Qualitative traits of two types of radish (*Raphanus sativus* L.) and their content of medically active substance sulforaphane in response to treatments with zeolite and nano-potassium

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Abstract

The experiment was carried out in the agricultural fields of the College of Agriculture/ University of Kerbala, during the winter agricultural season 2022-2023. The experiment included adding three levels of zeolite 0, 10, and 20 t ha^{-1} (Z0, Z1, Z2) and spraying three concentrations of nano-potassium 0, 1, 2 g L^{-1} (K0, K1, K2), and the treatments were applied on two types of radish, the Spanish black radish (B) and the local red radish (R), according to a factorial experiment with a randomized complete block design (RCBD) with three replications. The results showed a significant effect of zeolite and nano-potassium, either alone or in combination, in improving the chemical content of the two types of radish The treatment BZ1K1 was superior in both the percentage of carbohydrates in the roots 5.19% and the content of the roots of sulphate 68.00 mg $100g^{-1}$, while the treatment BZ2K1 had a significant effect on the content of the roots of the medically active substance sulforaphane, reaching 12.36 µg ml^{-1}. It can be concluded from this study that, in order to confront the environmental pollution, it is possible to rely on natural soil conditioners such as natural zeolite and reduce the quantities of added fertilizers through the optimal use of nano-fertilizers, but their long-term environmental and health effects should be concerned.

Keywords: Sulphate content, Sulforaphane content, Natural Zeolite.

Introduction

The winter vegetable radish (*Raphanus sativus* L.) belongs to the Brassicaceae family. It thrives in a temperate region and can withstand low temperatures, but it cannot withstand hot ones. India and central and western China are where it first appeared [1]. Radish is farmed for its leaves and roots, which are consumed raw, cooked, and juiced. It may grow to a height of 30 to 90 cm, and according to [2], its roots are robust and come in a variety of sizes, shapes, and colors. As a good source of sugars, dietary fiber, carbohydrates, proteins, vitamin A, vitamin B (B1, B2, B3, B5, B6, B9), and ascorbic acid, as well as minerals like calcium, potassium, phosphorus, iron, magnesium, sodium, zinc, and fluoride, radish contains a variety of substances that have both health and nutritional benefits. Black radish also includes glycosides (glucosinolates and isothiocyanates) as well as flavonoids, alkaloids, tannins, and phenolic compounds [3, 4].

The use of natural minerals as soil conditioners to enhance the physical and chemical characteristics of soil and boost water retention has grown in popularity in recent years along with interest in sustainable agriculture. One mineral that has a lot of promise for the development of clean agriculture is zeolite [5]. When glass-rich volcanic rocks mix with seawater, they change into zeolite, a natural mineral that may be created and manufactured [6]. By enhancing porosity and having the ability to exchange positive ions for negative ones, this mineral act to improve the physical traits of the soil. Its delicate structure also aids in soil aeration [7].

In recent years, nanotechnology has been applied in agriculture, particularly to enhance the usage and development of fertilizers [8, 9]. Nanomaterials are substances with particles between 1 and 100 nanometers in size. They are ideal for creating smart fertilizers because of their small size and high surface area after being encapsulated or chelated to make them slow to release and suitable for different stages of plant growth [10, 11]. Numerous studies have shown that using nano-fertilizers to achieve the desired results can reduce the excessive use of traditional chemical fertilizers and lessen their negative environmental effects. This is particularly true for nano-potassium, which is crucial to improving the quantitative and qualitative traits of many field and horticultural crops [12, 13].

This study seeks to show the impact of natural zeolite, nano-potassium, and their interactions on some qualitative aspects of the roots of two varieties of radish (Spanish black radish, local red radish), as well as their content of the medically active compound sulforaphane.

Materials and Methods

The experiment was conducted in the field of the College of Agriculture / University of Karbala during the winter of 2022-2023 on an area of 128.25 m^2. The field was divided into three blocks, each of which comprised 18 treatments. These blocks were to be divided into 54 experimental units, each of which had a 1 m^2 area and five planting lines with six plants per line. The experiment included three factors: two types of radish, adding three levels of zeolite stone (0, 10, and 20 t h^{-1}) (Z0, Z1, and Z2 respectively) and spraying with three concentrations of nano-potassium (0, 1, and 2 g L^{-1}) (K0, K1, K2, respectively).

Radish seeds were sown for both types on 1/11/2022, and zeolite was added before planting. As for nano-potassium, it was dissolved by distilled water and sprayed with two sprays, the first was 25 days after planting the seeds, and the second spray was two weeks after the first spray. The study was conducted as a factorial experiment utilizing a randomized complete block design (RCBD) with three replications, averages were compared at the probability level of 0.05, and the data were analyzed using the Genstat software. The following characteristics were estimated: fresh weight of the root system (g), dry weight of the root system (g), root content of total carbohydrates % [14], root sulphate content (mg 100 g^{-1} dry sample) [15], root content of the active ingredient Glucoraphanin Sulforaphane ((µg ml^{-1}) [16].

Results and Discussion

Fresh weight of roots (g)

The results of Table (1) show that the fresh weight of the root system was affected when zeolite was added, as the treatments Z1 and Z2 gave the highest averages of 141.4 and 113.8 g, respectively, when compared to the standard treatment that gave 86.4 g. The same table indicates that spraying with nano-potassium did not show any significant effect on this trait. As for the variety treatment, the black radish showed a significant superiority, and the highest average fresh weight of the root total was 175.7 g, compared to the red radish, which recorded 52.0 g.

The bilateral interaction between the two treatments of zeolite and the type of radish had a significant effect on this trait, as treatment BZ1 gave the highest values of 225.3 g, compared to treatment RZ0, which gave the lowest value of 41.5 g. The interaction between nano-potassium and the type, the treatment BK0 excelled and recorded the highest average amounted to 194.0 g, compared to the treatment RZ0, which recorded the lowest average amounted to 51.4 g. As for the binary interactions between zeolite and nano-potassium, the results showed the significant superiority of the Z1K0 treatment and gave the highest average fresh weight of the root system amounted to 177.8 g compared to the Z0K0 treatment, which recorded the lowest value of 61.0 g.

The triple interaction between the factors of the study indicated the superiority of treatment BZ1K0, which recorded the highest mean of the trait amounted to 293.3 g, compared to treatment RZ0K0, which recorded the lowest average fresh weight of root system amounted to 33.0 g.

Table (1): Root system fresh weight (g) of two types of radish under the influence of zeolite and nano-potassium.

Radish type (T)	Zeolite (Z) t ha^{-1}	Nano-Potassium (K) gm L^{-1}			Interaction Z × T	T Mean
		0 (K0)	1(K1)	2 (K2)		
Black (B)	0 (Z0)	89.0	117.7	186.8	131.2	175.7
	10 (Z1)	293.3	183.8	198.7	225.3	
	20 (Z2)	199.7	169.0	143.7	170.8	
Red (R)	0 (Z0)	33.0	47.8	43.8	41.5	52.0
	10 (Z1)	62.3	61.9	48.7	57.6	
	20 (Z2)	58.9	45.6	65.9	56.8	
K Mean		122.7	104.3	114.6	L.S.D 0.05 Z × T 45.21	L.S.D 0.05 T 26.10
L.S.D 0.05T × Z × K		78.40				
Interaction T × K	Black	194.0	156.8	176.4	L.S.D 0.05 T × K 45.21	
	Red	51.4	51.8	52.8	Z mean	
Interaction Z × K	0 (Z0)	61.0	82.7	115.3	86.4	
	10 (Z1)	177.8	122.9	123.7	141.4	
	20 (Z2)	129.3	197.3	104.8	113.8	
L.S.D 0.05						
Z × K		K			Z	
55.37		31.97			31.97	

Root dry weight (g)

The results of Table (2) show that the dry weight of the root system was affected by the addition of zeolite, so the treatments Z1 and Z2 gave the highest mean for the measured trait, which amounted to 27.0 and 22.7 g, respectively, compared to the control treatment, which recorded the lowest average of 18.1 g. While the same table shows that there is no effect of spraying with potassium nanoparticles on the dry weight of the root system. The table indicates that the type of radish had a significant effect, so the black radish recorded the highest mean for the trait, amounting to 32.0 g, compared to the red radish, which recorded 13.0 g .

The bilateral interaction between the two treatments of zeolite and the type of radish had a significant effect on this trait, as treatment BZ1 gave the highest average of 40.6 g compared to treatment RZ0 which gave the lowest average of 10.5 g. As for the interaction between nano-potassium and the type of radish, treatment BK0 excelled by giving the highest average dry weight of the root system amounted to 33.9 g compared to treatment RK0 which gave 11.3 g. The bilateral interactions between zeolite and nano-potassium treatments, the results showed their superiority significantly, as the Z1K0 treatment reached 32.5 g dry weight of the root system, compared to the control treatment, which recorded the lowest mean of the trait, which reached 12.7 g.

The BZ1K0 treatment, which measured 51.0 gm, outperformed the RZ0K0 treatment, which produced the lowest average dry weight of the root system, 7.9 g, according to the triple interaction between the study's components.

Table (2): The root dry weight (g) of two types of radish under the influence of zeolite and nano-potassium.

Radish type (T)	Zeolite (Z) t ha^{-1}	Nano-Potassium (K) gm L^{-1}			Interaction Z × T	T Mean
		0 (K0)	1(K1)	2 (K2)		
Black (B)	0 (Z0)	17.4	23.6	36.3	25.7	32.0
	10 (Z1)	51.0	34.9	36.0	40.6	
	20 (Z2)	33.0	29.6	26.8	29.8	
Red (R)	0 (Z0)	7.9	10.9	12.7	10.5	13.0
	10 (Z1)	14.1	15.2	11.1	13.4	
	20 (Z2)	11.8	14.8	19.0	15.2	
K Mean		22.5	21.5	23.6	L.S.D 0.05 Z × T 7.92	L.S.D 0.05 T 4.57
L.S.D 0.05T × Z × K		13.71				
Interaction T × K	Black	33.9	29.4	33.1	L.S.D 0.05 T × K 7.92	
	Red	11.3	13.6	14.5	Z mean	
Interaction Z × K	0 (Z0)	12.7	17.2	24.5	18.1	
	10 (Z1)	32.5	25.1	23.5	27.0	
	20 (Z2)	22.7	22.2	23.2	22.7	
L.S.D 0.05						
Z × K		K			Z	
7.92		5.60			5.60	

Carbohydrates in the roots %

According to Table 3, the control treatment recorded the lowest proportion of carbohydrates in the roots, at 3.08%, while the Z2 zeolite treatment recorded the greatest percentage, at 4.63%. Comparing the comparison treatment to the K1 nano-potassium treatment, which had the lowest percentage of carbohydrates at 3.89%, the K1 nano-potassium treatment had the greatest percentage of carbohydrates at 4.27%. The results of the radish-type treatments favored the black radish, which had the highest percentage of carbohydrates (4.29%), compared to the red radish, which had the lowest percentage (3.93%) of carbohydrates in its roots.

The same table showed that the binary interactions between the type of radish and the zeolite had a significant impact. The treatments BZ2, BZ1, and RZ1 excelled by giving them the highest averages for the percentage of carbohydrates in the roots, which amounted to 4.85%, 4.76%, and 4.47%, respectively, in contrast to the RZ0 treatment, which recorded 2.92%. The same data demonstrates that the nano-potassium and radish type overlapping treatments resulted in a considerable superiority, with the BK1 treatment outperforming the RK0 treatment and recording 4.47% compared to the RK0 treatment's 3.78% carbohydrate content in the roots. The Z1K1 treatment recorded the greatest percentage of carbohydrates in the roots, at a rate of 4.85%, in comparison to the control treatment, which recorded the lowest value of 2.82%, demonstrating the evident impact of the bilateral interactions between zeolite and potassium nanoparticles on plant growth.

The treatments BZ1K1 and BZ2K2, which recorded the highest percentage of carbohydrates in the roots, amounting to 5.19% and 5.15%, respectively, are clearly superior to RZ0K0, which recorded the lowest percentage of carbohydrates in the roots, amounting to 2.52%, according to the triple interaction of the study factors.

Table (3): The percentage of total carbohydrates (%) in the roots of two types of radish under the influence of zeolite and nano-potassium.

Radish type (T)	Zeolite (Z) t ha^{-1}	Nano-Potassium (K) gm L^{-1} 0 (K0)	1(K1)	2 (K2)	Interaction Z × T	T Mean
Black (B)	0 (Z0)	3.12	3.25	3.44	3.27	4.29
	10 (Z1)	4.44	5.19	4.67	4.76	
	20 (Z2)	4.45	4.96	5.15	4.85	
Red (R)	0 (Z0)	2.52	3.38	2.87	2.92	3.93
	10 (Z1)	4.38	4.51	4.53	4.47	
	20 (Z2)	4.45	4.38	4.39	4.40	
K Mean		3.89	4.27	4.17	L.S.D 0.05 Z × T 45.21	L.S.D 0.05 T 0.29
L.S.D 0.05T × Z × K		0.89				
Interaction T × K	Black	4.00	4.47	4.42	L.S.D 0.05 T × K 0.51	
	Red	3.78	4.09	3.93	Z mean	
Interaction Z × K	0 (Z0)	2.82	3.31	3.12	3.08	
	10 (Z1)	4.41	4.85	4.60	4.62	
	20 (Z2)	4.45	4.67	4.77	4.63	
L.S.D 0.05						
Z × K		K			Z	
0.63		0.36			0.36	

Sulphate content in root (mg 100 g^{-1} dry weight)

Table (4) indicates the superiority of the Z2 zeolite treatment by recording the highest average of the sulphate content in the roots of 56.05 mg 100 g^{-1} compared to the control treatment, which recorded the lowest average of 51.44 mg 100 g^{-1}. The results of the nano-potassium treatments show the superiority of the K2 treatment by giving the highest average of sulphate in the roots, amounting to 55.33 mg 100 g^{-1} compared to the comparison treatment, which gave the lowest average of 52.94 mg 100 g^{-1}. Radish type show superiority to the black radish treatment, which recorded 63.66 mg 100 g^{-1} compared to red radish, which recorded the lowest average of sulphate in the roots, amounting to 44.88 mg 100 g^{-1}.

The same table shows that the bilateral interaction between zeolite and radish type was significantly superior in treatment BZ1, which recorded the highest average of sulphate in the roots amounting to 65.33 mg 100 g^{-1}, when compared to treatment RZ0, which recorded the lowest average of 41.44 mg 100 g^{-1}. The same table shows that the bilateral interaction between nano-potassium and the type of radish was significantly superior in treatment BK1, where the highest average was recorded, amounting to 64.67 mg 100 g^{-1}, compared to treatment RK0, which recorded the lowest average of

43.33 mg 100 g^{-1}. Also, the interaction between zeolite and nano-potassium shows the superiority of the treatment. Z2K2 significantly increased and gave the highest mean of 58.33 mg 100 g^{-1}, compared to the comparison treatment, which recorded 50.17 mg 100 g^{-1}.

The triple interaction treatments of the study factors were significantly superior when treatment BZ1K1 recorded 68.00 mg 100 g^{-1}, which did not differ significantly from treatment BZ2K2 which recorded 67.00 mg 100 g^{-1} compared to treatment RZ0K0 which recorded the lowest average of sulphate in the roots amounted to 40.00 mg 100 g$_{-1}$.

Table (4): The root sulfate content (mg 100 g^{-1}) of two types of radish under the influence of zeolite and nano-potassium.

Radish type (T)	Zeolite (Z) t ha^{-1}	Nano-Potassium (K) gm L^{-1}			Interaction Z × T	T Mean
		0 (K0)	1(K1)	2 (K2)		
Black (B)	0 (Z0)	60.33	62.33	61.67	61.44	63.66
	10 (Z1)	65.33	68.00	62.67	65.33	
	20 (Z2)	62.00	63.67	67.00	64.22	
Red (R)	0 (Z0)	40.00	41.67	42.67	41.44	44.88
	10 (Z1)	42.67	45.00	48.33	45.33	
	20 (Z2)	47.33	46.67	49.67	47.89	
K Mean		52.94	54.56	55.33	L.S.D 0.05 Z × T 1.90	L.S.D 0.05 T 1.10
L.S.D 0.05T × Z × K		3.30				
Interaction T × K	Black	62.56	64.67	63.78	L.S.D 0.05 T × K	
	Red	43.33	44.44	47.89	Z mean	
Interaction Z × K	0 (Z0)	50.17	52.00	52.17	51.44	
	10 (Z1)	54.00	56.50	55.50	55.33	
	20 (Z2)	54.67	55.17	58.33	56.05	
L.S.D 0.05						
Z × K		K			Z	
2.33		1.34			1.34	

Glucoraphanin content (Sulforaphane) in root (µg ml^{-1})

The medically active substance Sulforaphane was significantly affected by zeolite, as shown in Table (5), and the treatment Z2 performed most effective giving the highest average of the active substance, amounting to 9.28 µg ml^{-1}, as opposed to the control treatment, which gave the lowest average, 4.29 µg ml^{-1}. The K2 nano-potassium therapy had the greatest average root content of the active ingredient, 8.36 µg ml^{-1}, according to the same data, which demonstrated its superiority to the control treatment, which had the lowest average of 6.02 µg ml^{-1}. The table shows that the black radish treatment outperformed the red radish treatment, which recorded a lower average root content of the active component at 6.29 µg ml^{-1}. The black radish treatment recorded the greatest average root content at 7.85 µg ml^{-1}.

The treatment BZ2 outperformed the control RZ0 by recording the greatest average active substance level of 10.80 µg ml^{-1} as opposed to 4.08 µg ml^{-1}, indicating that there is a considerable influence of the interactions between zeolite and the kind of radish, as shown by the same table. The bilateral interaction between nano-potassium and the type of radish showed a significant effect, so the BK2 treatment excelled by giving it the highest average root content of the active substance amounted to 8.47 µg ml-1, compared to the treatment RK0, which recorded the lowest value of 5.17 µg ml^{-1}. As for the bilateral interaction between the zeolite and nano-potassium treatments, the Z1K2 treatment showed the superiority of the treatment, which recorded the highest content of the active substance, amounting to 10.45 µg ml^{-1} compared to the comparison treatment, which gave the lowest content of 3.87 µg ml^{-1}.

Table (5): The Glucoraphanin (Sulforaphane) root content µg ml^{-1} of two types of radish under the influence of zeolite and nano-potassium.

Radish type (T)	Zeolite (Z) t ha^{-1}	Nano-Potassium (K) gm L^{-1}			Interaction Z × T	T Mean
		0 (K0)	1(K1)	2 (K2)		
Black (B)	0 (Z0)	4.29	4.45	4.76	4.50	7.85
	10 (Z1)	5.29	7.85	11.65	8.26	
	20 (Z2)	11.06	12.36	9.00	10.80	
Red (R)	0 (Z0)	3.45	4.13	4.66	4.08	6.29
	10 (Z1)	6.01	5.86	9.26	7.04	
	20 (Z2)	6.06	6.38	10.83	7.75	
K Mean		6.02	6.83	8.36	L.S.D 0.05 Z × T 0.009	L.S.D 0.05 T 0.005
L.S.D 0.05T × Z × K		0.016				
Interaction T × K	Black	6.88	8.22	8.47	L.S.D 0.05 T × K	
	Red	5.17	5.45	8.25	Z mean	
Interaction Z × K	0 (Z0)	3.87	4.29	4.71	4.29	
	10 (Z1)	5.65	6.85	10.45	7.65	
	20 (Z2)	8.56	9.37	9.91	9.28	
L.S.D 0.05						
Z × K		K			Z	
0.012		0.006			0.006	

Also, the results in the same table indicate that the triple interaction had a significant effect, as the treatment BZ2K1 had a significant effect and outperformed all treatments, and recorded the highest content of the root of the active substance amounted to 12.36 µg ml^{-1} compared to the treatment RZ0K0, which gave the lowest content of 3.45 µg ml^{-1}.

The treatment Z1 was successful and provided the highest averages in the fresh and dry weight of the root system, according to the results in tables (1, 2), while the treatment Z2 excelled, according to tables (3, 4, and 5), by recording the highest values in the percentage of carbohydrates in the roots and the content of sulphates and the medically active compound sulforaphane in the roots. The cause of these outcomes could be attributed to the fact that plants growing in soil that has zeolite added develop a robust root system deep in the soil, increasing the efficiency of water and nutrient absorption during the plant's growth phase and thereby increasing the metabolism of the plant internally, which promotes plant growth [17]. Additionally, the mineral zeolite works to provide elements that are adsorbed on its surface and within the mineral, which affects the soil's readiness for nutrients like nitrogen, phosphorus, potassium, iron, and magnesium and gradually releases them to the plant when it needs them, which leads to the cells multiply by increasing their division and elongation [17, 18]. This result is in line with [19]. The lack of superiority of treatment Z 2 in some traits may be due to genetic factors specific to the species.

The results indicated in Table (3) to the significant effect of spraying with nano-potassium on the studied traits. Treatment K1 excelled in the percentage of carbohydrates in the roots, while treatment K2 excelled in the root content of sulphate and the active substance sulforaphane. The reason may be due to the importance of potassium for its effective role in stimulating many enzymes and regulate the cell content of water, as it is responsible for closing and opening the stomata, and is considered an important factor for increasing the yield and improving its quality, as it transports processed carbohydrates to their storage places and contributes to increasing their volume [20]. Potassium also works to increase the stimulation of metabolic processes and the formation of amino acids, which have an essential role in the formation of glucosinolates, and these results are consistent with the findings of [21,22]. The findings demonstrated that black radish outperformed red radish in every feature. The genetic factors that control the absorption and accumulation of nutrients inside the plant may be the cause of the difference in genotypes between the two species in the rate at which carbohydrates are converted to glucosinolates. As a result, the plants' response to fertilization programs that are applied to improve both quantitative and qualitative production varies [17, 18].

The interaction of potassium fertilizer with zeolite mineral led to an increase in most of the studied characteristics, which can be attributed to the positive relationship between the mineral and nano-potassium, as potassium has an important role in transporting the products of the photosynthesis process, which affects the vital processes by activating enzymes that are associated with energy transfer processes and thus causing An increase in photosynthesis and the accumulation of its products, such as carbohydrates and sugars, in the storage parts [24]. At the same time, zeolite works to increase the readiness and absorption of nutrients necessary for the plant [17, 18].

It can be concluded from this study that natural zeolite can be included in the fertilization programs used for agricultural crops for its contribution to improving the quantitative and qualitative characteristics of the crop by improving the readiness of the elements and reducing their losses, which allows the use of smaller quantities of fertilizers to reduce environmental pollutants as a result of the excessive use of fertilizers. Nano fertilizers can also be used as a source of nutrients in small quantities, but this requires studying the long-term effects of these fertilizers.

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