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ORGANIC FERTILIZER AND NATURAL ZEOLITE EFFECTS ON MORPHOMETRIC TRAITS OF *BRASSICA NAPUS* L. POLLEN GRAINS

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SUMMARY

Morphological characteristics of *Brassica napus* pollen (polar axis, equatorial diameter, shape index, pattern of perforation of exines, and perimeter of perforation zones) were investigated by using scanning electron microscopy JEOL JSM-6390. The *B. napus* seeds were grown under various agroecological conditions, i.e., mineral fertilizer (NPK 60:60:60 t ha^{-1}) separately and together with zeolite, chicken droppings (10 t ha^{-1}) separately and together with zeolite (5 t ha^{-1}), and control (without fertilizers) from 2018 to 2020 at the Bunin Yelets State University, Yelets, Russia. It was found that pollen grains of *B. napus* had an oblong-ellipsoid shape. In the polar view, the pollen grains were circular with straight sides, though in the equatorial view, elliptical. For the polar axis of the pollen grains in fertilizer application treatments, the minimum values ranged from 24.59 to 27.76 µm, whereas the maximum readings were 40.13 to 42.12 µm compared with control (21.56 µm, 41.52 µm). The coefficient of variation for fertilizer applications ranged from 8.86% to 14.10% compared with control (14.85%) for the polar axis. For the equatorial axis of the pollen grains, the minimum values varied from 11.61 to 15.63 µm, whereas the range for the maximum values was 19.74 to 23.96 µm compared with the control (13.63 µm, 21.88 µm). For the equatorial axis among the fertilizer applications, the coefficient of variation varied from 8.47% to 12.01% compared with the control (18.35%). For the perimeter of the exine perforation of the pollen grains, the minimum values ranged from 1.68 to 1.95 µm, whereas the maximum values varied from 4.34 to 7.12 µm compared with the control (1.34 µm, 5.68 µm). The coefficient of variation varied from 24.48% to 33.19% compared with the control (34.78%) for the perimeter of exine perforation. The shape index of the pollen grains of the *B. napus* varied from 1.96 to 2.07 µm (correct unit of measure?) compared with the control (2.03 µm). Overall, with the use of organic fertilizers and zeolite, the morphometric parameters of pollen grains were significantly enhanced.

Keywords: *Brassica napus,* micromorphology, pollen grains, analytical scanning electron microscopy, agroecological experience

Key findings: Pure zeolite used separately and in combination with mineral fertilizers significantly influenced the morphological characteristics of the pollen grains of *Brassica napus* L. cv. Rif.

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INTRODUCTION

For the last two decades, the world production of rapeseed (*Brassica napus* L.) has grown steadily. The main manufacturers are China, India, Canada, and the European Union or EU (Carre and Pouzet, 2014). This culture of rapeseed production has been adapted in the end of 1900s in Western conditions of the USA (Jackson, 2000). Russian climatic conditions permit to grow the rapeseed plants in all the regions (Karpachev, 2009). The rapeseed productivity attracted crop breeders for many years. The seed development demands the constant improvement of this cultivation technology system in the concrete edaphoclimatic conditions taking into account the breed peculiarities and the reactions of different technological elements including both mineral and organic fertilizers.

Studies proved that the greatest impact on rapeseeds productivity is caused by the norm of nitrogenous fertilizer, while the minor impact is from previous production culture and the fertilizer type, as well as, the interaction between these factors (Rathke and Diepenbrock, 2005). The natural climatic conditions of the central European part of Russia are favorable for growing, not only early-ripening genotypes, but also medium-early cultivars and hybrids of spring rapeseed as the sum of active temperatures is 2200 °C–2350 °C, and rainfalls measure around 510-560 mm (Ram *et al*., 2015; Gulidova *et al*., 2017).

Intensified production of rapeseed raw material by increasing required resources, primarily fertilizers, plant protection measures, and means of complex mechanization, allows for enhancing crop yield and quality. To increase and stabilize the production of rapeseed, it is necessary to follow the path of expanding the areas in the northwestern regions, central chernozem, and non-chernozem zones of Russia (Vinogradov and Zubkova 2021, 2022b). However, the expansion of hectarage under winter and spring rape is dictated by the current economic situation. The availability of the genotypes with a shorter growing season and less sensitivity to heat deficiency, and moisture reserves in the soil, allows us to realize solutions for expanding the areas of rapeseed cultivation.

For example, the production of winter rapeseed in unconventional cultivation areas at the present stage of crop development urgently needs, not only the improvement of existing and well-known cultivation techniques, but also the development of new technological techniques taking into account the agrobiological properties of new genotypes, fertilizer systems with the inclusion of zeolite-containing rocks, tillage, planting times, and overall crop features (Mustafayev and Mazhaysky, 2018; Byshov *et al*., 2019; Zubkova *et al*., 2022a).

Numerous studies proved the good responsiveness of crops, including spring rapeseed, in obtaining a high seed yield yet, still maintaining good quality (Khabarova *et al*., 2018; Vinogradov *et al*., 2019, 2020). With fertilizer application and the use of biological products on fertile soils, such as chernozem, the yield of spring rapeseed can reach 30 c ha^{-1} (Nurhasanah and Ecke, 2016; Zubkova *et al*., 2020). Rapeseed can provide good yield and high-quality seeds under favorable growing conditions, and the role of each agrotechnical technique in growing seeds under varied crop rotations is vital. The main reason for crop fluctuations is the change in weather conditions during the growing season.

Spring rapeseed, unlike other grain crops, uses 1.5-2.0 times more macro- and microelements during the growing season, which might be the main reason for the costly vegetable oil (Zubkova *et al*., 2021a, b). Over the past two decades, breeders have carried out a lot of research work on breeding new cultivars and hybrids of rapeseed, and their technological properties have earned high appreciation. Yet, the features inherent in the new cultivars have not been fully studied. However, some analysis showed that the trend of improved quality of vegetable oils in the recent era is not due to the initial oilseed raw material, but also due to the use of the latest technologies implemented in the field, such as, new oil-extracting equipment, and technology of harvesting oilseeds (Vinogradov and Zubkova, 2018, 2021).

Organic animal waste is recognized as a valuable source of plant nutritional chemicals in farming systems and plays a certain role in soil improvement using organic substances (Schoenau and Davis, 2006). Therefore, the further development of rapeseed cultivation technologies using organic and mineral fertilizers is currently important. The application of zeolite-based fertilizers in oilseed production technologies, including in the production of spring rapeseed, contributes to an increase in seed productivity and quality (Vasileva, 2015). The increase in oilseed raw material due to the use of zeolite-containing material occurs due to the amorphous silicon included in mineral fertilizers, which is actively and quickly absorbed by living organisms and primarily by plants (Lesny *et al*., 2021).

Silicon, as an element of a living system, is an important macronutrient that plays an essential role in the growth and development of the plant body. Silicon promotes the active assimilation of calcium and phosphorus, which stimulates the development of plant growth, and the ripening of seeds (Churilov *et al*., 2019). The use of zeolite-containing fertilizers in growing crops increases the winter hardiness of plants, and resistance to pests, diseases, and adverse environmental factors. Silicon promotes the absorption of vitamins A, C, and E, and plays the role of an antioxidant. This active silicon is a water-soluble substance, effectively absorbed by the rhizosphere of plants (Zakharova *et al*., 2021).

The *Brassica napus* pollen grain is considered from the point of view of descriptive palynology, and bee pollen (Brindza and Brovarskyi, 2013; Redina *et al*., 2016; Brindza and Motyleva, 2018) and biochemistry (Piffanelli *et al*., 1998; Arabhanvi *et al*., 2015). Lo and Pauls (1992) studied the influence of the medium on the development of rape microspores under controlled conditions. The reaction of the pollens to various environmental conditions is studied in the tomato (Srivastava *et al*., 2012; Kumar *et al*., 2015) and the genus *Lysimachia* (Matyashuk *et al*., 2019). Environmental conditions have a direct impact on the formation and functioning of generative organs of plants. The parameters characterizing the viability of pollen grains are closely related to weather conditions like air temperature and humidity. In the central European part of Russia, the flowering phase of spring rapeseed is between late June toearly July and very often falls under unfavorable conditions (Zubkova *et al*., 2022a, b).

Studies on the effects of fertilizers on the morphology of *B. napus* pollen grains were not carried out in the past. Therefore, the recent study aimed to carry out a comparative study of the morphometric characteristics of *B. napus* pollen grown under diverse agroecological backgrounds.

MATERIALS AND METHODS

Plant material

The experiment in agrocenoses on spring rapeseed, *Brassica napus* L. cv. Rif (*B. napus*), to identify the efficiency of mineral fertilizers and natural zeolite was carried out in 2018–2020 at the Bunin Yelets State University, Yelets, Russia. The field experiment was conducted in the District Yelets, Lipetsk region of Russia, by following Dospekhov (2014). The climate of the study area is moderately continental. Its height above sea level is 166 m, and its coordinates are 52°62′N latitude and 38°50′E longitude. The experiment was set up according to the following treatments, i.e., a) NPK 60:60:60 (t ha^{-1}), b) chicken droppings (10 t ha^{-1}), c) NPK 60:60:60 (t ha^{-1}) + zeolite (5 t ha^{-1}), and d) chicken droppings (10 t ha^{-1}) + zeolite (5 t ha^{-1}), and e) control.

The pollen samples of *B. napus*, () obtained from the plots of different agroecological conditions, were studied. This said rapeseed cultivar was created by hybridization of the two cultivars, i.e., Rubezh and Magnum. Rapeseed cultivar Rif, namely, *Alternariosis fusariosis*, *and A. fomosis*, is high yielding and disease-resistant, and is characterized by no erucic acid in its seeds. Its plant height reaches 120 cm, and the average 1000-seed weight is around 2.8-3.5 g. The area of the experimental plots was 50 m^2, and the 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. For spring rapeseed,*B. 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. Seed sowing of the 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 units 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} (the active ingredient was lambdacyhalothrin = 50 g L^{-1}). The pollen grains were collected mechanically in the bud state from the flowers of rapeseed plants grown under different agroecological conditions.

Preparing pollen grains for analysis

For thorough studies of pollen grains morphology, samples were collected from mature flowers. The pollen grains were preliminarily dried at 30 °C – 40 °C and accurately pounded with a pestle to annual adhesion, and were put in the special carbonic scotch placed on the object table of the scanning electron microscope with a thin metal spreading rod.

Scanning electron microscopy (SEM)

Micro-pictures were taken on the scanning electron microscope (SEM) JEOL JSM-6390. The comparative morphological study of the pollen grains was performed according to the working rules on the SEM JEOL JSM-6390/LV (JEOL, Japan) in the conditions of low vacuum (P = 60 Pa), with the following zooming, i.e., 500 times—during the measurements, and 1,000–10,000 times—while taking the pictures of the exine sculpture features. Using the regime of low vacuum allows for performing the pollen study without its preliminary chemical treatment and receiving the undistorted data about the research object makes the process of the probe preparation easier.

Morphometric characteristics

The measurements of the pollen grain's linear dimensions were performed via 200 times repeatability in about 50 fields with the average value calculation. All measurements in the text dimensions are given in µm. The characterization of pollen grains was calculated by taking the following parameters, i.e., the polar axis (P-line connecting the proximal and distal pole), the equatorial axis (E-line perpendicular to the polar axis and located in the equatorial plane), SI–shape index (P/E), and perimeter of the exine perforation using the STIMAN program for SEM.

Statistical analysis

The recorded data were subjected to analysis of variance, and all the measurements were performed with n = 50. The calculated parameters were mean ± SE, standard deviation (SCO), min (minimum value), max (maximum value), and coefficient of variation (C.V.%) using mathematical formulas.

RESULTS AND DISCUSSION

The pollen grains morphology showed that pollen grains of *Brassica napus* L. cv. Rif was radially symmetrical and isopolar, and according to the localization, the apertures were zonotricolpate. Three compound apertures were according to distribution equidistant. The size, shape of pollen grains, and the number of apertures were documented in Figure 1. The shape of the pollen grains is oblong-ellipsoidal (Figure 1a-d). The apertures were long, and in the polar view, the pollen grains were circular with straight sides (Figure 1e-f). Petrov *et al.* (2017) reported that anthropogenic transformation of the environment can affect morphometric traits of the pollen grains in *B. napus* L.

In the obtained pictures, it can be seen that the pollen grains were 3-corporate. Colpi, with even edges and pointed / blunted ends, almost converge at the poles (Figure 1e-f). We even found 4-corporate pollen (Figure 1d), and that phenomenon has been called pollen heteromorphism (Nadot *et al*., 2000). Heteromorphism of pollen grains was found only in variants with the introduction of chicken droppings (10 t ha^{-1}) + zeolite (5 t ha^{-1}). Our previous studies allowed us to establish that in this variant, rapeseed plants developed more intensively even at the first stages of ontogenesis and the said variant was also characterized by the maximum yield of spring rape seeds (Zubkova *et al*., 2020).

The pollen exine ornamentation was reticulate. The obtained electronic photographs were consistent with the Palynological database (PalDat, 2020), and the results of micro sculpture of *B. napus* pollen and the hybrids of *Brassica oleracea* and *Brassica campestris*, provided in the previous study (Hossain *et al*., 1991). An important morphological characteristic is the size of pollen grains. The length of the polar axis (P) of rapeseed pollen, grown under various agroecological conditions, is shown in Figure 2. In the variants with mineral fertilizer (NPK 60:60:60: t ha^{-1}), and chicken droppings (10 t ha^{-1}), the size of the polar axis was lower than that in the control. The treatments with mineral fertilizer (NPK 60:60:60: t ha^{-1}) + zeolite (5 t ha^{-1}), and chicken droppings (10 t ha^{-1}) + zeolite (5 t ha^{-1}) were higher than those in the control, which is confirmed by the results of static treatment.

The variation limits the polar axis of pollen grain depending upon the type of treatment. In the application of four fertilizer treatments for the polar axis of pollen grains, the minimum values ranged from 24.59 µm (chicken droppings [10 t ha^{-1}]) to 27.76 µm (chicken droppings [10 t ha^{-1}] + zeolite [5 t ha^{-1}]), whereas the maximum readings were 40.13 µm (chicken droppings [10 t ha^{-1}]) to 42.12 µm (NPK 60:60:60 + zeolite [5 t ha^{-1}]) as compared with control (21.56 µm, the minimum values, and 41.52 µm, the maximum values) (Table 1). The coefficient of variation (C.V.) for all the fertilizer applications ranged

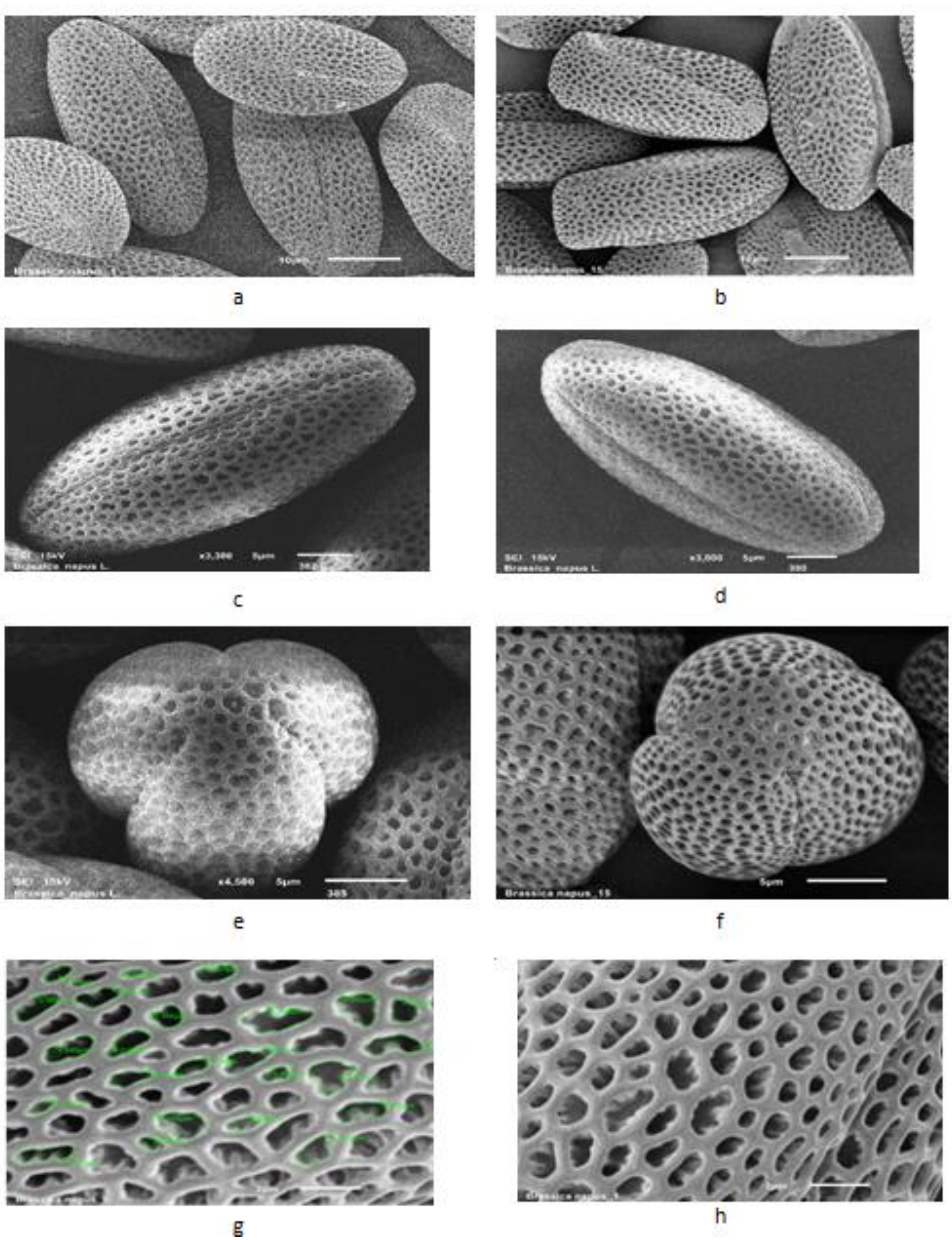

Figure 1 (a, b, c, d, e, f, g, h). Pollen grains of *Brassica napus* in different positions: a, b, c, d) General view of pollen, e, f) View from the pole, g) Pollen ornamentation of sporoderm, h) An example of measuring the perimeter of the exine perforation form.

Table 1. Morphometric characteristics of the *Brassica napus* L. cv. Rif (n = 200), μm.)., $\bar{x}$±Sx.

Morphometric characteristics	Mineral and organic fertilizers, and natural zeolite				
	NPK (60:60:60)	Chicken droppings (10 t ha^{-1})	NPK (60:60:60) + zeolite (5 t ha^{-1})	Chicken droppings (10 t ha^{-1}) + zeolite (5 t ha^{-1})	Control
Polar axis (P)					
Min.	24.59±0.14	24.59±0.21	26.81±0.22	27.76±0.15	21.56±0.11
Max.	41.63±0.22	40.13±0.35	42.12±0.33	41.04±0.28	41.52±0.21
C.V. (%)	11.28	14.10	9.95	8.86	14.85
Equatorial axis (E)					
Min.	11.61±0.12	13.93±0.11	14.44±0.12	15.63±0.11	13.63±0.11
Max.	19.74±0.13	20.62±0.14	20.56±0.16	23.96±0.13	21.88±0.21
C.V. (%)	10.17	12.01	8.85	8.47	18.35
The perimeter of the exine perforation					
Min.	1.95±0.69	1.91±0.38	1.68±0.44	1.68±0.28	1.34±0.50
Max.	7.13±0.62	4.34±0.42	5.18±0.26	5.18±0.41	5.68±0.71
C.V. (%)	32.40	33.19	25.31	24.48	34.78
Shape index (SI) of pollen grain (P/E)					
SI	2.03	2.11	1.96	2.07	2.02

from 8.86% to 14.10% compared with control (14.85%) for the polar axis of the pollen grains. In the variants of the treatments 60:60:60 + zeolite (5 t ha^{-1}) and chicken droppings (10 t ha^{-1}) + zeolite (5 t ha^{-1}), there was a tendency to increase the polar axis of pollen grains and to decrease the coefficient of variation. A similar pattern was observed in the size of the equatorial axis of the pollen grains. For the equatorial axis of the pollen grains, the minimum values varied from 11.61 μm (NPK 60:60:60) to 15.63 μm (chicken droppings [10 t ha^{-1}] + zeolite [5 t ha^{-1}]), whereas the range of the maximum values was 19.74 μm (NPK 60:60:60) to 23.96 μm (chicken droppings [10 t ha^{-1}] + zeolite [5 t ha^{-1}]) compared with control (13.63 μm, 21.88 μm) (Table 1). For the equatorial axis among all the fertilizer applications, the coefficient of variation varied from 8.47% to 12.01% compared with the control (18.35%). The results were consistent with the past findings, where the effects of processing plants with organic material (nanoscale calcite and seaweed extract) were reported to enhance the length of the polar and equatorial axes of grape pollen grains (Sabir, 2015).

For the perimeter of the exine perforation of the pollen grains, the minimum values ranged from 1.68 μm (NPK 60:60:60 + zeolite [5 t ha^{-1}], chicken droppings [10 t ha^{-1}] + zeolite [5 t ha^{-1}]) to 1.95 μm (NPK 60:60:60), whereas the maximum values varied from 4.34 μm (chicken droppings [10 t ha^{-1}]) to 7.12 μm (NPK 60:60:60) compared with control (1.34 μm, 5.68 μm) (Table 1). Among the fertilizer applications, the coefficient of variation varied from 24.48% to 33.19% compared with control (34.78%) for the perimeter of the exine perforation. The shape index (SI) of the pollen grains depends on the parameters of polar (P) and equatorial (E) axes. The shape index of the pollen grains of the tested species of *B. napus* varied from 1.96 (chicken droppings [10 t ha^{-1}]) to 2.07 (NPK 60:60:60 + zeolite [5 t ha^{-1}]) as compared with the control (2.03) (Figure 3, Table 1). The *B. napus* pollen is characterized by a high degree of perforation of the exine. The perimeter of the perforation areas was measured, and the results are presented in Figure 1g-h and Table 1. Pollen surrounded by a sculptural wall of exine plays an important role in protecting it from various environmental stresses and bacterial attacks in *Arabidopsis thaliana* (Zinkl *et al*., 1999). The same is also facilitated by favorable abiotic conditions, such as, additional water and nutrients for mother plants in *Ipomopsis aggregata* and *Linum lewisii* (Burkle and Irwin, 2009).

Analysis of variance confirmed significant differences between the pollen grain sizes (Figures 2–4). In pollen grains, the observed morphometric changes can be associated with the variation in plant metabolism in connection with the different variants. It is well established that the production of plant biomass is an important indicator for evaluating the plant tolerance to stress conditions (John *et al*., 2012). The balanced microelement composition ensures full-fledged physiological processes in plants and contributes to enhancing the productivity and quality of finished products. Boron,

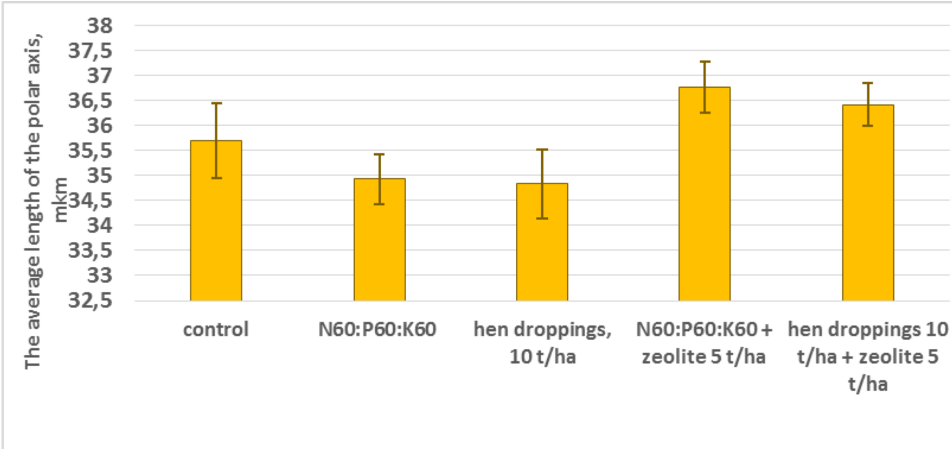

Figure 2. Sizes of the polar axis of the pollen grains of *Brassica napus* L. cv. Rif; vertical lines - standard deviation (±SD) of fifty independent experiments

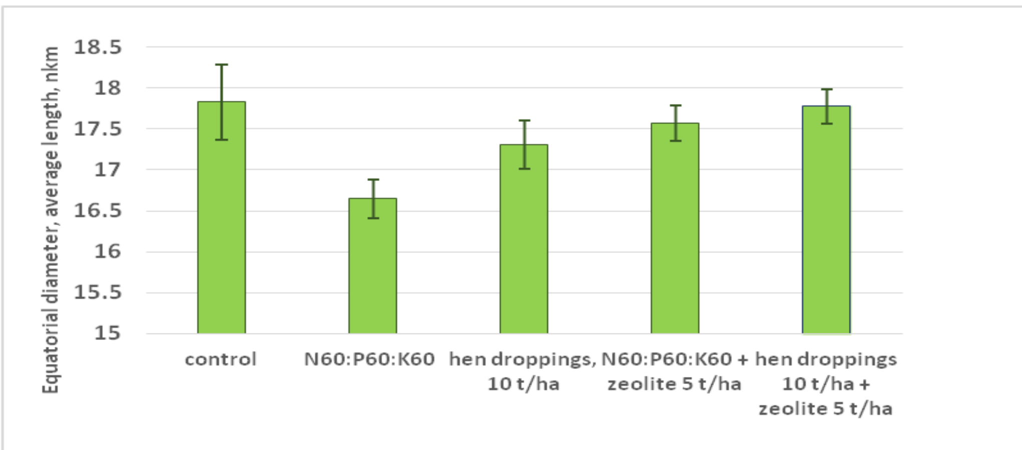

Figure 3. Sizes of the equatorial diameter of the pollen grains of *Brassica napus* L. cv. Rif; vertical lines - standard deviation (±SD) of fifty independent experiments.

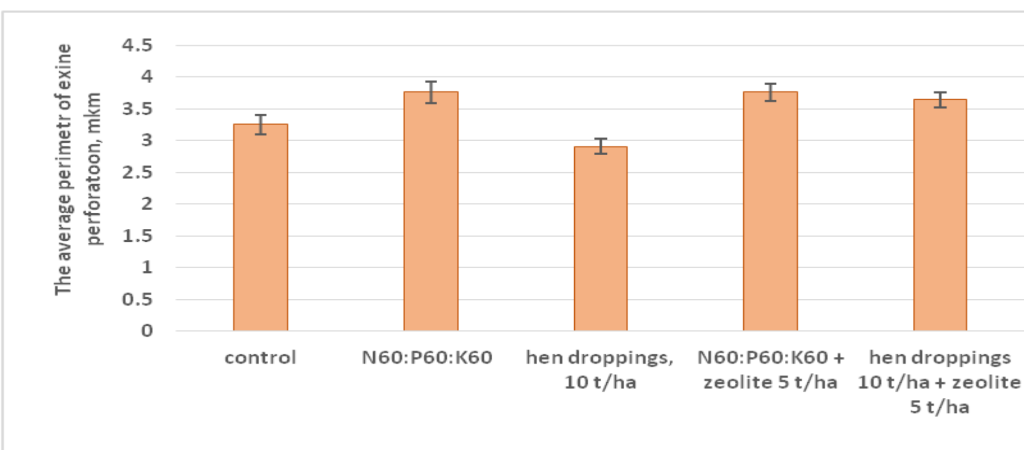

Figure 4. The perimeter of the recesses of exine of the pollen grains of *Brassica napus* L. cv. Rif; vertical lines - standard deviation (±SD) of fifty independent experiments.

molybdenum, manganese, zinc, and copper are vital trace elements for rapeseed crops (Martin *et al*., 2012; Pandey *et al*., 2018). Previous studies have also authenticated and proved that in the variants where plants were provided with trace elements, their development was more active compared with the control.

The saturation of plants with molybdenum occurred due to the introduction of a natural mineral into fertilizers, the average content of which in the mixtures was 3.04 wt% (Zubkova *et al*., 2021a, b). Molybdenum, as one of the micronutrients that plants need in very small amounts for normal growth, has also been proven to improve the growth parameters in different crop plants, such as, *Helianthus annuus* L. (Škarpa *et al*., 2013; Steiner and Zoz, 2015), *B. napus* L. (Liu *et al*., 2009), and other plants. Molybdenum deficiency is reported to have significant effects on pollen grains formation in maize, and the pollen grains were smaller and free of starch (Agarwala *et al*., 1978, 1979). Indeed, the microscopic studies revealed an increase in the size of pollen grains with the application of NPK 60:60:60 + zeolite (5 t ha^{-1}), and chicken manure (10 t ha^{-1}) + zeolite (5 t ha^{-1}). Mathematical processing revealed significant differences ($P \leq 0.05$) in all measured characteristics of pollen grains in the variant, chicken droppings (10 t ha^{-1}) + zeolite (5 t ha^{-1}).

CONCLUSIONS

Morphological characteristics of the spring rapeseed (*Brassica napus* L.) pollen grains, i.e., polar axis, equatorial diameter, shape index, the pattern of perforation of exines, and perimeter of perforation zones, were investigated in detail by using scanning electron microscopy. In the variants of rapeseed grown in control and with mineral fertilizers, the differences between these indices were not significant. However, in the variants with the use of organic fertilizer and zeolite, the changes in the morphometric parameters of pollen were significantly different. Rapeseed pollen is characterized by a high degree of perforation of the exine. The greatest influence of zeolite and chicken droppings application was recorded on the perimeter of exine perforation zones. Thus, the effects of zeolite and chicken droppings on the morphometric parameters of spring rapeseed pollen grains were established.

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