THE RADIOLOGICAL IMPACT OF THE USE OF PHOSPHATE FERTILIZERS IN GERMINATION AND GROWTH OF *ERUCA SATIVA* (ROCKET PLANT)

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Abstract. Various types of phosphate fertilizers could alter the soil's physical and chemical properties, such as pH, electric conductivity (EC), and organic matter, phosphorus, and potassium contents. These changes will subsequently affect plant growth and crop yield. In this study, we have examined the application of three types of commonly used phosphate fertilizers, namely monoammonium phosphate (MAP), diammonium phosphate (DAP), and triple superphosphate (TSP), on the growth and yield of rocket (*Eruca sativa* Mill) plants. Rocket is frequently consumed as a salad ingredient in many countries, including Saudi Arabia. We have compared the effects of the three types of phosphate fertilizers on soil composition, pH, and electric conductivity, as well as their ultimate effects on rocket plant growth and yield. In addition, GC-MS analysis was used to isolate compounds from the seeds of treated plants using methanol extracts. Various compounds with different concentrations have been detected. Serval unique compounds were also detected in each treatment. Moreover, we measured the radon activity accumulated in the soil due to the use of these fertilizers. Our results indicate that monoammonium phosphate is the best phosphate fertilizer, as it resulted in the highest rocket plant growth and the lowest accumulated radon activity in the soil.

Keywords: Brassicaceae, erucic acid, photosynthetic pigments, radon activity, GC-MS

Introduction

The terrestrial environment is filled with natural radioactivity, which exists in many geological formations such as phosphate rocks (Alhawsawi et al., 2021) and soils as well as in air and plants (Ibrahim et al., 1993; Aly Abdo et al., 1999). These environmental components contain a measurable amount of radioactivity. In this regard, three major industries require further attention in the terms of their scope and handled materials: the oil and gas industry, the zircon industry, and the phosphate and fertilizers industry (Kant et al., 2006).

Using fertilizers in agriculture has become essential worldwide. Fertilizers play an important role in increasing crop production as well as improving nutrient deficits and soil properties (Chauhan and Chauhan, 2014b; Korany et al., 2021). The physiology and metabolism of plants are altered by the use of different fertilizers in terms of uptake of different elements and radionuclide. The use of fertilizers in soil may cause an increase in the radionuclide content, causing high uptake of radionuclide (Chauhan and Chauhan,

2014a). The radionuclide (uranium, thorium, radium, and polonium) can be transferred from soil to root and translocate/accumulate in various parts of plants (roots, stems, leaves, and grains). ²²⁶Radium appears to have the greatest potential amount of radionuclide that can translocate and accumulate in plant shoot tissue (Knight, 1983).

The phosphate and associated fertilizers industry has an added complication because it has two distinct sources of raw material: igneous or sedimentary origin. The source material contains isotopes from natural uranium and thorium decay chains (Kant et al., 2006). Accordingly, phosphate fertilizer is one of the most important sources of ionizing radon from radionuclide (Kadi and El-Eryani, 2012; Ugolini et al., 2020).

Redistribution of natural radionuclide in the environment takes place through mining and processing of phosphate ore. Radionuclide becomes available in ore products such as phosphogypsum and phosphoric acid, which is the starting material for DAP, TSP and nitrogen, phosphorus, and potassium (NPK) fertilizers (Kadi and El-Eryani, 2012; Szajerski, 2020; Korany et al., 2021).

The specific metabolic character of the plant species may lead to accumulation of radionuclides in their organs, which may further depend upon the physicochemical characteristics of the soil. Therefore, there may be an increase in risk to the human population via the food chain.

Alpha- and gamma-spectrometry have been used by Saueia and Mazzilli (2005) to study radionuclide concentrations in phosphogypsum, phosphate fertilizers, and phosphate rocks in Brazil. They concluded that MAP and DAP fertilizer samples derived directly from phosphoric acid showed low activities for ²²⁶Ra, ²²⁸Ra, and ²¹⁰Pb, but significant concentrations of uranium and thorium up to 822 and 850 Bq kg⁻¹ were recorded, respectively.

Transfer of uranium due to its dissolution in water causes the emission of radon gas. Radon and its decay products are among the main causes of lung and other types of cancers (Gao et al., 2008). Increasing radionuclide in soils and/or groundwater may result in human consumption of radiation through the food chain or drinking water (Alhawsawi et al., 2021) and, consequently, its deposition in bone tissue (Laich, 1991). Continuous irradiation of the human skeleton by ²²⁶Ra may cause biological damage such as bone sarcoma (Marovic and Sencar, 1995).

Radon is a radioactive gas, which is created during the radioactive decay of ²³⁸U. There are many natural and industrial sources of radiation to which humans are exposed, such as cosmic rays, terrestrial radionuclides, and radon and its daughters. Most (60%) comes from natural sources of radiation. Natural radiation that penetrates us from the earth's crust varies from one place to another on the earth's surface, depending on geology and topography (UNSCEAR, 2000).

Radon is an invisible, odourless, tasteless radioactive gas. It is formed by the disintegration of radium, which is a decay product of uranium. Additionally, it emits alpha particles called radon daughters or "progeny". Inhalation of radon gas and its daughters will expose the lung tissue to short-lived alpha-emitting radionuclides, which will increase the risk of lung cancer.

Eruca sativa Mill (commonly known as rocket) is an edible annual plant from the Brassica family. It is widely used as a salad vegetable. Rocket leaves have astringent, antiphlogistic, diuretic, tonic, depurative, emollient, laxative, digestive, stimulant, and rubefacient effects (Yaniv et al., 1998). *Eruca* seeds contain a large amount of thio-functionalized glucosinolates along with erucic acid, which is used in cosmetics, detergents, and polymer production (Ahh et al., 2002; Lazzeri et al., 2004). Fertilization

with nitrogen, phosphorous, and potassium to improve plant growth and production has been increasing in the past 60 years (Burt et al., 2009). The use of phosphate fertilizers on agricultural farms has enhanced gamma-ray activity concentration on the farms and therefore the dose absorbed by the public (Nasim-Akhtar and Tufail, 2007).

Agricultural fields are essential around the world, as they help to increase the productivity of crops, and soil properties. The radionuclides (uranium, thorium, radium, and polonium) can be transferred from soil to roots and thence to other parts of plants (stems, leaves, and grains), as well as leach into drinking water and by both routes can be ingested by humans (Alhawsawi et al., 2021). Thus, radon concentration in agricultural fields is important due to the health risk and must be considered when designing control strategies.

The present study aims to estimate alpha radioactivity in plants grown under three different types of phosphate fertilizers (monoammonium, diammonium, and triple superphosphate) and investigate the effect of alpha radioactivity on plant growth and yield, as well as effective compounds in *Eruca* seeds' methanol extract, as estimated by GC-MS.

Material and methods

Plant material and growth condition

A pot experiment was conducted under greenhouse conditions at the Research and Training Station, King Faisal University, Al-Ahsa, Kingdom of Saudi Arabia. A temperature between 32 and 36°C and relative humidity of 47–56%, with a 14-h average photoperiod, was recorded. The experiments contained four treatment groups, the control and three types of phosphorus fertilizers, namely monoammonium phosphate, diammonium phosphate, and triple superphosphate. Seeds of *Eruca sativa* were planted in a 25-cm-diameter plastic pot containing 8 kg of sand mixed with 8 g of the corresponding fertilizer. In this study, we tested the effect of three phosphate fertilizers on growth, yield, and soil radon concentrations using a technique based on the CR-39 SSNTDs (Solid State Nuclear Track Detectors made from CR-39 materials). A plastic radiation detector CR-39 was placed 5 cm below the soil surface in each pot. Each treatment consisted of 10 replicates (pots). The pots were arranged in a completely randomized block design. Every 2 months, the plants were fertilized with nitrogen and potassium.

Vegetative growth characteristics

Sixteen weeks after sowing, six randomly selected plants from each treatment were harvested. The plants' height, number of leaves, longest root length using Pit excavations technique (Voorhees, 1989), and dry weight of leaves and roots were measured and recorded.

Chemical analysis in leaves

Photosynthetic pigments determination

The third bottom fresh leaves from six randomly selected plant (Sixteen weeks after sowing) were excised. The amounts of Chl. a, Chl. b and carotenoid were measured as described by the Association of Official Analytical Chemists A.O.A.C (1984) using

80% acetone as a solvent. The measurement of absorbance was performed using the Agilent 8453 ultraviolet (UV) visible spectrophotometer.

Mineral composition

The harvested leaves and root samples were air-dried at room temperature. Dried leaves, and root samples obtained from six randomly selected plants from each treatment were pulverized separately. A 0.5 g powder-dried sample was digested in a 50-ml volumetric flask with 2.5 ml of concentrated sulfuric acid (H₂SO₄, 95–97%). The digestion was performed on a hotplate at approximately 270°C. Hydrogen peroxide (H₂O₂) solution was added bit-by-bit until the digestion was completed (a clear solution was obtained) (Piper, 1942). After digestion, deionized water was added to achieve a final volume of 50 ml in the volumetric flask. The resultant clear solution was then used for N, P, and K composition determination according to the methods mentioned below.

1. Nitrogen determination was carried out using the modified micro-Kjedahl method, as described by Jackson (1967).

2. The percentage of P was estimated calorimetrically according to the method of Murphy and Riley (1962).

3. K concentrations (mg/100 g) were determined using an Atomic Absorption Flame Photometry model Shimadzu-AA7000 according to the method of Mazumdar and Majumder (2003). Soil analyses were performed according to Page et al. (1982).

GC/MS analysis

Six months after sowing, seeds from the remaining four plants from each treatment group were harvested and air-dried at room temperature. One hundred fifty grams of *E. sativa* air-dried seed powders were extracted with methanol (3.83%) by a Soxhlet apparatus at 80°C for 36 hours. The gas chromatography–mass spectrometry (GC-MS) was performed at the Department of Chemistry, Faculty of Science, King Faisal University, using a GC 1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–35MS (30 m × 0.25 mm × 0.25 µm film thickness). The separation conditions and method for identification of the separated components were as described by Alkuwayti et al. (2019).

Radon concentrations of phosphate fertilizers in the soil using a technique based on the CR-39 SSNTDs

In order to conduct the current radon measurement survey, sixteen CR-39 (Interacts, Italy) detectors were installed in the studied area. A CR-39 detector (area $1 \times 1 \text{ cm}^2$) was installed carefully in each pot. Eight sue detectors CR-39 were measured after 80 days, and the rest of the samples were measured after 120 days (*Figure 6*), during which, α particles emitted by radon and their daughters bombarded the CR-39 track detectors. After the irradiation period, the detectors were developed in NaOH solution 6.25 N for 6 hours at 70°C. After chemical etching, the track densities were determined by an optical microscope 400X. The radon concentration in Bq/m³ was determined by the following equation (ICRP, 1993; Mohamed et al., 2015).

$$CRn = \rho x \times 1000/Ft \tag{Eq.1}$$

where ρ is the track density in tracks cm⁻², *F* is the calibration factor for the dosimeter used in the survey in the units of tracks cm⁻² kBq⁻¹m³h⁻¹, and t is the exposure time in hours.

Statistical analysis

Data were statistically analysed using ANOVA/MANOVA in Statistica 6 software (Statsoft, 2001). The significance of differences among means was detected using the least significant difference test (LSD) at p = 0.05.

Results and discussion

Vegetative growth

All fertilizers tested significantly increased the growth of *Eruca* plants up to two times increase in the plant height, leaf number, and root length as compared to control (*Table 1*). Among the three fertilizers, MAP had the highest enhancement effects in all the above-mentioned vegetative growth parameters, followed by DAP and TSP. However, the application of all three types of tested fertilizers did not bring any significant increase in the yield, as seen in the dry weight of leaves from the fertilizer-treated plants. Indeed, a decrease in dried leaf weight was observed in the plants fertilized by MAP and TSP as compared to control. In contrast to the dried leaf weight, the plants treated with MAP and TSP showed a significant increase in dried root weight. Thomas and Rengel (2002) also observed that MAP or DAP resulted in greater canola plant growth as compared with TSP. Nutrient analysis of the shoots also indicated that these differences in growth were most likely the result of differences in nitrogen nutrition. The authors suggested that an increase in soil pH due to DAP application reduced the fixation of phosphate, which increased the availability of phosphate.

Table 1. Effect of different types of fertilizers on plant height (cm), leave number (n), root length (cm) and dry weight (g/plant) of leaves and root.), phosphorus content (%) in leaves and roots

Treatment	Plant height (cm)	Leave number (n)	Longest root length (cm)	Dry weight of leaves (g/plant)	Dry weight of root (g/plant)	Phosphorus % in the leaves	Phosphorus % the root
Control	$27.5 \ b \ \pm$	11.25 c \pm	16.1 c±	2.4 a±	0.7 b±	$0.46 c \pm$	$0.44 \text{ b}\pm$
Monoammonium phosphate	45.7 a±	33 a±	37.5 a±	1.6 a±	1.3 a±	0.58 b±	0.56 a±
Diammonium phosphate	44.7 a±	24.5 b±	27.3 b±	2.9 a±	0.5 b±	$0.56 b\pm$	0.53 a±
Triple Super phosphate	41.8 a±	27.7 a±	25.3 b±	1.9 a±	0.9 b±	0.60 a±	0.49 b±

Values are given as average of three replicates. Different letters (a, b and c) represent significance at p=0.05 level

AlKhader et al. (2013) noted significant differences in lettuce plants' fresh and dry weights (MAP>SSP>DAP>PR) in a greenhouse trial with MAP, DAP, single superphosphate (SSP), and phosphate rock (PR). However, field experimentation with lettuce plants by the same group indicated that the above-mentioned fertilizers did not

bring any significant effect to the plants' fresh and dry weights (MAP=SSP=DAP>PR). Under both greenhouse and field conditions, plant height and leaf surface area were significantly affected by the phosphate fertilizer type and rate of application. Fertilizer application also produced a higher total leaf number than the control treatment under greenhouse conditions. The authors suggested that MAP produced more $H_2PO_4^-$, which is more readily available to plants than the other P forms. In the high-pH calcareous soil of the greenhouse trial, MAP had higher solubility than DAP. The dissolution of DAP produced ammonia, which reduced the P availability to the plant (Bohn et al., 1979; Mengel, 1987).

Chemical analysis of leaves

All three types of fertilizer increased the photosynthetic pigments chlorophyll a, b and carotenoids (*Table 2*) and the phosphorus percentage in the leaves and roots (*Table 1*) of the treated plants. These increases are consistent with the observed increase in plant growth. Among the three types of fertilizers, triple superphosphate gave the highest increase in all types of photosynthetic pigments, followed by monoammonium phosphate and diammonium phosphate. As for phosphorus contents, it is interesting to observe that among the three types of fertilizers, the triple superphosphate gave the highest phosphorus percentage in the leaves, but the lowest phosphorus percentage in the roots. Consistent with the photosynthetic pigments results, diammonium phosphate had less effect on the increase of phosphorus contents in leaves and roots than the monoammonium phosphate fertilizer. Our results are in agreement with Constigan (1984), who stated that triple superphosphate had little effect on lettuce dry weight but contributed to higher P concentrations within the plants.

Treatment	рН	EC (ms/cm)	Organic matter %	Total N%	Available P%	Available K (mg/100g)	Chl a mg/100g F.W.	Chl b mg/100g F.W.	Carotenoids pigments mg/100g F.W.
Control	5.86 a	1.09 a	0.215 c	0.0125 b	3.435 b	12.26 a	57.34 c	21.73 c	65.9 a
Monoammonium phosphate	6.01 a	1.13 a	0.265 c	0.024 a	4.33 a	14.125 a	71.25 b	29.55 b	80.37 a
Diammonium phosphate	5.99 a	1.20 a	0.43 b	0.0335 a	4.975 a	15.73 a	63.62 c	22.27 c	74.03 a
Triple Super phosphate	5.85 a	1.27 a	0.515 a	0.0287 a	5.4 a	16.435 a	80.37 a	36.32 a	93.33 a

Table 2. Soil properties, chemical compositions of soil and pigments content (chlorophyll a, chlorophyll and Carotenoids)

Values are given as average of three replicates. Different letters (a, b and c) represent significance at p=0.05 level

Chemical analysis of soil

The properties and mineral composition of soil from the control and fertilizer-treated soils were analysed at the end of the experiment, and the results are shown in *Table 2*. All types of fertilizers showed no significant differences in soil pH, EC of the soil as well as available K content. Among the three fertilizers, TSP had the highest impact on available phosphorus and organic matter content, while DAP phosphate had the highest impact on total nitrogen.

GC-MS analysis

The GC-MS results (*Table 3*) indicated that 5-(methylthio)-Pentanenitrile was the major compound in all treatment groups. Its composition varied from 34.73% (diammonium phosphate treatment) to 55.52% (triple superphosphate treatment). This compound belongs to the glucosinolate compounds, which are rich in the Brassicaceae family and Eruca sativa (Chiang et al., 1998; Chaudhary et al., 2018). In addition, two other compounds, namely pentadecanoic acid and 9-Octadecenal, (Z)-/Olealdehyde/cis-9-Octadecenal/Z-9-Octadecenal, were also found in small quantities in all the treatment groups (Figures 1-4). The pentadecanoic acid composition varied from 4.49% (monoammonium phosphate treatment) to 6.92% (triple superphosphate treatment), while that of 9-Octadecenal, (Z)-/Olealdehyde/cis-9-Octadecenal/Z-9-Octadecenal varied from 1.2% (triple superphosphate treatment) to 3.27% (diammonium phosphate treatment). The second major compound was different among the treatment groups. 7-Tetradecenal, (Z)/Z-7-Tetradecenal/(7Z)-7-Tetradecenal, being the second major component in the control (24.3%) and monoammonium phosphate (18.41%) treatments, was not detected in the diammonium phosphate or triple superphosphate treatments. On the other hand, cis-9-hexadecenal, being the second major component in the diammonium phosphate (25.84%) and triple superphosphate (17.03%) treatments, was not detected in the control and monoammonium treatments. The control and triple superphosphate contained similar amounts of 9.9treatments Dimethoxybicyclo[3.3.1]nona-2,4-dione (approximately 1.5%) and erucic acid (approximately 3-4.94%), while the monoammonium phosphate and diammonium phosphate treatments contained similar amount of 2,5-Dimethyl-[1,3]dioxane-4carboxaldehyde (3-5%) and E,E,Z-1,3,12-Nonadecatriene-5,14-diol (2-3%). Several unique components were detected in each treatment, as shown in Table 4.

Radon concentration

Relation between the track density and radon concentration is highly correlated as shown from *Figure 5*. The data in *Figure 6* show the values of radon concentration under different types of studied fertilizers. Radon concentration for the control was 117 Bq/m³, while radon concentration for soils treated with MAP, DAP, and TAP was 136.5 Bq/m³, 169 Bq/m³, and 533 Bq/m³, respectively. These results were after an exposure time of 80 days. However, after 120 days of exposure, the radon concentration was less than that of 80 days of exposure. The maximum value of 164.9 Bq/m³ was obtained by treatment with TSP. The minimum value was 65.1 Bq/m³, with MAP. The value of 82.5 Bq/m³ was recorded for DAP treatment. TSP gave higher results than DAP and MAP treatments.

Data in *Table 2* indicated that the concentration of chlorophyll a was higher than chlorophyll b in plants treated by TSP. This increase is consistent with the observed increase recorded by Marcu et al. (2013). In addition, data in *Table 1* showed significant decrease in leaves and root dry weight in plants treated by TSP. Our results are in agreement with Foyer and Noctor (2016), Beyaz et al. (2016), Sengupta et al. (2013), and Marcu et al. (2012) who stated that plants exposed to radiation stress showed significant decrease in in different growth parameters in different plant species. Bayrak et al. (2018) mentioned that relatively large contamination of natural radionuclides in phosphate fertilizers contaminate the agricultural land and the environment during cultivation and have the potential to transfer to the living beings.

Treatment No.	Control	(%)	Monoammonium phosphate	(%)	Diammonium phosphate	(%)	Triple superphosphate	(%)
1	Pentanenitrile, 5-(methylthio)-	51.42	Pentanenitrile, 5- (methylthio)-	48.22	Pentanenitrile, 5-(methylthio)-	34.73	Pentanenitrile, 5-(methylthio)-	55.52
2	3(2H)-Isothiazolone, 2-methyl-	6.76	2,5-Dimethyl- [1,3]dioxane-4- carboxaldehyde	5.28	2,5-Dimethyl-[1,3]dioxane-4- carboxaldehyde	3.41	3(2H)-Isothiazolone, 2- methyl-	6.37
3	Pentadecanoic acid	6.69	Pentadecanoic acid	4.49	Pentadecanoic acid	6.01	Pentadecanoic acid	6.92
4	7-Tetradecenal, (Z)- Z-7- Tetradecenal (7Z)-7- Tetradecenal	24.3	7-Tetradecenal, (Z)- Z- 7-Tetradecenal (7Z)-7- Tetradecenal	18.41	cis-9-Hexadecenal	25.84	Oxacycloheptadec-8-en-2-one, (8Z)	8.13
5	9,9- Dimethoxybicyclo[3.3.1]nona- 2,4-dione	1.57	cis-1,2- Cyclododecanediol	1.29	9-Octadecenal, (Z)- Olealdehyde cis-9-Octadecenal Oleylaldehyde Z-9-Octadecenal (9Z)-9- Octadecenal	3.27	cis-9-Hexadecenal	17.03
6	9-Octadecenal, (Z)- Olealdehyde cis-9-Octadecenal Oleylaldehyde Z-9- Octadecenal (9Z)-9- Octadecenal	1.46	9-Octadecenal, (Z)- Olealdehyde cis-9- Octadecenal Oleylaldehyde Z-9- Octadecenal (9Z)-9- Octadecenal	2.55	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	9.58	9,9- Dimethoxybicyclo[3.3.1]nona- 2,4-dione	1.42
7	Erucic acid	4.93	3- Cyclohexylnonadecane	17.29	(R)-(-)-14-Methyl-8-hexadecyn-1- ol/14-Methyl-8-hexadecyn-1-ol	14.07	9-Octadecenal, (Z)- Olealdehyde cis-9- Octadecenal Oleylaldehyde Z- 9-Octadecenal (9Z)-9- Octadecenal	1.2
8	.gammaSitosterol	2.87	E,E,Z-1,3,12- Nonadecatriene-5,14- diol	2.47	E,E,Z-1,3,12-Nonadecatriene-5,14- diol	3.09	Erucic acid	3.41

 Table 3. Chemical constituents of Eruca sativa methanol extract in control and each treatment



Figure 1. GC-MS of Eruca sativa methanol extract in control



Figure 2. GC-MS of Eruca sativa methanol extract in monoammonium phosphate



Figure 3. GC-MS of Eruca sativa methanol extract in diammonium phosphate



Figure 4. GC-MS of Eruca sativa methanol extract in triple ammonium phosphate

Treatment	Component	Percentage (%)
Control	gamma-Sitosterol	2.87
Managementer al accelerate	3-Cyclohexylnonadecane	17.29
Monoammonium phosphate	cis-1,2-Cyclododecanediol	1.29
Diammonium phosphoto	(R)-(-)-14-Methyl-8-hexadecyn-1-ol/14-Methyl-8- hexadecyn-1-ol	14.07
	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)- diepoxy-	9.58
Triple super phosphate	Oxacycloheptadec-8-en-2-one, (8Z)	8.13

Table 4. Unique GC-MS components in control and each treatment of Eruca sativa methanolextract



Figure 5. Relation between the track density and radon concentration are a good correlation (R=0.99) has been Slop of the linear relation is 12.65



Figure 6. Radon concentration in soil treated with different types of fertilizers

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Conclusion

All three types of phosphate fertilizer enhanced the growth of *Eruca sativa*; among them, monoammonium phosphate resulted in the highest plant growth. However, no significant increase in yield was observed with any of the fertilizer treatments. Consistent with the plant growth enhancement, an increase in photosynthetic pigments contents was also observed in the presence of all three types of phosphate fertilizers. All examined fertilizers improved the soil quality by increasing the organic matter, total nitrogen, as well as available phosphorus and potassium contents in the soil. TSP led to the most organic matter, as well as available phosphorus and potassium contents in the soil. Subsequently, plants grown with TSP also contained the highest phosphorus content in their leaves. The result from the radon activity measurement indicated that soil treated with TSP accumulated the highest radon activity. Taken together with the results from our study, we thus propose the use of MAP due to its low radon activity accumulation in soil and highest growth enhancement.

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