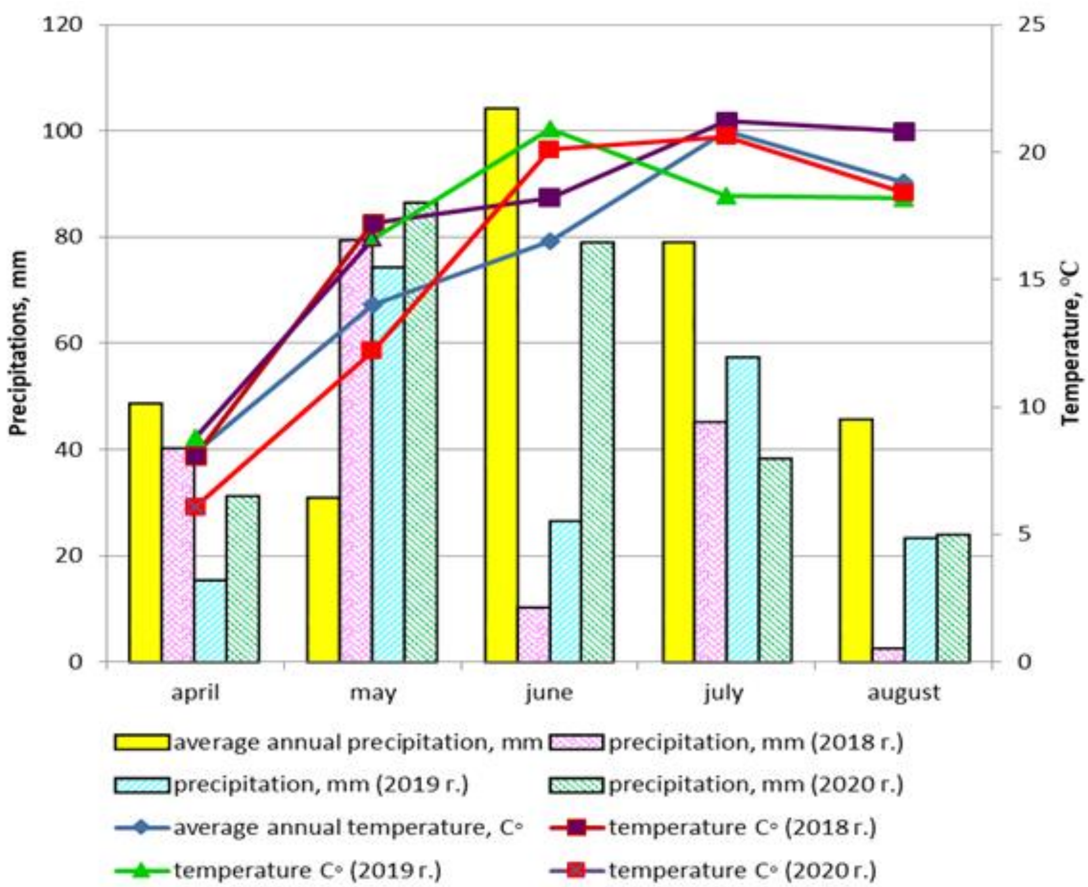

Figure 1. Weather conditions during the spring rapeseed crop seasons of 2018-2020.

registration plots were 40 m^2. The experiment was repeated four times. Natural zeolites of the Terbunsky origin (Lipetsk region, Russia) were used in the research. Zeolites had the following average mineral composition (% weight): sodium-Na (0.1), manganese-Mg (0.9), aluminum-Al (9.4), silicon-Si (21.3), phosphorus-P (0.4), sulfur-S (0.3), potassium-K (1.6), calcium-Ca (0.8), iron-Fe (2.3), cobalt-Co (9.5), nitrogen-Ni (3.4), copper-Cu (0.3), zinc-Zn (1.1), and molybdenum-Mo (1.2).

For spring rapeseed (*Brassica napus* L.), the crop production practices were carried out according to the recommendations made generally for the forest-steppe zone of the Central Federal District (Karpachev, 2009). Seed sowing of rapeseed was done in the third week of April, at a depth of 2 cm, row spacing of 12.5 cm, and a seeding rate of 2.0 million unit ha^{-1}. Winter wheat was the previous crop on the experimental fields. Care for the rapeseed crop consisted of treating against weeds with weedicide Galion @ 0.3 l ha^{-1} (active ingredients i.e., clopyralid – 300 g L^{-1} + picloram – 75 g L^{-1}). Against the cruciferous flea and rapeseed flower beetle, the crop was treated with the insecticide Karate Zeon @ 0.15 L ha^{-1} (active ingredient was lambda-cyhalothrin – 50 g L^{-1}).

The rapeseed's biochemical characteristics of plants were studied during the phase of the rosette in May, the micro-morphological characteristics of leaves during its full flowering in July,and the spring rapeseed ripening in August. Spring rapeseed was harvested in the third week of August.

Data recorded

The data were recorded on the micro-morphological parameters of the spring rapeseed (*Brassica napus* L.) i.e., the microstructure of abaxial and adaxial leaf surfaces, the size of stomata, photosynthetic pigments in leaves, the biochemical composition including trace element in the leaves, and the seed yield of spring rapeseed grown with the use of mineral fertilizers, natural zeolite only, and mineral fertilizers plus zeolite, and control (without fertilizers).

Visualization of the leaf surface and cross-sections

In spring rapeseed (*Brassica napus* L.), the microstructure of adaxial and abaxial leaf surfaces, and the number and size of stomata were determined by using an analytical electron microscope 'EVO 50 XVP' (Carl Zeiss). To study the morphology of abaxial and adaxial leaf surfaces, sections of 5 × 5 mm were taken from the left and right of the central middle ribs of 10 leaves and placed on carbon tape mounted on a microscope table. The cross-sections of the leaves were 0.5-1 mm thick and were fixed on an adhesive base. The leaves were not pretreated, since the microscopy was carried out under low vacuum conditions (60 Pa), and the deformation of the cross-sections was not significant.

Analysis of photosynthetic pigments

In spring rapeseed (*Brassica napus* L.) plant leaves, the content of photosynthetic pigments were determined according to the standard method (Klein and Klein, 1974). Leaves were taken in the flowering phase from the middle part of 20 plants from 1100 h to 1400 h (the period in a day where pigment content in a plant is highest) with subsequent averaging. Measurements of photosynthetic pigments content — chlorophyll *a* and *b*, as well as, carotenoids were determined using a KFK-5M spectrophotometer (Russia). Carotenoids were determined at λ = 440.5 nm, chlorophyll *a* at λ = 665 nm, and chlorophyll *b* at λ = 649 nm. Each pigment extract was measured in tenfold replicates. The concentrations of chlorophylls *a* and *b* in the extract were calculated using the Vernon formula (Klein and Klein, 1974).

$$C_{\text{chlorophyll } a} = 13.7 \cdot D_{665} - 5.76 \cdot D_{649}$$

$$C_{\text{chlorophyll } b} = 25.8 \cdot D_{649} - 7.6 \cdot D_{665}$$

To determine the concentration of carotenoids (mg/l) in the total extract of pigments, the Wettstein formula was used (Klein and Klein, 1974).

$$Scar = 4.695 \cdot D440.5 - 0.268\ (Ca + b)$$

where Ca + *b* is the total content of chlorophylls *a* and *b* in solution (mg/l).

D_{665}, D_{649}, and $D_{440.5}$ are the indicators of optical density of an alcohol solution at the corresponding wavelengths (665, 649, and 440.5 nm).

The content of pigments in the extract was determined in the test material, considering the volume of the extract and the weighed portion of the sample according to the following formula:

$$A = V \cdot C / (P \cdot 1{,}000)$$

where C is the concentration of pigments (mg/l), V is the volume of the extract (ml), P is the weight of plant material (g), and A is the pigment content in plant material (mg/g of crude weight).

Trace elements in rapeseed leaves

Determination of microelements in spring rapeseed plants was done using the atomic absorption method on a spectrophotometer 'Spectrum-5' in an acetylene-air flame (Pupyshev, 2009). The mineralization of the plant samples was done through the method of dry ashing. Mobile forms of elements were extracted with 1 ml nitric acid.

Preparing rapeseed leaves for analysis

Leaves for analysis were selected in dry weather from 5 to 9 plants in each experimental area. The plant material was carefully freed from dust and soil particles and brought to an air-dry state. Medium samples were crushed, sieved, and placed into marked bags with a weight of 3 gper bag.

Chemicals

All the chemical substances chosen for the analysis were certified solutions for analysis and were bought from Sigma Aldrich (USA) and Merck KgaA (Germany).

Statistical analyses

All the analyses were performed in triplicate. Results were expressed as mean values (n = 3) and as standard deviation (SD). For statistical analysis, the XLSTAT package - v. 2016 was used (Microsoft Excel, v. 2016).

RESULTS AND DISCUSSION

Scanning electron microscopy

The micro sculpture of the spring rapeseed (*Brassica napus* L.) plant leaf surface was noticeably affected by different treatments of fertilizers used in the experiment (Figure 2). The leaf surface was found smoother in the variants where only zeolite was used as fertilizers (Figures 2 C, D) and zeolite in combination with mineral fertilizer (Figures 2 E, F), as compared to the control treatment, where it was more folded and wrinkled. An important property of natural minerals is their ability to retain and rationally consume moisture and nutrients during the growing season, thereby creating favorable conditions in the soil-plant interaction system (Loboda, 2000; Ryakhovskaya and Ginatulina, 2009). By adding clinoptilolite to the soil, the water retention capacity was increased by 50% on slope soils and by 70% on lowland soils (Xiubin and Zhanbin, 2001).

Consequently, the rapeseed plants using zeolite were provided with moisture throughout the growing season, which was observed in the structure of the leaf surface. In addition, the stomata were found larger in the rapeseed plants treated with natural zeolite compared to the control. The stomatal ridges were well delineated and the stomatal gap was visible (Figures 2 G, H). The effects of successive soil moisture decrease in plants were studied, which revealed that the stomata were restored at the initial level of activity while the cotton species only after a few days (Pallas *et al*., 1967). Within the same species, the different leaves can have different leaf surface properties and these properties may vary in the same leaf between the adaxial and abaxial sides (Eglinton and Hamilton, 1967).

The adaxial side of the spring rapeseed (*Brassica napus* L.) plant leaf was characterized by a greater number of stomata compared to the abaxial side (Figure 3). The maximum number of stomata was observed in the variant treated with natural zeolite plus mineral fertilizers. However, on average there were 537 pieces/mm^2 on the adaxial surface and 480 pieces/mm^2 on the abaxial surface of the leaf. This indication was increased in the variants treated with the zeolite because it was probably associated with a smoother leaf surface. It is a well-known fact that parameters of photosynthesis were significantly improved through the use of various types of plant nutrition (El-Mogy *et al*., 2019). The said past studies further revealed that the introduction of mineral fertilizers separately and in combination with a natural ameliorant contributed to an increase (22%) in photosynthetic pigments as compared to the control (Figure 4).

Carotenoids are a widespread class of pigments. Their function in nature is not limited to working with light and plays an important role in metabolism in eukaryotes (Ladygin and Shirshikova, 2006; Borisenko and Zholobova, 2015). The maximum carotenoids (0.374 mg/g of crude weight) were observed in the variant where zeolite was used with $N_{60}P_{60}K_{60}$. However, the minimum magnitude

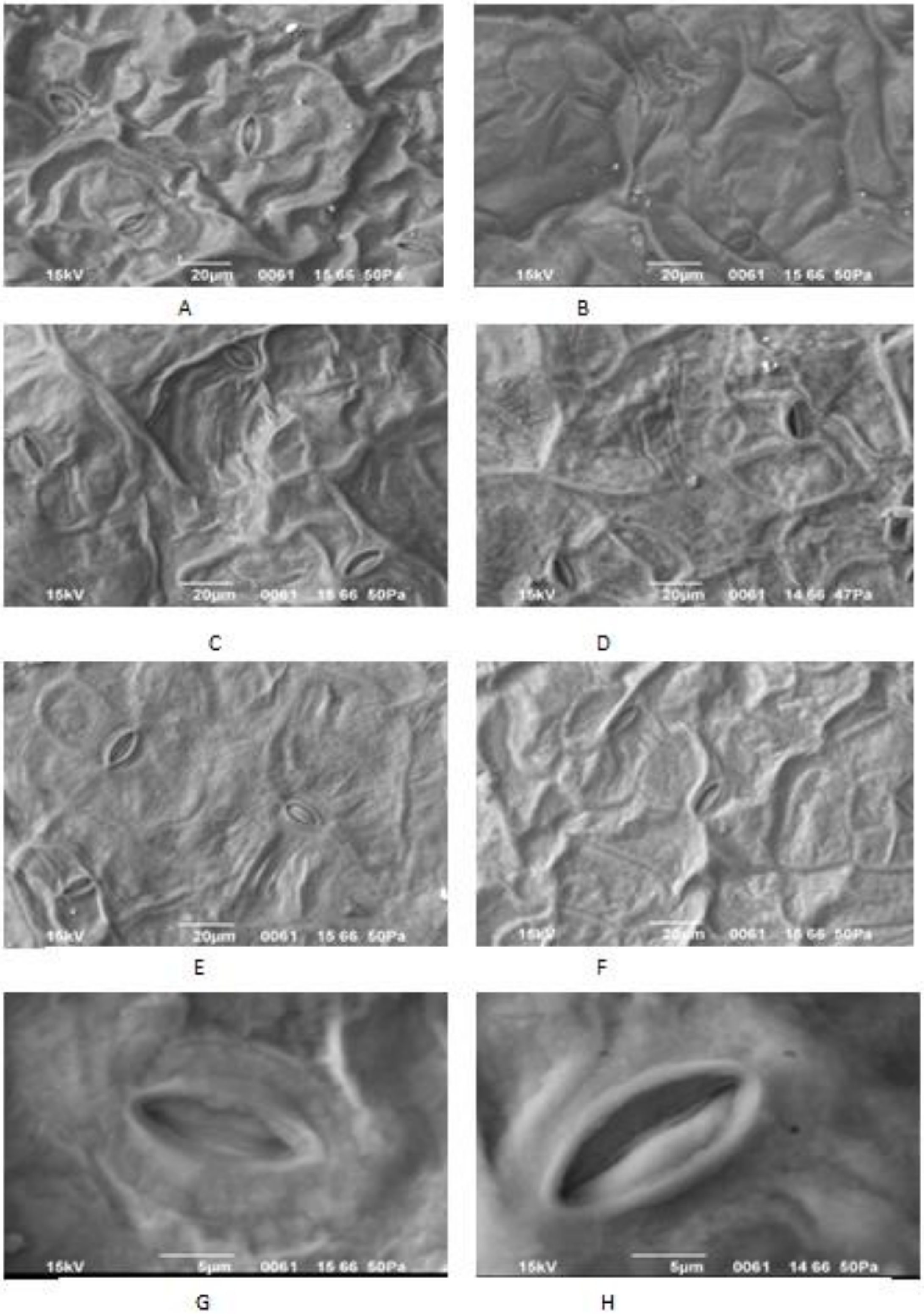

Figure 2. Features of micro-sculpture of the abaxial (A, C, E) and adaxial (B, D, F) sides of the spring rapeseed leaf. A and B are leaves of the control variant; C and D are leaves from plots of 5 t ha^{-1} of natural mineral; E and F are leaves from NPK plots + 5 t ha^{-1} of natural mineral; G and H are stomata of the adaxial side of the leaf (G-control, H - NPK + 5 t ha^{-1}).

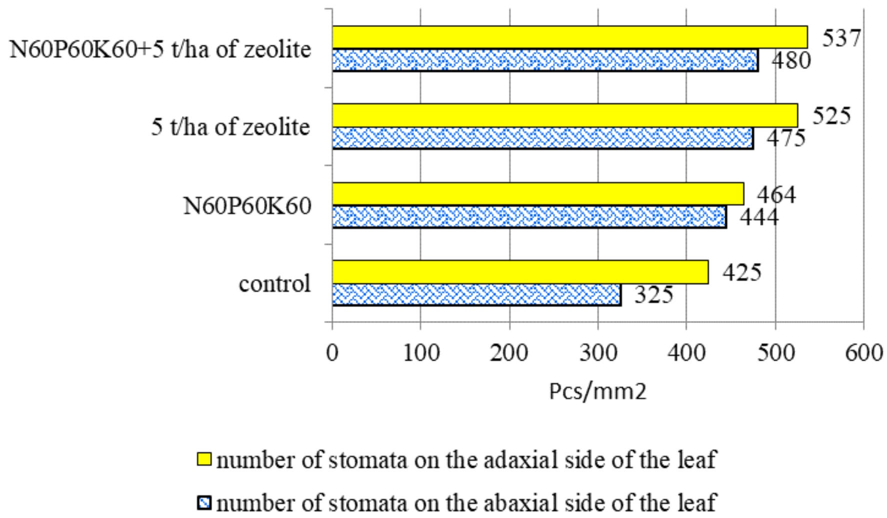

Figure 3. The number of stomata in the spring rapeseed leaves.

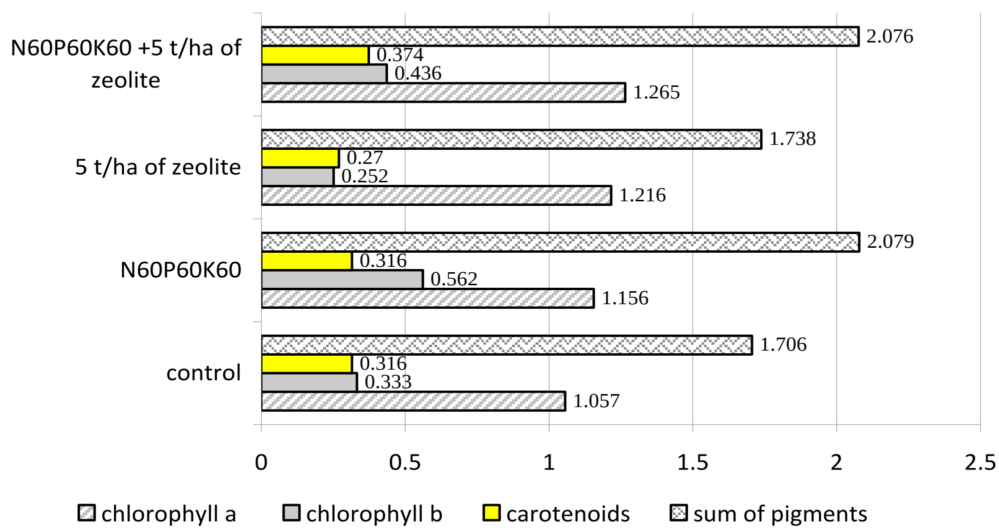

Figure 4. The content of photosynthetic pigments in spring rapeseed plants (mg g^{-1} of crude weight).

of carotenoids (0.270 mg/g of crude weight) was observed in the variant treated with zeolite only. Chlorophyll content in rapeseed plants ranged from 1.057 mg/g of crude weight (control) to 1.265 mg/g of crude weight ($N_{60}P_{60}K_{60}$ + Zeolite 5 t ha^{-1}). There was a high correlation between chlorophyll *a* and the number of stomata on the abaxial side (r = 0.95), and adaxial side (r = 0.98) of the rapeseed leaf.

It has been validated that a balanced composition of trace elements in a plant provides the basis for high and stable yield. However, the excessive accumulation of trace elements in plant cells can lead to the materialization of their toxicity. Therefore, the content of trace elements should also be monitored in plant parts (Byshov *et al*., 2020). It should also be noted that natural mineral fertilizer zeolite exhibited adsorption activity concerning the studied micro-elements (Mn, Fe, Zn, Ni, and Co). However, their highest accumulation was noted in the spring rapeseed variant with $N_{60}P_{60}K_{60}$ and decreased by adding natural zeolite.

In the course of the studies carried out to determine the content of trace elements in plants according to the phases of spring rapeseed (*Brassica napus* L.) development, it was found that the content of the studied trace elements (Mn, Fe, Zn, and Ni) was decreased by the harvesting phase. The exception was cobalt (Co), the content of which increased by the time of rapeseed ripening. The present results were consistent with the previous studies, where it has been proven that rapeseed is a co-concentrating plant, absorbing nutrients throughout the growing season, and reaching a maximum ratio in the ripeness phase (Novikova, 2005). Cobalt participates in the plant growth processes and cellular reproduction of leaves, and increases the total water content of plants (Kabata-Pendias, 2010).

The concentration of the element Co in the rosette phase ranged from 0.058 g kg^{-1} of dry matter (control) to 0.158 g kg^{-1} of dry matter ($N_{60}P_{60}K_{60}$ + zeolite 5 t ha^{-1}). However, at the time of ripening, the Co content was increased in these variants by 36.2% and 76.9%, respectively. With a lack of nickel (Ni), urea accumulated in leaf tissues, resulting in cell destruction. In cultivated plants, the nickel deficiency occurred when its content becomes less than 0.10 mg/kg of dry matter. However, in the present studies, the lack of Ni was not found in the different variants of the study (Figure 5).

Manganese (Mn) is an essential trace element that plays an important functional role in plant metabolism. Mn participates in a spectrum of enzyme-catalyzed reactions, including redox reactions, phosphorylation, decarboxylation, and hydrolysis (Schmidt and Husted, 2019). However, in the present studies, the Mn content ranged from 19.4 to 24.75 g/kg of dry weight depending upon the treatments and the phases of spring rapeseed crop development.

The importance of iron (Fe) for plant growth was established about 50 years earlier than the discovery of other trace minerals. However, iron deficiency is characterized by a yellow color in the young leaves (Ma and Ling, 2009). The amount of Fe ranged from 39.98 mg/kg of dry weight (control + the full ripeness phase) to 54.48 mg/kg of dry weight ($N_{60}P_{60}K_{60}$ + zeolite 5 t ha^{-1} + the rosette phase) in the spring rapeseed (*Brassica napus* L.). The highest accumulation of Fe was observed at the time of maturation in the spring rapeseed variant treated with $N_{60}P_{60}K_{60}$ (52.11 mg/kg of dry weight), which exceeded the control by 30.3%, and $N_{60}P_{60}K_{60}$ + zeolite 5 t ha^{-1} by 2.5% (Figure 6).

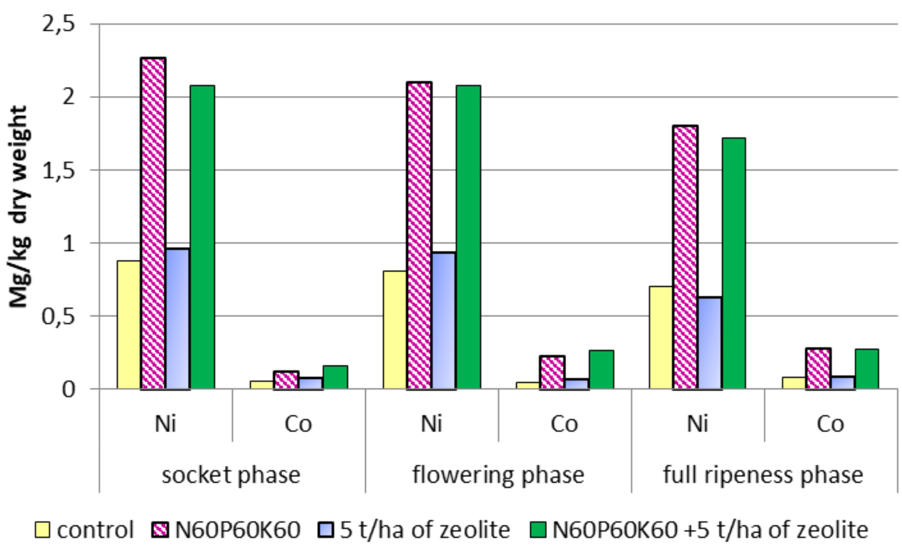

Figure 5. Nickel (Ni) and Cobalt (Co) content in the spring rapeseed plants.

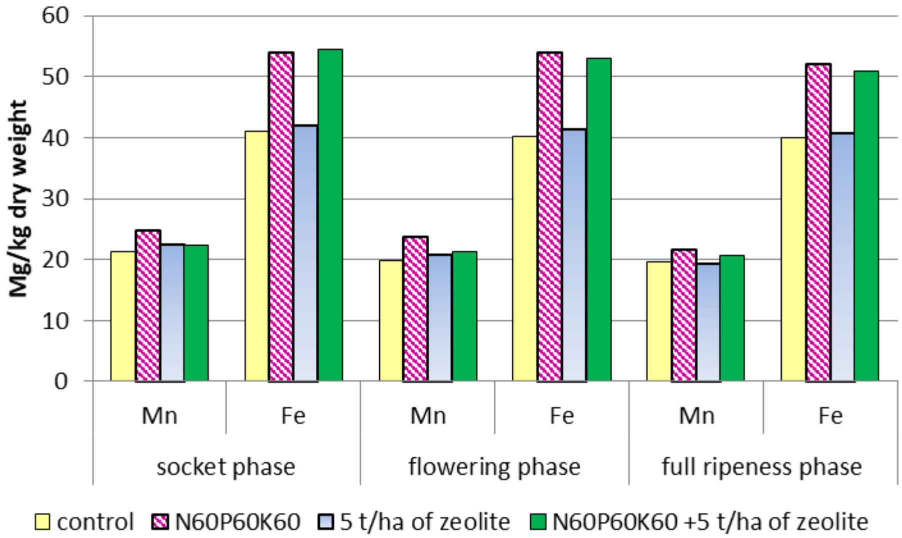

Figure 6. Manganese (Mn) and Ferrous iron (Fe) content in the spring rapeseed plants.

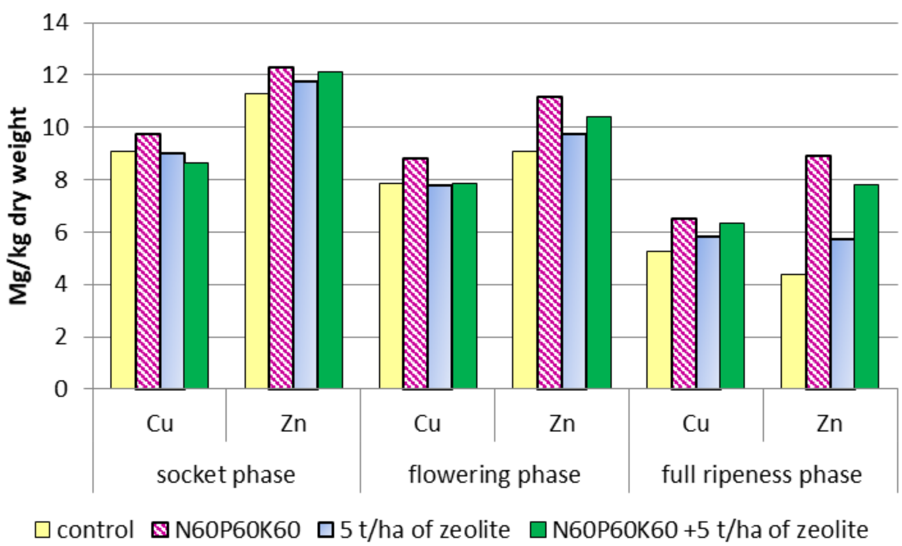

Figure 7. Copper (Cu) and Zinc (Zn) content in the spring rapeseed plants.

Zinc (Zn) is a vital trace element for plants that is actively involved in many biochemical processes (Kabata-Pendias, 2010). The maximum concentration of zinc was observed in plants at the rosette phase of spring rapeseed ranging from 8.65 mg kg^{-1} of dry weight ($N_{60}P_{60}K_{60}$ + zeolite 5 t ha^{-1}) to 9.74 mg kg^{-1} of dry weight ($N_{60}P_{60}K_{60}$). In the full ripeness phase of plants, Zn content decreased with a range from 4.37 mg kg^{-1} of dry weight (control) to 8.90 mg kg^{-1} of dry weight ($N_{60}P_{60}K_{60}$) (Figure 7). The accumulation of the studied elements in spring rapeseed plants at the stage of full maturation can be represented with the following decreasing series i.e., Fe>Mn>Zn>Cu>Ni>Co. The correlation coefficients showed a high relationship between all the studied trace elements (Table 1).

All the fertilizer treatments contributed an additional yield to the spring rapeseed crop. Natural zeolite only (dose of 5 t ha^{-1}) provided an increase in plant productivity by 0.35 t ha^{-1}, variant $N_{60}P_{60}K_{60}$ caused an increase by 0.91 t

Table 1. Correlation matrix for five trace elements (Fe, Cu, Zn, Ni, Co, and Mn) in the spring rapeseed at the ripening phase.

Elements	Fe	Cu	Zn	Ni	Co
Mn	0.94	0.84	0.92	0.94	0.92
Fe		0.93	0.96	0.99	0.99
Cu			0.99	0.89	0.93
Zn				0.93	0.95
Ni					0.99

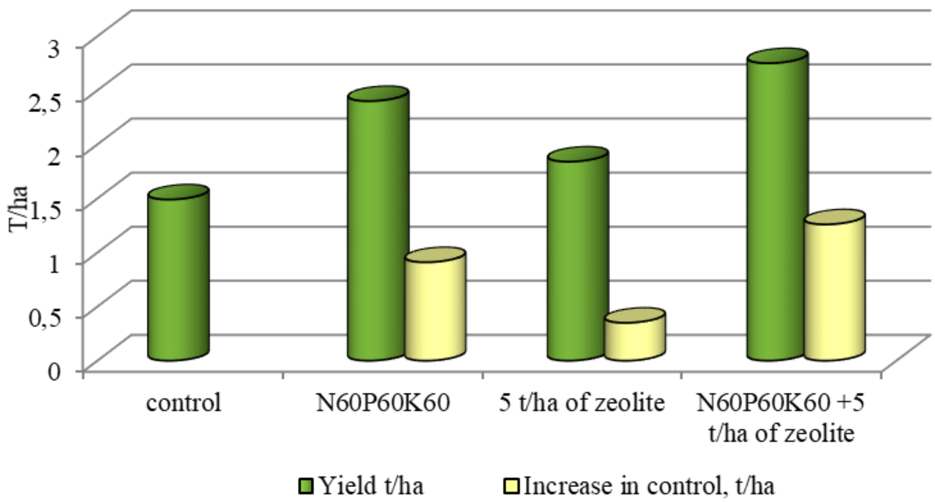

Figure 8. Average spring rapeseed yield (t ha^{-1}) during 2018-2020. LSD_{05} interaction of factors AB (0.22 t ha^{-1}).

ha^{-1}, and the use of zeolite in combination with $N_{60}P_{60}K_{60}$, by 1.26 t ha^{-1} compared with the control (0.22 t ha^{-1}) (Figure 8). A high correlation (r = 0.95) was observed between the yield and the photosynthetic pigments. On average, moderate to high correlation was observed between the yield and number of stomata on the abaxial side (r = 0.73) and adaxial side (r = 0.60) of the spring rapeseed leaf.

CONCLUSIONS

Scanning through electron microscopy, the spring rapeseed (*Brassica napus* L.) plant leaves showed a smoother surface in the variants treated with natural zeolite only and in combination with mineral fertilizers. The adaxial side of the rapeseed plant leaf was characterized by a greater number of stomata than the abaxial side. However, the maximum number of stomata was observed in the variant with the use of natural zeolite plus mineral fertilizers. There was a high correlation between chlorophyll *a* and the number of stomata on the abaxial side (r = 0.95) and the adaxial side (r = 0.98) of the leaf. In the microelement analysis of rapeseed plants, the highest accumulation of microelements (Mn, Fe, Zn, Ni, and Co) was noted in the variant treated with $N_{60}P_{60}K_{60}$ while the microelements decreased when added with natural zeolite. In spring rapeseed, the maximum yield (2.75 t ha^{-1}) was recorded in the variant of zeolite plus mineral fertilizer, which exceeded the control by 1.26 t ha^{-1}. A high correlation (r = 0.95) was found between the yield and the photosynthetic pigments.

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