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# Combined Application of Boron and Zinc Improves Seed and Oil Yields and Oil Quality of Oilseed Rape (*Brassica napus* L.)

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**Abstract:** Oilseed crops require several micronutrients to support their physiological functions and reproductive phases. A deficiency of these nutrients can significantly reduce the yield and oil quality of oilseed crops. Soil application of micronutrients can reduce their deficiency and improve plant growth, yield, and oil quality. Oilseed rape (*Brassica napus* L.) is an important oilseed crop that produces oil with low levels of saturated fat and high levels of beneficial omega-3 fatty acids, which renders it a widely used cooking oil. However, the yield and oil quality of oilseed rape are significantly affected by the deficiency of boron (B) and zinc (Zn). This two-year field study determined the influence of sole and combined soil application of B and Zn on the physiological attributes of plants, seed and oil yields, and oil quality under semiarid climatic conditions. Nine different B and Z combinations, i.e., B0 + Zn0 (control), B0 + Zn8, B0 + Zn10, B1 + Zn0, B1 + Zn8, B1 + Zn10, B2 + Zn0, B2 + Zn8, and B2 + Zn10 (kg $ha^{-1}$), were included in the study. Sole and combined application of B and Zn significantly altered physiological attributes, seed and oil yields, and oil quality. The highest values for plant height, number of siliques per plant, number of seeds per silique, 1000-seed weight, seed and oil yields, oil quality (higher stearic acid, palmitic acid, oleic acid, linoleic acid, linolenic acid, and lower erucic acid), and physiological traits (protein concentration, soluble sugar concentration, chlorophyll concentration, photosynthesis and transpiration rates, and stomatal conductance) were recorded with the combined application of 2 + 8 kg $ha^{-1}$ B and Zn, respectively, during both years of this study. The lowest values of yield- and oil-quality-related traits and physiological attributes were recorded for the control treatment. A dose-dependent improvement was recorded in B and Zn contents in leaves, and the highest values were recorded with the combined soil application of 2 + 10 kg $ha^{-1}$ B + Zn, respectively. It can be concluded that 2 + 8 kg $ha^{-1}$ B + Zn should be applied to oilseed rape for higher seed and oil yields and better oil quality under semiarid climatic conditions.

**Keywords:** antioxidant enzymes; oil profile; seed yield; photosynthesis; semiarid climate

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## 1. Introduction

Oilseeds, alongside sugar and cereal crops, are essential components in ensuring a well-balanced and nutritious diet for humans. Vegetable oil is a significant source of essential fatty acids and vitamin E, which are vital for the proper physiological functioning of the human body [1,2]. The production of edible oil is of utmost importance in fulfilling the domestic demand and industrial requirements of a country. Additionally, it holds promising potential to emerge as a significant contributor to employment opportunities [3]. Pakistan is currently importing 2.917 million tons of edible oil, with a total value of PKR 574.199 billion (USD 3.419 billion) annually. Edible oil production in Pakistan during 2020–2021 was 0.374 million metric tons. The anticipated total yield of edible oil extracted from various crops was 3.291 million metric tons [3]. Due to a significant shortage of edible oil in Pakistan, it is imperative for the government to augment the cultivation of oilseed crops to address the edible oil shortage issue [3].

Oilseed rape (*Brassica napus* L.) is an important oilseed crop on a global scale [4]. It is positioned in third place following soybean and palm oil in terms of area under cultivation and holds fifth position in terms of oil production [5]. Oilseed rape oil exhibits superior nutritional properties due to its lower content of erucic acid and saturated fats, which are present in concentrations of 2% and 6%, respectively [6]. The seeds typically contain approximately 40–45% oil [7], with 6–14% linolenic acid and 50–66% oleic acid [8]. The oil has the required profile of saturated fatty acids (7%), higher unsaturated fatty acid contents, i.e., oleic acid (~61%) and linoleic acids (8%) [9], and lower erucic acid, glucosinolates, and cholesterol. Therefore, this oil is considered safe for human consumption [10,11]. It is cultivated on 2418 thousand hectares in Pakistan, which produce 2256 thousand tons of seeds and 374 thousand tons of oil [3]. It is an inexpensive oil that helps individuals recover from malnutrition and improves their health, making it ideal for populations residing in developing nations [12]. Climate extremes, such as heat and drought stress [13–15], soil salinity [16], limited light availability [17], and waterlogging [18], are the major constraints significantly reducing the yield and oil quality of oilseed rape.

Insufficient availability of nutrients, particularly micronutrients, is another significant constraint in oilseed rape production [19]. Micronutrients play a crucial role during the vegetative and reproductive growth of plants [20]. High-yielding cultivars produce low yields despite NPK application, which can be attributed to the insufficient utilization of micronutrients [21]. The optimal yield potential of high-yielding cultivars can be realized through the application of micronutrients in conjunction with macronutrients [22]. Chaudry et al. [23] reported that the application of micronutrients, specifically zinc (Zn) and boron (B), significantly increased wheat yield compared to the control group, whether applied individually or in combination, while Mandal et al. [24] indicated a significant correlation between the application of fertilizer and the physiological growth process.

Zinc is a vital micronutrient required for the growth and development of crop plants. It is a constituent of carbonic anhydrase and elicits aldolase, both of which are imperative for carbon metabolism [25]. Additionally, it is a constituent of diverse biomolecules, including lipids and proteins, and functions as a cofactor for auxins, thereby exerting a significant influence on nucleic acid degradation [26]. Zinc is an essential constituent of numerous enzymes and is compulsory for their activation. Consequently, Zn deficiency limits carbohydrate digestion, induces injuries to the pollen tube, and reduces yield [27]. The primary cause of Zn deficiency in crops is the reduced solubility of Zn in soils, which is somewhat more significant than the reduction in the overall amount of Zn [28]. The efficacy of Zn application in improving crop yield and quality has been reported in earlier studies [29]. Soil application of Zn to oilseed rape has been found to increase branching, the number of pods, and seed production [30]. A significant proportion (80% of rainfed area) of Pakistani soils exhibits Zn deficiency due to higher soil pH levels [31]. Zinc deficiency in the topsoil significantly hampers agricultural productivity. The decrease in Zn levels in plants induces the generation of reactive oxygen species (ROS) within plant tissues, which disrupts the integrity of cell membranes and impedes the normal functioning of cells [32].

Boron is another essential micronutrient and necessary for the growth of crop plants. However, it can be observed that B levels are rapidly decreasing in Yermosols or Aridisols [33]. Boron deficiency is the second most important micronutrient constraint in crop production after zinc [34]. Boron is an important component for various biological processes in plants, including but not limited to the growth and development of pollen tubes, the maintenance of membrane integrity, seed production, and pollination [35]. The primary roles of B include the breakdown of nucleic acids, carbohydrates, proteins, indole acetic acid, and phenol, which are involved in the synthesis of plant cell walls and the maintenance of membrane integrity [36]. Additionally, B plays a key role in cellular division and the control of carbohydrate and protein metabolism, and these processes influence the reproductive phase and development of seeds [37]. Boron deficiency in soil leads to the erratic growth of seedlings and reduced photosynthesis [38]. Furthermore, B deficiency limits root elongation and deforms flowers and fruits due to inadequate cell division in the meristematic region. Conversely, a sufficient B supply improves root development [39]. Nevertheless, an excess or deficiency of B impedes physiological and morphological functions in plants [40].

Zinc is a crucial micronutrient for human beings and plays a significant role in various biological processes, including protein, lipid, and nucleic acid metabolism and gene transcription. Zinc plays important roles in reproduction, immune response, and wound healing [41]. Therefore, Zn must be included in the diet for these processes. Boron is important for the promotion of bone health, the facilitation of hormonal equilibrium, and the provision of defense against oxidative damage and inflammation in both plant and animal organisms [42]. Although B is not essential for human health, its deficiency could negatively affect these processes. An enhancement in these micronutrient levels in daily dietary intake is particularly important in rural areas of developing nations, where populations are already at risk of micronutrient deficiencies [43]. Hence, the introduction of fortified foods with micronutrients is necessary for these areas [44]. Enhancing the bioavailability of micronutrients in crucial food grains through biofortification could be a promising strategy to cope with micronutrient deficiency. The integration of micronutrient-efficient cultivars and appropriate agronomic techniques presents a promising opportunity to enhance the yield and quality of arable crops [45,46]. Nonetheless, the implementation of agronomic strategies is comparatively more rapid and dependable in contrast to genetic biofortification [21,47]. The implementation of biofortification techniques in oilseed crops, specifically targeting the incorporation of nutritionally significant elements such as B and Zn, has emerged as a promising strategy to address the issue of micronutrient deficiency in developing nations [48]. Agronomic biofortification of micronutrients can be achieved through three primary approaches, i.e., seed priming, foliar, and soil application. These methods are characterized by their rapid nutrient delivery, economic feasibility, and ease of implementation [21,47]. Soil application of micronutrients is a widely employed agronomic technique to enhance both the yield and nutritional quality of food crops [33,49]. Nevertheless, the excessive application of micronutrients can lead to crop toxicity by disrupting the equilibrium between soil solution and adsorption sites [50,51]. Usman and Mohamed [52] observed that the application of Zn, either alone or in combination with B elevated Zn levels in plants. An increased Zn concentration leads to higher flowering and a reduction in fruit drop [53].

High-yielding cultivars of oilseed rape have been introduced in Pakistan to improve the production of edible oil in the country. However, there is a significant gap between the potential yield of these cultivars and the actual yield. Numerous studies have investigated the impact of B and Zn on crop yields, either individually or in combination. However, the potential synergistic or antagonistic effects of soil-applied B and Zn (either alone or in combination) on the growth and yield of oilseed rape have never been studied. Additionally, the underlying physiological mechanisms have not been explored in the semiarid environments characterized by calcareous soils. Hence, the current study investigated the impacts of soil-applied B and Zn (alone or in combination) on the growth, physiological, and biochemical attributes; seed and oil yields; and oil quality of oilseed rape. It was hypothesized that the growth, physiological, and biochemical attributes; seed and oil yields; and oil quality of oilseed rape would be significantly altered by the soil application of B and Zn. It was further hypothesized that the combined application of B and Zn would improve the growth, physiological, and biochemical attributes; seed and oil yields; and oil quality of oilseed rape compared to their individual application. The results of this study would help to improve the seed and oil yields and oil quality of oilseed rape in semiarid environments characterized by calcareous soils.

## 2. Materials and Methods

### 2.1. Experimental Site

The experiment was carried out at the Agronomic Farm, University of Sargodha, Pakistan (32.08° N, 72.67° E, 193 m asl), for two consecutive seasons, i.e., 2020–2021 and 2021–2022. The experimental site is characterized by a subtropical semiarid climate, with an average annual precipitation of 400 mm. The average minimum temperature observed during the coldest month is 14.3 °C, whereas the average minimum temperature during the warmest month is 39.2 °C. Physicochemical analysis of the experimental soil was conducted for two consecutive years, and the results are presented in Table 1. The experimental soil had a higher soil pH and was deficient in boron and zinc, both of which limit the growth and productivity of oilseed rape.

**Table 1.** Physiochemical properties of experimental soil during 2020–2021 and 2021–2022.

| Soil Properties | Values | | Analytical Method and Reference |
|---|---|---|---|
| | 2020–2021 | 2021–2022 | |
| Physical composition [54] | | | |
| Sand (g kg$^{-1}$) | 470 ± 3.2 | 468 ± 3.3 | Bouyoucos hydrometer method [54] |
| Silt (g kg$^{-1}$) | 239 ± 2.4 | 239 ± 2.2 | |
| Clay (g kg$^{-1}$) | 289 ± 1.5 | 289 ± 1.4 | |
| Textural class | Loam–clay loam | | |
| Chemical composition | | | |
| Saturation % | 40.22 ± 1.18 | 40.72 ± 1.15 | [55] |
| pH | 7.6 ± 0.04 | 7.7 ± 0.02 | [56] |
| ECe (µS cm$^{-1}$) | 15.42 ± 22.2 | 16.82 ± 28.76 | [56] |
| Soil organic matter (g kg$^{-1}$) | 7.40 ± 0.61 | 7.40 ± 0.32 | Walkley and Black method [57] |
| Total soil N (mg kg$^{-1}$) | 4.09 ± 8.14 | 4.14 ± 7.32 | Modified Kjeldahl method [58] |
| Extractable P (mg kg$^{-1}$ soil) | 7.39 ± 0.11 | 7.73 ± 0.31 | Olsen's method [59] |
| Available potassium (mg kg$^{-1}$) | 271 ± 12.12 | 273 ± 11.14 | Flame photometric method [60] |
| Hot-water-soluble boron (mg kg$^{-1}$) | 0.33 ± 0.08 | 0.37 ± 0.12 | Hot water extraction [61] |
| DTPA-extractable zinc (mg kg$^{-1}$) | 0.46 ± 0.11 | 0.51 ± 0.14 | DTPA soil test [62] |

The values presented are means (n = 4) ± standard errors of the means.

### 2.2. Experimental Details and Crop Husbandry

Experimental field was irrigated prior to seedbed preparation, and fine seedbed was prepared once soil reached an appropriate moisture level for tillage practices. Two with moldboard plough, followed by two cultivations utilizing a narrow tine cultivator were performed. Finally, two plankings were conducted to prepare the fine seedbed. The seeds of oilseed rape cultivar 'G 97' were procured from a local market, treated with fungicide ((thiophenate methyl) 2.5 g per kg seed), and sown using a manual drill at a depth of 2 cm by keeping seed rate at 2.5 kg $ha^{-1}$. The rows and plants were maintained at 0.45 m and 0.1 m distances, respectively. Sowing was conducted during the final week of October each year. Thinning was carried out at the 2 to 4 true leaf stage, approximately 3 to 4 weeks after sowing for maintaining plant-to-plant distance. Nine different combinations of B and Zn, i.e., B0 + Zn0 (control), B0 + Zn8, B0 + Zn10, B1 + Zn0, B1 + Zn8, B1 + Zn10, B2 + Zn0, B2 + Zn8, and B2 + Zn10 (kg $ha^{-1}$), were included in this study.

The experiment was conducted according to randomized complete block design with four replications, and a net plot size of 4 m $\times$ 2.25 m. The first irrigation was applied 30 days after sowing. Subsequent irrigations were applied at flowering, silique formation, and seed formation. Tensiometer (Model RM 627) was utilized to maintain plant available soil moisture at 70% during the study.

Granular zinc sulfate (33%) and boric acid (17.5%) were used as sources of Zn and B, respectively. The calculated amounts of B and Zn according to the treatments were applied at the time of sowing. Nitrogen (N), phosphorus (P), and potassium (K) were applied at a rate of 23, 23, and 12 kg $ha^{-1}$, respectively, utilizing urea, diammonium phosphate, and sulfate of potash as the sources. The entire amount of K and P and one-third N were applied during crop sowing. The remaining N was applied in three splits at flowering, silique development, and seed formation.

Weeding was carried out twice to manage the weed species. Manual weeding was conducted twice within a period of 2 to 5 weeks after sowing, prior to the closure of the crop canopy. All cultural and management practices were consistently implemented in accordance with the crop requirements across all plots. The siliques were manually detached from the plants at maturity and subsequently threshed in mid-April.

### *2.3. Data Collection*

Plant height, yield, and yield-related traits; B and Zn contents in leaves; seed and oil yields; oil quality traits; and physiological and biochemical traits were recorded according to standard procedures described below.

### *2.4. Plant Height and Yield-Related Traits*

The heights of ten mature randomly selected plants in each treatment were measured using a meter rod. Yield components, i.e., number of siliques per plant, number of seeds per silique, and 1000-seed weight, were recorded at maturity. The siliques were manually separated from the plants, counted, and subsequently subjected to manual threshing to record number of seeds per silique. The weight of 1000 seeds was measured using an analytical balance (Model Number HC2204). The seed yield was measured by harvesting and threshing all plants in each experimental yield. The seeds obtained from each plot after threshing were weighed on electronic balance (PL 3200+ L Japan), and the weight per plot was subsequently converted into tons per hectare using unitary method.

### *2.5. B and Zn Contents in Leaves*

The leaves were collected, rinsed with distilled water, and dried in an oven at 65 °C for 48 h to determine B and Zn concentrations. The leaves were ground using an electric grinder. Boron was determined by converting the leaves into ash, utilizing a muffle furnace operating at 550 °C for 6 h. The resulting ash was treated with 0.36 N $H_2SO_4$. Azomethine-H method facilitated the determination of B concentration on a spectrophotometer at 420 nm [63]. Leaf powder (0.5 g) was digested in a mixture of $HNO_3$ and $HClO_4$ with a ratio of 2:1 for 6 h to determine Zn contents [64]. The distilled water was used to bring the volume of the mixture to 25 mL. The concentration of Zn was recorded by utilizing atomic absorption spectroscopy (AA-6300, Shimadzu, Japan).

*2.6. Estimation of Oil Yield and Oil Quality Traits*

The seeds were oven-dried at 45 °C for 24 h and ground to powder. Oil extraction was conducted using 3.5 g seed powder. The excess fat was removed from the samples by using Soxhlet apparatus maintained at 60 °C for 8 h. Each specimen required 180 g petroleum ether solution. Afterward, seed specimens were subjected to a thermal treatment of 50 °C for 24 h to extract the oil. Oil yield was computed through the multiplication of the seed yield by the oil content. The fatty acid composition, encompassing palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linolenic acid (C18:3), linoleic acid (C18:2), and erucic acid (C22:1), was determined by using gas chromatography of methyl esters [65].

*2.7. Determination of Protein and Soluble Sugar Contents*

Leaf soluble protein and total soluble sugars (TSSs) were quantified by using fresh leaf extract (0.1 g) in potassium phosphate buffer (50 mM and a pH value of 7.5). The extract was centrifuged at 15,500 rpm (25,155 relative centrifugal force (RCF) or g force) for 15 min at 4 °C after filtering through four layers of cheese cloth. Supernatant was collected and kept at 4 °C. Bradford [66] protein dye-binding technique was used to determine the amount of leaf soluble protein using bovine serum albumin as the reference protein. TSSs were analyzed in a Cecil CE 2021 spectrophotometer using an anthrone reagent [67].

*2.8. Estimation of Chlorophyll Contents, Photosynthesis, Transpiration Rate, and Stomatal Conductance*

Chlorophyll contents were determined from the fully flourishing third younger leaf by using chlorophyll meter (Model, SPAD-502: Konica Minolta Sensing: Inc., Osaka, Japan) [68]. Four young leaves were randomly chosen 30 days after sowing and sequentially placed in an infrared gas analyzer (IRGA). Stomatal conductance, photosynthesis, and transpiration rates were measured between 11:00 and 12:00 a.m. The IRGA chamber was programmed to take readings under the conditions specified by Zekri [69] and Moya et al. [70]. The conditions were 403.3 mmol $m^{-2}$ $S^{-1}$ molar flow rate, 99.90 KPa atmospheric pressure, 6.0 to 8.9 millibar vapor pressure, 1711 mol $m^{-2}$ $S^{-1}$ photosynthetically active radiation, leaf temperature of 28.40 to 32.40 °C, ambient temperature of 22.40 to 27.90 °C, and ambient $CO_2$ concentration of 352 mol $mol^{-1}$ [71].

*2.9. Antioxidant Enzymes*

2.9.1. Extraction

The enzyme extraction procedure was conducted at 4 °C using 200 mg leaf samples. The samples were homogenized in a pre-chilled mortar and pestle using 3 mL of ice-cold 50 mM sodium phosphate buffer (pH 7.0), 0.1 mM EDTA, and 1% *w*/*v* polyvinyl pyrrolidone (PVP). The mixture was centrifuged at 15,000 rpm (25,155 RCF or g force) for 20 min at 4 °C. The supernatant was utilized as a raw enzyme extract. The enzyme assays were conducted under ambient conditions, and the enzymatic activity was quantified using a spectrophotometer.

2.9.2. Peroxidase (POD) Activity

A procedure reported by Ullah et al. [72] was slightly modified to determine peroxidase (POD) activity. The 1 mL reaction mixture contained 40 mM phosphate buffer, 15 mM guaiacol, and 5 mM $H_2O_2$ (pH 6.8). After the reaction mixture settled, reaction was initiated by adding $H_2O_2$, and the increase in absorbance at 470 nm was measured for 1 min. The POD activity was measured in accordance with its 25 $mM^{-1}$ $cm^{-1}$ extinction coefficient.

2.9.3. Superoxide Dismutase (SOD) Activity

The SOD activity was determined by inhibiting the photochemical reduction of nitroblue tetrazolium (NBT) [73]. A superoxide-generating system of 14.3 mM methionine, 82.5 mM NBT, and 2.2 mM riboflavin was added to the reaction mixture (3 mL) of 50 mM phosphate buffer (pH 7.8) and 0.1 mM EDTA. The reaction was initiated by adding 100 cc of unprocessed enzyme. The free-radical-induced NBT reduction was tested in a reaction medium containing all the components except the enzyme. Six 15W fluorescent bulbs were used as the light source, and the tubes were kept there for 30 min. Turning off the light halted the reaction. The entire mixture of reactants was incubated in the dark as a dark blank, along with 100 mL of enzyme extract. The reduction in NBT was evaluated by monitoring the shift in absorbance at 560 nm. To calculate enzyme units, measurements from the dark blank were used. One unit of SOD was defined as the quantity of the enzyme that resulted in a 50% inhibition of NBT reduction under the test conditions. The unit of measurement for enzyme activity was $mg^{-1}$ protein.

### 2.10. Data Analysis

The collected data of all the recorded traits were analyzed by using one-way analysis of variance (ANOVA). The normality was tested by Shapiro–Wilk normality test, which indicated a normal distribution. Therefore, statistical analysis was performed on original data. The differences among years were analyzed by *t*-test, which were significant. Therefore, data from each year were analyzed, presented, and interpreted separately. SAS software (Version 9.1; SAS Institute, Cary, NC, USA) was used for statistical analysis. Least significant difference post hoc test at 95% probability was used to separate treatment means where ANOVA denoted significant differences [74]. SigmaPlot was used for the graphical presentation of the data (SigmaPlot 2008).

## 3. Results

### 3.1. Growth, Yield, and Boron and Zinc Contents

Growth, yield, yield-related attributes, and B and Zn contents in leaves were significantly ($p < 0.05$) affected by sole and combined application of B and Zn during 2020–2021 and 2021–2022 (Tables 2 and 3). Taller plants (10 and 13% during 2020–2021 and 2021–2022, respectively) were recorded with B2 + Zn8 treatment compared to control treatments (Table 2). Nevertheless, B1 + Zn8, B1 + Zn10, and B2 + Zn0 treatments were on par with B2 + Zn8 during first year. The lowest plant height was noted for the control treatment during both years of this study (Table 2).

**Table 2.** The impact of soil-applied boron and zinc on plant height, number of siliques per plant, number of seeds per silique, and 1000-seed weight of oilseed rape grown under field conditions during 2020–2021 and 2021–2022.

| Treatments | Plant Height (cm) | | Number of Siliques Plant$^{-1}$ | | Number of Seeds Silique$^{-1}$ | | 1000-Seed Weight (g) | |
|---|---|---|---|---|---|---|---|---|
| | 2020–2021 | 2021–2022 | 2020–2021 | 2021–2022 | 2020–2021 | 2021–2022 | 2020–2021 | 2021–2022 |
| Control | 122.20 ± 2.46 E | 117.97 ± 2.21 F | 200.21 ± 2.60 F | 194.63 ± 4.21 G | 13.02 ± 1.04 E | 10.94 ± 0.92 E | 2.63 ± 0.09 C | 2.44 ± 0.11 E |
| B0 + Zn8 | 125.67 ± 0.87 DE | 126.17 ± 1.40 E | 260.74 ± 2.89 AB | 253.41 ± 9.42 B | 15.14 ± 0.48 CD | 15.23 ± 0.58 C | 2.86 ± 0.15 BC | 2.64 ± 0.09 D |
| B0 + Zn10 | 127.43 ± 1.59 CD | 128.37 ± 1.78 CD | 204.94 ± 2.08 E | 198.45 ± 3.02 F | 15.21 ± 0.50 CD | 15.07 ± 0.66 C | 3.26 ± 0.41 AB | 2.84 ± 0.11 BC |
| B1 + Zn0 | 129.13 ± 1.30 BCD | 124.77 ± 1.45 E | 237.45 ± 4.16 CD | 243.78 ± 5.23 C | 15.33 ± 0.69 CD | 15.04 ± 0.71 C | 3.23 ± 0.38 AB | 2.94 ± 0.08 B |
| B1 + Zn8 | 132.20 ± 2.04 AB | 126.57 ± 1.15 DE | 249.14 ± 7.37 BC | 244.15 ± 4.41 C | 16.22 ± 0.71 BC | 16.15 ± 1.02 BC | 3.03 ± 0.31 ABC | 2.64 ± 0.09 D |
| B1 + Zn10 | 131.15 ± 1.01 ABC | 124.97 ± 1.49 E | 230.47 ± 1.15 D | 230.32 ± 8.78 D | 18.21 ± 0.50 B | 17.33 ± 0.48 B | 2.93 ± 0.45 ABC | 2.74 ± 0.15 CD |
| B2 + Zn0 | 132.40 ± 1.16 AB | 132.57 ± 2.35 B | 232.97 ± 6.36 CD | 218.95 ± 7.86 E | 13.44 ± 0.64 E | 11.47 ± 0.71 DE | 3.00 ± 0.32 ABC | 2.84 ± 0.12 BC |
| B2 + Zn8 | 135.60 ± 1.33 A | 134.97 ± 2.12 A | 268.73 ± 11.18 A | 274.32 ± 10.31 A | 21.45 ± 0.66 A | 21.63 ± 1.95 A | 3.30 ± 0.12 A | 3.34 ± 0.11 A |
| B2 + Zn10 | 129.60 ± 1.89 BCD | 129.97 ± 1.90 C | 229.44 ± 6.69 D | 241.96 ± 4.54 C | 14.94 ± 0.82 DE | 12.86 ± 0.55 D | 3.10 ± 0.25 AB | 2.74 ± 0.13 CD |
| $LSD_{0.05}$ | 4.72 | 2.02 | 17.20 | 3.43 | 2.18 | 1.71 | 0.43 | 0.10 |

The values following B and Zn indicate the amount of applied B and Zn kg $ha^{-1}$. The values are means ± standard errors. Means followed by different letters within a column are significantly different ($p \leq 0.05$) from each other.

**Table 3.** The impact of soil-applied boron and zinc on seed yield and boron and zinc contents in leaves of oilseed rape grown under field conditions during 2020–2021 and 2021–2022.

| Treatments | Seed Yield (t ha$^{-1}$) | | B Content in Leaves (mg kg$^{-1}$) | | Zn Content in Leaves (mg kg$^{-1}$) | |
|---|---|---|---|---|---|---|
| | 2020–2021 | 2021–2022 | 2020–2021 | 2021–2022 | 2020–2021 | 2021–2022 |
| Control | 1.82 ± 0.05 D | 1.79 ± 0.11 D | 10.25 ± 0.09 G | 10.23 ± 0.21 H | 27.46 ± 0.87 I | 27.42 ± 1.14 I |
| B0 + Zn8 | 2.01 ± 0.06 CD | 1.79 ± 0.10 D | 11.13 ± 0.14 F | 11.09 ± 0.22 G | 32.13 ± 1.02 F | 32.09 ± 0.98 F |
| B0 + Zn10 | 2.13 ± 0.13 BC | 1.99 ± 0.09 BC | 11.37 ± 0.17 E | 11.33 ± 0.15 F | 34.18 ± 0.55 D | 34.16 ± 0.55 D |
| B1 + Zn0 | 1.85 ± 0.09 D | 1.73 ± 0.15 D | 14.12 ± 0.15 D | 14.08 ± 0.15 E | 29.66 ± 0.45 H | 29.63 ± 0.34 H |
| B1 + Zn8 | 2.02 ± 0.17 CD | 2.01 ± 0.08 BC | 14.24 ± 0.17 D | 14.21 ± 0.18 E | 33.53 ± 0.25 E | 33.51 ± 0.52 E |
| B1 + Zn10 | 2.07 ± 0.19 BC | 1.92 ± 0.19 CD | 14.57 ± 0.22 C | 14.53 ± 0.25 D | 35.58 ± 1.22 C | 35.56 ± 0.44 C |
| B2 + Zn0 | 2.26 ± 0.23 B | 2.17 ± 0.12 B | 17.09 ± 0.14 B | 17.04 ± 0.10 C | 30.13 ± 0.48 G | 30.08 ± 0.38 G |
| B2 + Zn8 | 2.88 ± 0.24 A | 2.44 ± 0.14 A | 17.22 ± 0.13 B | 17.19 ± 0.23 B | 37.46 ± 1.21 B | 37.43 ± 1.14 B |
| B2 + Zn10 | 2.10 ± 0.17 BC | 2.03 ± 0.15 C | 17.46 ± 0.11 A | 17.42 ± 0.11 A | 39.42 ± 1.15 A | 39.39 ± 1.11 A |
| $LSD_{0.05}$ | 0.20 | 0.19 | 0.13 | 0.10 | 0.11 | 0.12 |

The values following B and Zn indicate the amount of applied B and Zn kg ha$^{-1}$. The values are means ± standard errors. Means followed by different letters within a column are significantly different ($p \leq 0.05$) from each other.

Soil application of B2 + Zn8 improved the number of siliques per plant by 25 and 29% during 2020–2021 and 2021–2022, respectively, compared to the control treatment. The B0 + Zn8 treatment followed B2 + Zn8 for the number of siliques per plant, while the control treatment resulted in the lowest number of siliques per plant during both years. The highest and lowest number of seeds per silique were recorded for the B2 + Zn8 and control treatments, respectively. The B2 + Zn8 treatment improved the number of seeds per silique by 38% and 52% compared to the control during 2020–2021 and 2021–2022, respectively (Table 2). Similarly, B1 + Zn10 followed B2 + Zn8 for the number of seeds per silique during both years. The highest and lowest 1000-seed weight and grain yields were observed for the B2 + Zn8 and control treatments, respectively, during both years of this study. Soil application of B2 + Zn8 improved 1000-seed weight by 20% and 27% during 2020–2021 and 2021–2022, respectively, compared to the control treatment (Table 2). The seed yield of plants fertilized with B2 + Zn8 was improved by 37% and 27% during 2020–2021 and 2021–2022, respectively, compared to the control (Table 3).

The B and Zn contents in the leaves were linearly increased with their application doses. The highest and lowest B and Zn contents in the leaves were noted for the B2 + Zn10 and control treatments, respectively, during both years (Table 3). Soil application of B2 + Zn8 resulted in the second-highest values for B and Zn contents in leaves during both years of this study (Table 3).

### *3.2. Oil Yield and Quality*

Combined and sole application of B and Zn significantly improved oil yield and quality during both years of study (Tables 4 and 5). The highest and lowest values for oil yield, stearic acid, palmitic acid, and oleic acid were recorded for the B2 + Zn8 and control treatments, respectively, during both years of the study (Table 4).

Soil application of B2 + Zn8 resulted in the highest (54% and 53% higher than control treatment during 2020–2021 and 2021–2022, respectively) oil yield during both years (Table 4). Similarly, stearic acid was improved by 31% and 30%, palmitic acid by 22% and 21%, oleic acid by 33% and 34%, linoleic acid by 26% and 24%, and linolenic acid by 38% and 40% with soil-applied B2 + Zn8 during 2020–2021 and 2021–2022, respectively, compared to the control treatment (Table 5). The B2 + Zn10 and B2 + Zn0 treatments followed B2 + Zn8 for linoleic acid and linolenic acid during both years of this study. However, erucic acid was significantly ($p \leq 0.05$) reduced by 62% and 63% with the application of B2 + Zn8 during 2020–2021 and 2021–2022, respectively (Table 5).

**Table 4.** The impact of soil-applied boron and zinc on oil yield, stearic acid, palmitic acid, and oleic acid in the oil of oilseed rape grown under field conditions during 2020–2021 and 2021–2022.

| Treatments | Oil Yield (t ha$^{-1}$) | | Stearic Acid (mg g$^{-1}$) | | Palmitic Acid (mg g$^{-1}$) | | Oleic Acid (mg g$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|
| | 2020–2021 | 2021–2022 | 2020–2021 | 2021–2022 | 2020–2021 | 2021–2022 | 2020–2021 | 2021–2022 |
| Control | 0.54 ± 0.07 D | 0.55 ± 0.02 H | 4.53 ± 0.45 G | 4.51 ± 0.32 G | 12.24 ± 0.67 H | 12.23 ± 0.91 H | 152.93 ± 5.78 E | 150.93 ± 15.65 E |
| B0 + Zn8 | 0.67 ± 0.06 C | 0.58 ± 0.02 G | 5.11 ± 0.42 F | 5.09 ± 0.39 F | 13.46 ± 0.30 G | 13.43 ± 0.32 G | 181.88 ± 4.58 D | 179.88 ± 11.45 D |
| B0 + Zn10 | 0.76 ± 0.09 B | 0.69 ± 0.07 E | 5.24 ± 0.38 F | 5.22 ± 0.44 F | 13.83 ± 0.19 F | 13.81 ± 0.14 F | 184.08 ± 5.66 D | 181.86 ± 12.60 D |
| B1 + Zn0 | 0.72 ± 0.07 BC | 0.78 ± 0.11 B | 5.76 ± 0.31 E | 5.74 ± 0.22 E | 14.43 ± 0.21 D | 14.41 ± 0.10 D | 195.09 ± 11.23 CD | 193.09 ± 10.91 CD |
| B1 + Zn8 | 0.77 ± 0.09 B | 0.79 ± 0.12 B | 5.92 ± 0.23 DE | 5.89 ± 0.24 DE | 14.66 ± 0.29 C | 14.63 ± 0.09 C | 196.75 ± 13.61 CD | 195.75 ± 9.87 CD |
| B1 + Zn10 | 0.66 ± 0.05 C | 0.64 ± 0.04 F | 6.13 ± 0.15 C | 6.11 ± 0.14 C | 14.13 ± 0.14 E | 14.11 ± 0.15 E | 191.86 ± 9.90 CD | 190.86 ± 11.56 CD |
| B2 + Zn0 | 0.77 ± 0.04 B | 0.76 ± 0.02 C | 6.33 ± 0.19 B | 6.31 ± 0.18 B | 15.48 ± 0.16 A | 15.46 ± 0.22 A | 215.15 ± 12.23 AB | 213.15 ± 14.56AB |
| B2 + Zn8 | 1.18 ± 0.08 A | 1.19 ± 0.07 A | 6.54 ± 0.17 A | 6.52 ± 0.20 A | 15.63 ± 0.17 A | 15.61 ± 0.32 A | 229.24 ± 10.65 A | 227.24 ± 10.71 A |
| B2 + Zn10 | 0.73 ± 0.07 BC | 0.72 ± 0.02 D | 6.03 ± 0.14 CD | 6.02 ± 0.12 CD | 15.15 ± 0.23 B | 15.13 ± 0.18 B | 203.04 ± 13.67 BC | 201.04 ± 12.44 BC |
| $LSD_{0.05}$ | 0.07 | 0.01 | 0.19 | 0.20 | 0.17 | 0.19 | 16.45 | 17.40 |

The values following B and Zn indicate the amount of applied B and Zn kg ha$^{-1}$. The values are means ± standard errors. Means followed by different letters within a column are significantly different ($p \leq 0.05$) from each other.

**Table 5.** The impact of soil-applied boron and zinc on linoleic acid, linolenic acid, and erucic acid contents in the oil of oilseed rape crop grown under field conditions during 2020–2021 and 2021–2022.

| Treatments | Linoleic Acid (mg g$^{-1}$) | | Linolenic Acid (mg g$^{-1}$) | | Erucic Acid (mg g$^{-1}$) | |
|---|---|---|---|---|---|---|
| | 2020–2021 | 2021–2022 | 2020–2021 | 2021–2022 | 2020–2021 | 2021–2022 |
| Control | 40.64 ± 2.24 G | 39.62 ± 3.12 F | 16.33 ± 3.35 D | 15.33 ± 2.12 D | 0.87 ± 0.03 A | 0.85 ± 0.08 A |
| B0 + Zn8 | 45.39 ± 1.12 F | 44.37 ± 1.01 E | 22.36 ± 1.12 C | 21.36 ± 1.06 C | 0.74 ± 0.14 ABC | 0.72 ± 0.10 AB |
| B0 + Zn10 | 46.50 ± 1.15 EF | 45.50 ± 1.90 DE | 22.77 ± 1.07 C | 22.71 ± 2.21 BC | 0.82 ± 0.11 AB | 0.80 ± 0.06 A |
| B1 + Zn0 | 49.51 ± 1.05 CD | 47.51 ± 2.12 CD | 23.38 ± 0.45 C | 22.38 ± 2.09 BC | 0.64 ± 0.15 BCD | 0.63 ± 0.13 BC |
| B1 + Zn8 | 50.39 ± 2.12 BCD | 49.39 ± 2.02 BC | 24.63 ± 2.45 ABC | 23.63 ± 2.11 ABC | 0.53 ± 0.13 CD | 0.51 ± 0.11 C |
| B1 + Zn10 | 48.29 ± 1.98 DE | 46.29 ± 2.33 DE | 23.06 ± 0.34C | 22.06 ± 0.91 C | 0.69 ± 0.11 BCD | 0.67 ± 0.07 BC |
| B2 + Zn0 | 52.39 ± 2.45 AB | 51.39 ± 2.11 AB | 26.33 ± 1.50 A | 25.33 ± 1.05 A | 0.49 ± 0.02 E | 0.47 ± 0.03 D |
| B2 + Zn8 | 54.75 ± 1.65 A | 52.75 ± 1.01 A | 26.69 ± 1.91 A | 25.69 ± 1.11 A | 0.33 ± 0.10 E | 0.31 ± 0.07 D |
| B2 + Zn10 | 51.83 ± 1.16 BC | 50.83 ± 0.94 AB | 25.63 ± 1.08 AB | 24.63 ± 1.19 AB | 0.40 ± 0.09 E | 0.38 ± 0.08 D |
| $LSD_{0.05}$ | 2.49 | 2.52 | 2.53 | 2.51 | 0.22 | 0.24 |

The values following B and Zn indicate the amount of applied B and Zn kg ha$^{-1}$. The values are means ± standard errors. Means followed by different letters within a column are significantly different ($p \leq 0.05$) from each other.

### *3.3. Physiological Parameters*

Sole and combined application of B and Zn significantly altered protein, soluble sugar, and chlorophyll concentrations. Protein, soluble sugar, and chlorophyll concentrations in leaves were significantly increased under the combined application of B and Zn. The highest values of these traits were recorded with the application of B2 + Zn8 during both study years, while the control treatment resulted in the lowest values (Table 6).

**Table 6.** The impact of soil-applied boron and zinc on protein concentration, soluble sugar concentration, chlorophyll concentration, and photosynthesis rate of oilseed rape grown under field conditions during 2020–2021 and 2021–2022.

| Treatments | Protein Concentration ($mg\ g^{-1}$ FW) | | Soluble Sugar Concentration ($mg\ g^{-1}$ FW) | | Chlorophyll Concentration (SPAD Value) | | Photosynthesis Rate ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$) | |
|---|---|---|---|---|---|---|---|---|
| | 2020–2021 | 2021–2022 | 2020–2021 | 2021–2022 | 2020–2021 | 2021–2022 | 2020–2021 | 2021–2022 |
| Control | 20.19 ± 0.53 F | 20.17 ± 0.90 F | 20.19 ± 1.03 F | 20.17 ± 0.88 F | 34.65 ± 1.41 G | 34.61 ± 1.12 G | 8.51 ± 0.31 C | 8.49 ± 0.78 C |
| B0 + Zn8 | 21.62 ± 0.88 E | 21.59 ± 0.67 E | 21.62 ± 0.43 E | 21.59 ± 0.41 E | 36.58 ± 1.14 F | 36.55 ± 1.64 F | 9.48 ± 1.03 BC | 9.46 ± 0.98 BC |
| B0 + Zn10 | 21.86 ± 0.73 E | 21.83 ± 0.55 E | 21.86 ± 0.33 E | 21.83 ± 0.52 E | 38.92 ± 1.01 DE | 38.88 ± 1.21 DE | 9.88 ± 0.90 BC | 9.84 ± 0.78 BC |
| B1 + Zn0 | 22.36 ± 0.21 CD | 22.33 ± 0.15 CD | 22.36 ± 0.19 CD | 22.33 ± 0.19 CD | 39.60 ± 1.71 DE | 39.58 ± 1.49 DE | 10.18 ± 0.23 B | 10.15 ± 0.31 B |
| B1 + Zn8 | 22.51 ± 0.18 C | 22.49 ± 0.11 C | 22.51 ± 0.17 C | 22.49 ± 0.24 C | 40.37 ± 1.14 D | 40.34 ± 1.12 D | 10.62 ± 0.45 B | 10.60 ± 0.41 B |
| B1 + Zn10 | 22.20 ± 0.61 D | 22.18 ± 0.13 D | 22.20 ± 0.24 D | 22.18 ± 0.19 D | 38.25 ± 2.31 EF | 38.21 ± 1.01 DE | 9.64 ± 0.97 BC | 9.62 ± 0.89 BC |
| B2 + Zn0 | 24.10 ± 0.22 AB | 24.07 ± 0.22 AB | 24.10 ± 0.25 AB | 24.07 ± 0.22 AB | 45.36 ± 1.43 B | 45.33 ± 1.93 B | 13.62 ± 1.12 A | 13.58 ± 1.45 A |
| B2 + Zn8 | 24.16 ± 0.11 A | 24.13 ± 0.09 A | 24.16 ± 0.17 A | 24.13 ± 0.11 A | 48.25 ± 2.12 A | 48.20 ± 1.10 A | 14.18 ± 0.94 A | 14.16 ± 1.33 A |
| B2 + Zn10 | 23.89 ± 0.20 B | 23.86 ± 0.11 B | 23.89 ± 0.20 B | 23.86 ± 0.08 B | 43.27 ± 1.78 C | 43.25 ± 1.22 C | 12.84 ± 1.78 A | 12.81 ± 1.83 A |
| $LSD_{0.05}$ | 0.26 | 0.27 | 0.26 | 0.27 | 1.81 | 1.80 | 1.75 | 1.73 |

The values following B and Zn indicate the amount of applied B and Zn kg $ha^{-1}$. The values are means ± standard errors. Means followed by different letters within a column are significantly different ($p \leq 0.05$) from each other.

Soil applied B at 2 kg $ha^{-1}$ alone or in combination with Zn significantly improved the photosynthesis rate. The highest increase in the photosynthesis rate was noted with B2 + Zn8 during both years compared to the control treatment (Table 6). Stomatal conductance and transpiration rate were significantly improved by 91% and 93% and 48% and 49% with B2 + Zn8 compared to the control during 2020–2021 and 2021–2022, respectively (Figures 1 and 2).

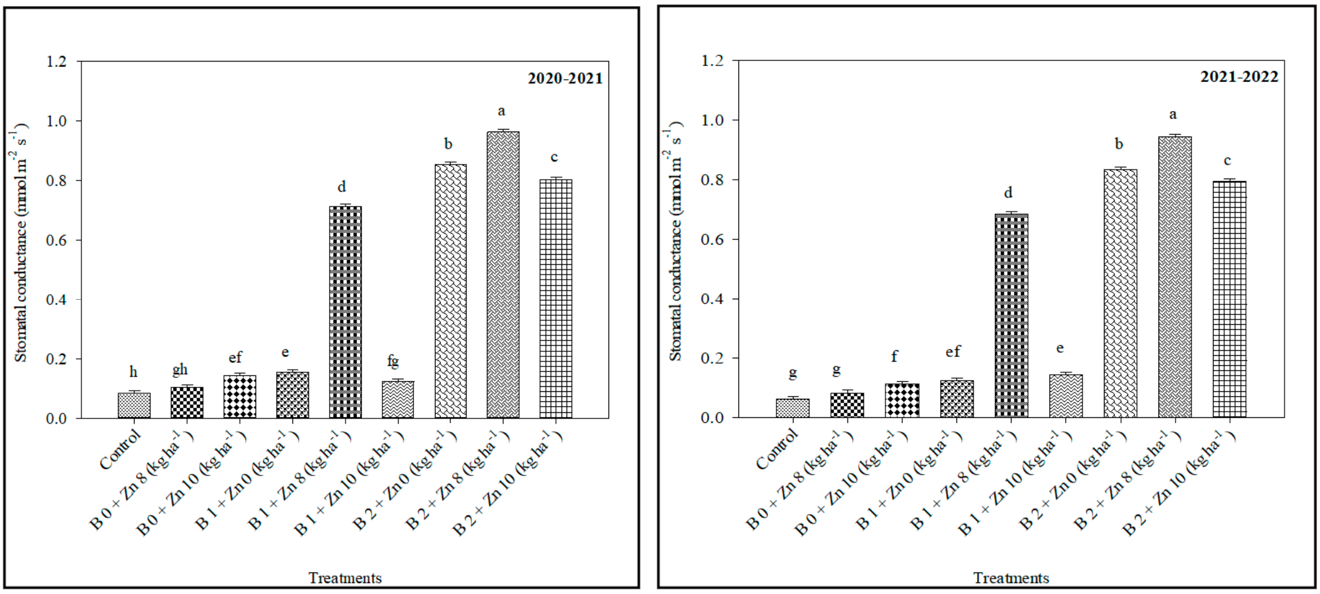


**Figure 1.** The influence of sole and combined application of boron and zinc on stomatal conductance of oilseed rape plants grown under field conditions during 2020–2021 and 2021–2022. The error bars are standard errors of the means (n = 4). Different letters on the bars indicate significant differences among treatment means ($p \leq 0.05$).

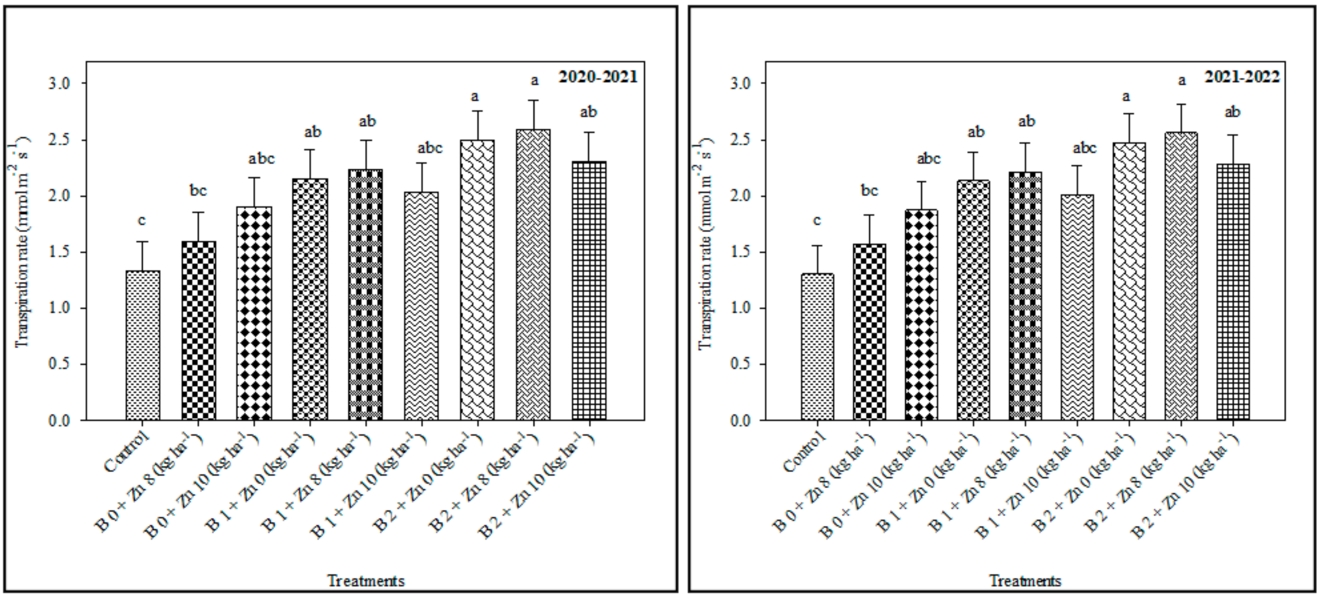


**Figure 2.** The influence of sole and combined application of boron and zinc on transpiration rate of oilseed rape plants grown under field conditions during 2020–2021 and 2021–2022. The error bars are standard errors of the means (n = 4). Different letters on the bars indicate significant differences among treatment means ($p \leq 0.05$).

### *3.4. Antioxidant Enzyme Activities*

Boron and zinc deficiency caused significant oxidative stress in plants, leading to higher activities of POD and SOD enzymes (Figures 3 and 4). The highest and lowest activities of POD and SOD were noted with the control and B2 + Zn8 treatments, respectively. The activities of both enzymes were decreased with an increasing concentration of B and Zn, indicating that plants did not experience oxidative stress when both nutrients were available in sufficient amounts.

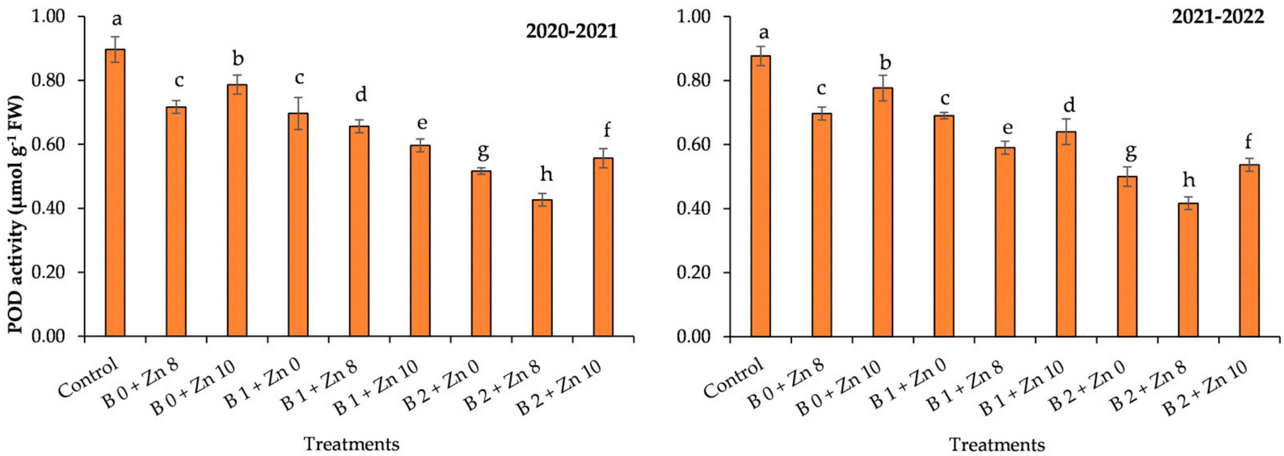


**Figure 3.** The influence of sole and combined application of boron and zinc on the activity of POD enzyme in oilseed rape plants grown under field conditions during 2020–2021 and 2021–2022. The error bars are standard errors of the means (n = 4). Different letters on the bars indicate significant differences among treatment means ($p \leq 0.05$).

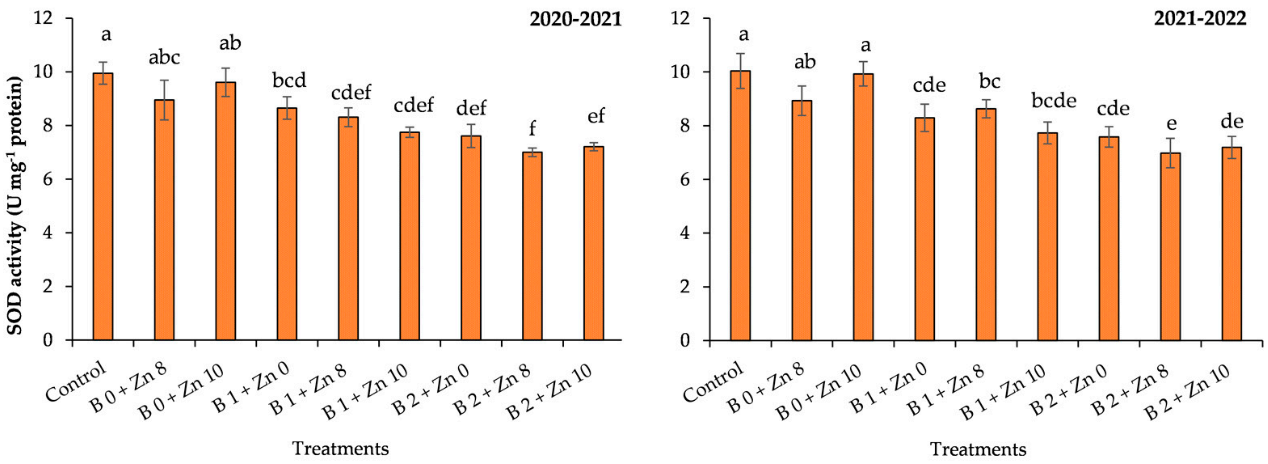


**Figure 4.** The influence of sole and combined application of boron and zinc on the activity of SOD enzyme in oilseed rape plants grown under field conditions during 2020–2021 and 2021–2022. The error bars are standard errors of the means (n = 4). Different letters on the bars indicate significant differences among treatment means ($p \leq 0.05$).

## 4. Discussion

Micronutrients are required in low amounts by plants, and their deficiencies exert significant negative impacts on the growth, yield, and quality of the produce. Sole and combined application of B and Zn significantly affected growth, seed yield, yield attributes, B and Zn contents in leaves, and oil yield and quality, as well as the physiological and antioxidant characteristics of oilseed rape, as hypothesized. Similarly, the combined application of B and Zn resulted in higher improvements in these traits compared to their individual application. The highest improvements in all traits were recorded with the B2 + Zn8 treatment during both years of this study. Overall, the combined application of B and Zn significantly increased the yield and yield-related attributes of oilseed rape in the current study. This could be attributed to the higher synthesis of assimilates, their improved mobility to potential sinks, and enhanced efficiency in pollination and seed development. Kanwal et al. [75] reported that optimum B and Zn application to the root zone caused a significant and consistent increase in the yield and yield-related traits of oilseed rape. Our results are in agreement with Aref [76] and Rehman et al. [33] who reported that the synergistic impact of these two micronutrients improved plant height, seed yield, and yield characteristics under low B and Zn availability in the soil. Plants uptake the required amount of B if B and Zn are supplemented in B-deficient soils, which improves plant development [77]. Additionally, the soil application of B and Zn resulted in a higher photosynthetic rate and chlorophyll content, ultimately leading to an increase in dry matter and subsequent growth and production [33,78]. Boron plays a crucial role in the synthesis of chlorophyll molecules required for photosynthesis. Sufficient B concentration in the current study probably facilitated the synthesis of chlorophyll, thereby resulting in enhanced leaf pigmentation and vitality in the current study. Zinc is involved in the activation of enzymes that are essential for the process of chlorophyll synthesis. The higher chlorophyll and photosynthesis under the B2 + Zn8 treatment are thought to be the result of enzyme activation, which resulted in better chlorophyll synthesis and subsequently improved photosynthesis.

The application of optimum B and Zn doses significantly improves the growth and yield of crop plants [79]. B and Zn application has been found to have a significant impact on the translocation of photoassimilates from the source (i.e., leaves) to other plant parts and the promotion of pollen tube elongation, ultimately resulting in an increased number of siliques [80,81]. Rehman et al. [49] and Potarzycki and Grzebisz [82] reported that B + Zn application improved the number of siliques per plant due to the higher number of flowers, the appropriate development of pollen and the pollen tube, pollination, and seed formation. It is well described that the application of B and Zn increases the number of seeds per silique and 1000-seed weight [33,83]. Several research studies have reported that the combined application of B and Zn can enhance seed production by increasing yield characteristics [33,84]. The deficiency of these nutrients impedes the development of the petiole and peduncle cells, resulting in diminished growth, seed yield, and yield characteristics [85]. Furthermore, a lower plant height at varying B + Zn concentrations may indicate a sensitive differentiation between deficiency and toxicity, thereby impeding the growth and yield characteristics of the plant without any apparent symptoms [86]. The reduction in plant growth, seed yield, and yield traits observed in the control group or low/higher doses of B + Zn can be attributed to a decrease in enzymatic reactions that regulate cell division and elongation. Conversely, excessive levels of B + Zn can lead to an imbalance of various enzymes, ultimately resulting in a reduction in plant height, seed yield, and yield traits [80]. The application of B2 + Zn10 resulted in enhanced uptake of both B and Zn by the leaves, indicating a synergistic relationship between these nutrients. However, the higher/excessive consumption of B and Zn reduced the growth and yield characteristics.

The observed enhancement in the yield and quality of oil under B2 + Zn8 may be attributed to the participation of micronutrients in the synthesis of elevated levels of fatty acid compounds [87,88]. The combined application of B and Zn may have contributed to the increase in oil content in seeds through the potential impact on the protein content of leaves. Therefore, the optimal utilization of B and Zn has the potential to enhance the seed oil content [29,89]. The combined application of B and Zn resulted in a significant improvement in the yield and quality of oil in the current study. These findings suggest that a synergistic relationship exists between B and Zn [90]. Although B and Zn are crucial for the growth and development of oilseed rape, their application does not directly affect the fatty acid composition of oilseed crops. These micronutrients play a crucial role in facilitating diverse enzymatic processes that are integral to photosynthesis. The increased photosynthetic activity has the potential to result in the elevated production of carbon precursors that can be utilized for the synthesis of fatty acids. The improved fatty acid profile of the oilseed rape oil in the current study can be linked to improved photosynthesis and, subsequently, the production of carbon precursors, which are utilized by plants for the synthesis of fatty acids.

The highest protein concentration, soluble sugar concentration, chlorophyll concentration, photosynthesis rate, stomatal conductance, and transpiration rate were recorded under the B2 + Zn8 treatment during both years. Zinc plays a crucial role in the structural composition and catalytic mechanisms of proteins and enzymes, thereby significantly contributing to plant development and exhibiting a favorable impact on growth. Consequently, Zn deficiency in plants may result in a reduction in protein and soluble sugar concentration in grains and lower amino acids in plant parts [91]. Higher protein concentration and soluble sugar concentration observed under B2 + Zn8 could impede the mobility of antioxidant enzymes, which causes oxidative destruction to protein and soluble sugar [32]. Photosynthesis in plants involves the absorption of light to produce organic compounds. The application of B2 alone increased leaf area, potentially leading to an improvement in the production of indole acetic acid. This hormone promotes chlorophyll concentration and the photosynthetic rate, which might be attributed to the higher photosynthesis rate in the current study [92]. Boron plays a crucial role in the maintenance of structural integrity, particularly in relation to the vascular tissues responsible for the transportation of water. The regulation of B levels has the potential to impact stomatal conductance and the intra-plant movement of water. Sufficient zinc concentrations have the capacity to regulate stomatal conductance and effectively manage transpiration. The optimum Zn and B supply in the current study improved the protein concentration, soluble sugar concentration, chlorophyll concentration, photosynthesis rate, stomatal conductance, and transpiration rate in the current study by improvements in these processes.

Zinc efficiently improves chlorophyll concentration through enzyme activation, photosynthesis rate, and the movement of photosynthate to seeds [93]. Our results corroborate the findings of Aref [76], who found that the synergistic effect of B and Zn increases chlorophyll contents and the photosynthesis rate. In the current experiment, B and Zn application improved chlorophyll contents and the photosynthesis rate, while higher rates of B + Zn reduced chlorophyll concentration, which might be attributed to the toxicity of these nutrients. Similar outcomes were obtained by Akta et al. [94] who concluded that an increase in B and Zn contents decreased the chlorophyll contents in leaves. Lower stomatal conductance and transpiration rates were recorded in Zn- and B-deficient plants during both years in the current study, which might be attributed to the destruction of the vascular bundle [95]. An impairment of the xylem vessels decreases the movement of water from the roots to the leaves, thereby impacting the stomatal conductance and transpiration rate. On the other hand, phloem impairment can impede the transportation of essential nutrients and sugars, thereby exerting an indirect influence on stomatal conductance and photosynthetic processes. Reduced transpiration rate and stomatal conductance under B and Zn deficiency in the current study are due to these processes. Pinho et al. [96] also concluded that a linear relationship persists among stomatal conductance and transpiration rates and micronutrient availability. Han et al. [97] stated that B application (2 kg $ha^{-1}$) increased stomatal conductance and reduced intercellular $CO_2$ absorption. Enhancements in physiological characteristics may lead to better crop growth due to the initiation of various physiological processes that result in increased seed production and improved oil quality [28,98,99]. However, B and Zn deficiencies cause yellowing of the foliage and necrosis [100].

In present study, peroxidase (POD) and superoxide dismutase (SOD) activity was lower under B2 + Zn8, indicating that there was low oxidative stress in this treatment. Antioxidant enzymes play an active role in reducing the detrimental effects of ROS species on photosynthesis and photorespiration. The highest POD and SOD activities were recorded for the control treatments, indicating that deficiency of B and Zn caused oxidative stress in plants. Nevertheless, B and Zn application lowered oxidative stress, as evidenced by the reduced activities of these enzymes.

## 5. Conclusions

It can be concluded that the combined application of B and Zn (2 and 8 kg $ha^{-1}$, respectively) in soil could be successfully used to improve the yield, yield-related attributes, and oil quality of oilseed rape. Significantly higher oil yield and quality were recorded under the B2 + Zn8 treatment. The combined application of B and Zn (2 and 8 kg $ha^{-1}$, respectively) significantly improved the physiological characteristics and activities of antioxidant enzymes. Therefore, successful enhancements in the yield, yield-related characteristics, and oil quality of oilseed rape can be achieved through the combined application of B and Zn at rates of 2 and 8 kg $ha^{-1}$, respectively, under semiarid climatic conditions.

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**Data Availability Statement:** All data are provided within the manuscript.

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