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Full Length Research Paper

Influence of selenium on the antioxidant activity of Mas cotek (*Ficus deltoidea*) as affected by soil acidity and phosphorus (P) fertilization

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A glasshouse experiment was carried out to investigate the effect of selenium on the antioxidant activity of *Ficus deltoidea* (Mas cotek) at the selenium rates of 0, 10, 20, 40, and 50 g Se/ha in limed (2 t/ha $CaCO_3$) and unlimed soils treated with and without phosphorus (P) fertilizer (100 kg P_2O_5/ha). The experimental set up was a complete randomized factorial design with four replications. Two months old seedlings were transplanted into polybags containing 10 kg soil and after 4 months the plants were harvested for selenium and antioxidant analyses. Selenium in the plant tissue was extracted using the dry ashing method and determined with the graphite furnace atomic absorption spectrophotometer. Meanwhile, for biochemical analyses, the antioxidant activity was determined by measuring the free radical scavenging effect with 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) in the extract of the dried leaf sample. From this study, selenium content in Mas cotek was significantly increased with selenium application, and the highest selenium uptake was recorded when lime, P fertilizer, and 50 g Se/ha were applied. In terms of the antioxidant activity, Se, in general, augments antioxidant compound in Mas cotek leaves, and these compounds are induced more effectively by higher Se concentration in the soils. In short, for maximum antioxidant activity and Se content, without a major loss in yield, the recommended rate is 50 g Se/ha when lime and P fertilizer were applied.

Key words: Soil-plant-antioxidant relationship, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), selenite (Se^{4+}) fertilization, acid soil, liming.

INTRODUCTION

Selenium (Se) was first recognized as an essential micronutrient for humans and animals in the late 1950s (Pedrero and Madrid, 2008). It is regarded as an antioxidant in animal and human nutrition (Mora et al., 2008). Selenium participates in a large number of biological processes in the human body. This element plays a prominent role in glutathione peroxides (GSH-Px) selenoproteins for the cellular antioxidant defense system, the prevention of DNA damage, cancer initiation and cancer progression. The anti-carcinogenic activities of some Se forms against colon, lung, and skin, and other types of cancer have been stated (Pezzarosa et al., 2007). The role of Se in the detoxification of heavy metals such as mercury and lead are also important for the human body. Certain Se compounds have been claimed to prevent carcinoma, slow the aging process, and enhance sexual activities (Maihara et al., 2004).

The antioxidant properties justify the increasing interest in growing Se-enriched plants, which represents an

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important source of this element in the human diet. Selenium can enter the food chain through plants from the soils naturally or due to anthropogenic activities. Selenium content in soil determines the amount of Se in plants that are grown in that soil, and on top of that, the site conditions affecting the bioavailability of the element are also important. Selenium is hardly fixed in the soil and highly available for the plants where the soil pH is high and precipitation and leaching is low, whereas high acidity, high level of sulfur, and complexation with heavy metals, iron, and aluminium decreases Se availability (Giray and Hincal, 2004). Pyrzynska (2008) stated that diet with less than 0.1 µg/g Se result in deficiency, while regular consumption of food containing more than 1 µg/g Se results in toxicity. There is no published information on the status of Se in Malaysian soils. However, the unpublished data of preliminary study on baseline heavy metals levels of Malaysian soils (Zarcinas et al., 2004) indicate that the Se concentration of the Malaysian soils ranges from 0.008 to 7 mg/kg with mean concentration of 0.7 mg/kg. Selenium concentrations in plants are also quite low (0.016 to 1.392 mg/kg) with mean concentration of 0.09 mg/kg, which can lead to Se deficiency in human being. Therefore, a strategy should be developed to increase its intake either through consumption of plants or in the form of liquid infusion of herbal teas.

The market for medicinal plants and spices in Malaysia is expected to increase to 71400 tonnes within the next two years. It is estimated that the annual growth rate will be 9% by the year 2011. Mas cotek is one of the medicinal plants that has been identified to meet the requirements of the market. With growing interest in medicinal plants as a source of new pharmaceutical products and the increasing demand for medicinal plant products in Malaysia, it is expected that Mas cotek which is commonly used as herbal teas can provide a good source of Se supply. Moreover, a previous study (Hu et al., 2002) demonstrated that tea leaves were a safe and effective source of supply of the human Se intake. The role of Se as an antioxidant suggests that Se addition to the soil may improve the plant quality. Plants take up part of the supplemented Se applied to soil and metabolize it into organically bound Se, which is safer than the dietary supply of Se (Hu et al., 2002). In Se-deficient areas, the Se content of plants can be increased by adding selenite or selenate to the soil. Selenate is weekly adsorbed on the soil and also easily leached. In contrast, selenite reacts strongly with soil constituents and remains in the soil over a long period representing a long-term source of bioavailable Se (Mora et al., 2008). Due to its slow release characteristics, the selenite is considered more economical than selenate. Therefore, selenite was preferred in the present study to investigate its uptake and to understand its influence on the antioxidant property of Mas cotek.

A study by Mora et al. (2008), shows that the application of lime to ameliorate soil acidity can reduce Se sorption by soil and therefore, increase Se uptake by plants. On the other hand, the use of fertilizers containing phosphate (the most limiting nutrient in highly weathered acid tropical soil) should be expected to raise Se availability to plants, because phosphates strongly compete with Se for sorption sites in the soil system (Mora et al., 2008). Uptake and accumulation of Se by plants also is determined by its concentration and chemical forms, the presence of competing ions, and the affinity of a particular plant to absorb and metabolize Se (Pyrzynska, 2008). Plants vary considerably in their ability to tolerate Se. To date, there is no evidence of Se accumulation and tolerance in Mas cotek. Moreover, the contribution of Se to the antioxidative system of selenite-treated Mas cotek plants has not been reported. Hence, the main objective of this study was to determine the Se content and its influence on the antioxidant activity as affected by soil acidity and phosphorus (P) fertilization of Mas cotek.

MATERIALS AND METHODS

Screening of Se concentration in commercial Mas cotek herbal teas

To confirm the low Se content in plants grown in the highly weathered Malaysian soils, 15 commercial Mas cotek herbal teas were purchased randomly in the local market to screen for Se levels. The selected products did not contain any added ingredients, that is, they were a 100% Mas cotek foliar tissue as stated in the labels of the products. Selenium concentrations were extracted by the dry ashing method and taken up in 10% HNO_3 acid solution (Leo and James, 1973), and then, were determined with the Perkin Elmer Zeeman 4100ZL graphite furnace atomic absorption spectrophotometer.

Glasshouse experiments

Mas cotek from the female plants were chosen as they have large and thick leaves and were commonly used for herbal tea products in the market. Mas cotek plants were cultivated under an open shelter glasshouse condition. This experiment was conducted using the topsoil (0 to 20 cm) of Bungor Series (kaolinitic, iso-hyperthermic, Typic Paleudult) which is a common mineral soil in Malaysia and has a low Se level of 0.78 mg/kg.

Treatments consisted of two levels of lime (0 and 2 t/ha $CaCO_3$), two levels of P (0 and 100 kg P_2O_5/ha), and five levels of Se (0, 10, 20, 40, and 50 g Se/ha) in a completely randomized factorial design with four replicates. Each polybag was filled with 10 kg of soil and all the samples were fertilized with urea (100 kg N/ha) and muriate of potash (100 kg K_2O/ha). The liming rate of 2 t/ha $CaCO_3$ is the recommended rate for mineral soils of Malaysia (Shamsuddin, 1989), while the rates of N, K, and P fertilizer were based on the recommendation of Malaysia Agriculture Research Development Institute (MARDI) for the cultivation of Mas cotek. Eight weeks-old Mas cotek plants were transplanted into polybags containing 10 kg of soil. Selenium was added as sodium selenite and was applied in the solution form to the soil. Initial weight of each polybag (soil + plant) was recorded to see the influence of Se on plant growth. The plants were watered daily with deionized water during the whole growth stage (4 months). One of the Mas cotek plants was sent to the Herbarium Biodiversity Unit, Institute of Bioscience (IBS), Universiti Putra Malaysia, Selangor, Malaysia to be registered (ACP0066).

Before harvesting, the weight of all polybags (soil + plant) was again determined in order to obtain the data on the increment of fresh weight of the plant. Fresh and dry matter weight were then determined. Plants samples were dried at 65°C for 48 h to determine the dry weight and ground using a grinder to 1 mm (Leo and James, 1973) for total Se concentrations and biochemical analysis.

Plant analysis

Selenium content of Mas cotek *(Ficus deltoidea)*: Plant samples were dry ashed (Leo and James, 1973). Plant leaves were weighed in porcelain crucibles and placed in a muffle furnace. The temperature was increased till 500°C and maintained until white or grayish white ash was obtained. Subsequently, the crucible was taken out of the furnace and left to cool. The ash was moistened by adding a few drop of deionized water, and 2 ml concentrated HCl. Then, 10 ml of 20% HNO_3 was added and the crucible was placed in the water bath for 1 h. The mixture was then transferred into a 100 ml volumetric flask and was made up to volume with deionized water. Finally, the solution was shaken and filtered through Whatman No. 2 filter paper. Total Se concentration was analyzed by using the Perkin Elmer Zeeman 4100 ZL graphite furnace atomic absorption spectrophotometer. The Se content was calculated using the formula, Se content in plant (µg/plant) = Se concentration in leaves (µg/g) × dry weight of leaves (g).

Antioxidant activity of Mas cotek *(F. deltoidea)*: The influence of Se on the antioxidant ability were determined by measuring the free radical scavenging effect with 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) in the extracts from the dried leaf samples according to the method of Wong et al. (2006). Also, the extraction of antioxidant compounds was conducted employing the method of Wong et al. (2006). A total of 0.5 g dried leaves was placed in 150 ml conical flask and the flask was covered with aluminium foil. The conical flasks containing the samples were placed on an orbital shaker at room temperature for 1 h in the dark. After 1 h, the samples were filtered using a Whatman No. 1 filter paper and the extracts were stored in the freezer at -80°C.

DPPH free radical scavenging assay was measured using DPPH free radical test. The initial absorbance of DPPH in methanol was measured using a Spectramax+ 384 spectrophotometer at the wavelength of 515 nm until the absorbance remained constant. A total of 40 µl of extracts was added to 3 ml of 0.1 mM methalonic DPPH solution. The mixture was incubated at room temperature for 30 min before the change in absorbance at 515 nm was measured. The percent of inhibition was calculated using the following formula.

Inhibition (%) = [(A515 of control – A515 of sample)/A515 of control] × 100

where A stand for absorbance at wavelength of 515 nm.

Soil analysis

Extractable Se of the soil: At the end of the experiment, available Se from each treatment was extracted using the dilute double acid extractant (0.05 N HCl + 0.025 N H_2SO_4) (Mehlich, 1953). For the extraction, 5 g air-dried sieved soil (less than 2 mm fraction) was weighed into a conical flask and 25 ml extracting solution (0.05 N HCl + 0.025 N H_2SO_4) was added. The mixture was shaken at 180 rpm for 15 min and filtered with Whatman No. 42 filter paper. Selenium present in the filtered solutions was then determined by using the Perkin Elmer Zeeman 4100ZL graphite furnace atomic absorption spectrophotometer.

Soil pH: Soil pH was determined using radiometer 827 pH Lab meter in a 1:2.5 (w/v) soil to distilled water ratio (Black et al., 1965).

Extractable P: Phosphorus was extracted by the Bray and Kurtz 2 method (Bray and Kurtz, 1945) and analyzed by colorimetric method using the Lachat auto-analyzer instrument; QuikChem Series 8000 at a wavelength of 882 nm.

Statistical analysis

Original data were subjected to analysis of variance (ANOVA) and significant differences of the means of treatments were separated using Tukey test at 0.05 significance level of probability. Contrast analyses were also conducted using SAS 9.1 Software (Copyright © 2002-2003 by SAS Institute Inc Cary, NC, USA) to determine any significant difference in terms of Se content and its antioxidant activity between plants grown in limed and unlimed soils and with and without P fertilizer addition. No transformation of the original data was performed.

RESULTS AND DISCUSSION

Result of the preliminary study of Mas cotek indicated that herbal teas (Table 1) are quite low in Se concentration (0 to 0.26 µg/g) with mean concentration of 0.08 ± 0.09 µg/g. In India, according to a study by Manjusha et al. (2007), Se content in their herbal teas is 0.19 ± 0.02 µg/g which is higher compared to the results in this study. This result confirms the claim that Se concentrations in plants grown in highly weathered tropical soil of Malaysia is very low. However, in Australia, Se content in its tea infusion is 0.005 µg/g (Marro, 1996). Other than that, a study by Choi et al. (2009) also state that in Korean green tea's and black tea drinks, Se content is already below the detection limit of analytical instrument (< 0.001 µg/g).

Selenium content in Mas cotek

The chemical properties of the soil samples used in this study before the treatments were applied showed that its original pH is 4.3, its extractable P concentration is 5.20 mg/kg, and its Se concentration is 0.09 mg/kg.

Effect of Se fertilizer rates on Se content in Mas cotek

The Se content in Mas cotek foliar tissue after fertilization of sodium selenite in soil is as shown in Figure 1. The Se content in Mas cotek tissue was significantly increased with selenite application. These results show that the more Se added, the higher the Se content, and the highest content (6.09 µg/plant) was found in plants treated with 50 g Se/ha when lime and P fertilizer were added.

Effect of soil acidity on Se content in Mas cotek

According to the treatments mentioned earlier, there are

Table 1. Selenium concentration (µg/g) in Mas cotek herbal teas.

Brand name of Mas cotek herbal tea	Selenium conc. in herbal teas (µg/g)
KB Teh Herba Premium	0.23
Petani The Mas Cotek	0.12
D'Mas 100% Mas Cotek	0.05
Polens Mas Cotek Herbal Tea	0.21
Herbal care Minuman Botani Mas cotek	0.15
Natureen Teh Mas Cotek	nd
N'Apis Mas Cotet Uncang	nd
Pilah Best Emas Cotek	nd
Gen 2F Mas Cotek	nd
Herbagus Teh Mas Cotek	0.05
aml Botanical Herbs Teh Herba Mas cotek	nd
Nani's Teh Herba Mas Cotek	nd
Minuman herba Mas Chotek (crystal liz resources)	0.11
Wd Herbs Warisan Dakam the D'Mas	0.26
Suria Minuman Botanical Mas Cotek	0.03

Nd: not detectable or below limit of detection.

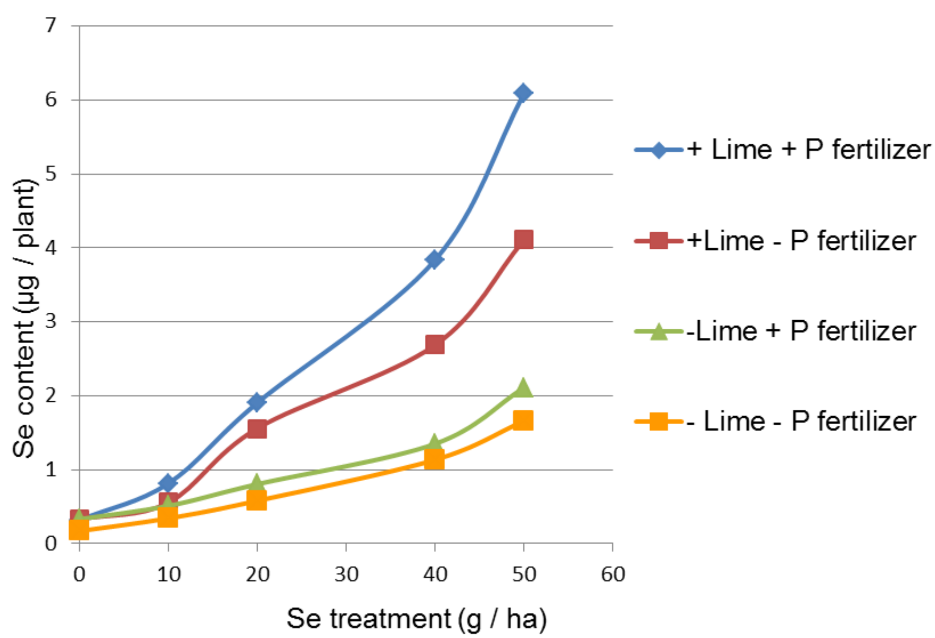

Figure 1. Selenium content in foliar samples of Mas cotek grown at various Se rates in limed and unlimed soils treated with P fertilizer.

plants that are grown on limed and unlimed soil. Lime additions to the soil indirectly increase the soil pH. In this study, the pH of limed soil was increased from 4.03 to 6.16. A study by Goh and Lim (2004) stated that at an increasing pH (pH 3 to 7), the adsorption of Se to the soil decreased. In fact, the adsorption of selenite to the soils fell from 83% at pH 3 to 59% at pH 7 according to the Goh and Lim (2004). Thus, Se availability is higher and may result in an increase in Se content of the plant. However, as observed in Figure 1, there was no difference in Se content among plants growing with 0 and 10 g Se/ha irrespective of the level of soil acidity or P applied. Nevertheless, when 20 g Se/ha and more of Se were applied, Se content was higher in the plants growing in the limed soils. From the soil pH data taken, limed soil has soil pH range from 5.93 to 6.60 (Mean = 6.16 ± 0.21), while in unlimed soil, its soil pH is quite low which is from 3.74 to 4.36 (Mean = 4.03 ± 0.21) only. This

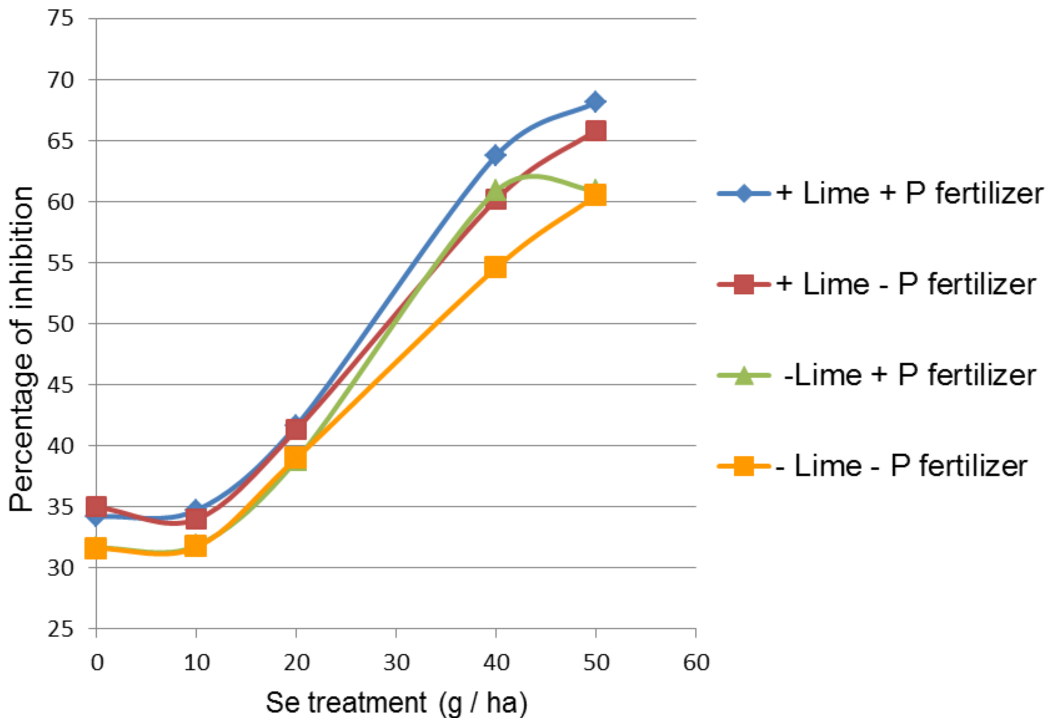

Figure 2. Antioxidant activities (percentage inhibition) of Mas cotek grown at various Se rates in limed and unlimed soils treated with P fertilizer.

means that at higher pH, Se adsorption to soil was probably lower as compared to the unlimed soils and Se availability to the plant has increased.

Effect of P fertilizer on Se content in Mas cotek

Figure 1 shows that the Se content in Mas cotek foliar tissue progressively increased with P fertilizer addition irrespective of the level of soil acidity. A study by Nakamaru et al. (2006) on the effect of phosphate concentration on the Se sorption onto soils shows that the added phosphate inhibited the Se sorption in the soils. These observations suggested that selenite was more easily taken up by Mas cotek, thereby increasing its foliar Se content.

Antioxidant activity of Mas cotek

Effect of Se rates on the antioxidant activity of Mas cotek

The antioxidant activity of the Mas cotek foliar extracts determined by DPPH method significantly increased when 20 g Se/ha or more Se were applied (Figure 2). Below this rate of application, there was no significant difference in the antioxidant activity.

Effect of soil acidity and P fertilizer on antioxidant activity of Mas cotek

There was no significant difference in antioxidant activity between plants grown in limed and unlimed soil. The antioxidant activities were also not affected by the addition of P fertilizer. The antioxidant activities were related to selenite application rates rather than lime and/or P fertilizer additions.

Weight increment of Mas cotek

Effect of Se rates on Mas cotek growth

Selenium may have beneficial biological functions, acting as an antioxidant at low concentration and stimulating plant growth, whereas at higher concentrations it acts as a pro-oxidant, reducing the yield (Pezzarossa et al., 2007). As shown in Figure 3, increasing Se supplied can result in reduction in plant weight. The more Se added, the lower the increment of plant weight.

Effect of soil acidity and P fertilizer on Mas cotek growth

Figure 3 shows that the lowest increment in plant weight

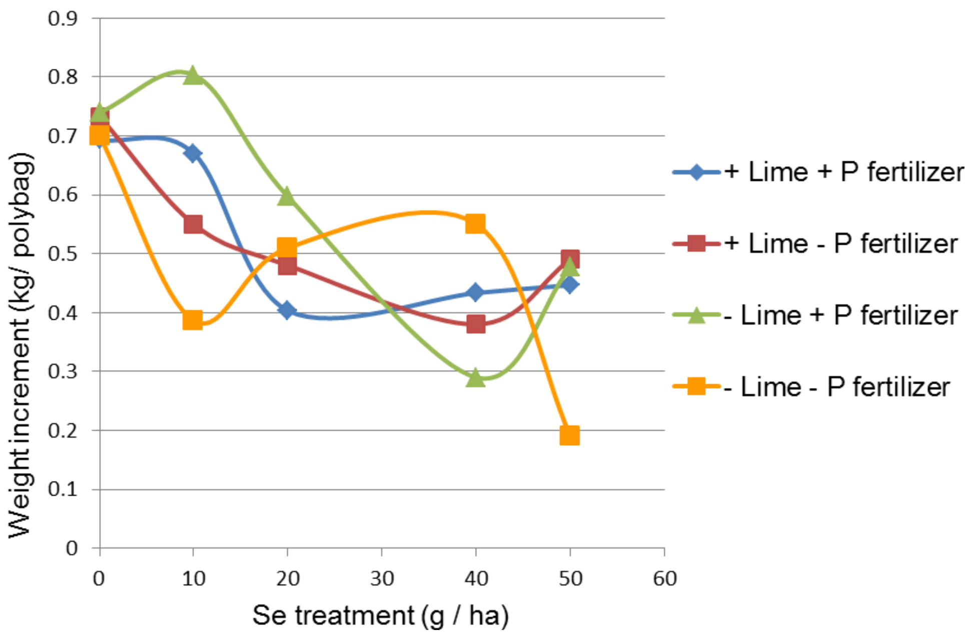

Figure 3. Increment in weight (kg) of Mas cotek grown at various Se rates in limed and unlimed soils treated with P fertilizer.

was recorded in unlimed soil when no P was added. There was no significant difference in weight increment of Mas cotek grown in limed and unlimed soils. A similar result was found on the effect of P fertilizer on the plant growth. Taking into consideration all the parameters factored in this study, we recommend that the most appropriate application rate for the cultivation of Mas cotek is 50 g Se/ha with lime and P fertilizer addition.

Conclusion

It is concluded that Se uptake by Mas cotek significantly increased with selenite application to the soils. However, the Se uptake by Mas cotek is higher if lime and P fertilizer were applied to the soil. In terms of antioxidant activity, Se in general augments antioxidant compounds in Mas cotek leaves. These compounds were more effectively induced by higher Se concentration in the soils. It is recommended that farmers fertilize Mas cotek with Se at the rate of 50 g Se/ha with lime and P fertilizer addition. As a result, both the Se intake by humans and the antioxidant activity of herbal teas can be increased, although this rate will gradually affect plant growth.

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