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Review Article

Influence of Exogenous Application of Selenium on Glucosinolate Accumulation in Broccoli

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ABSTRACT

Several studies focused towards improving the quality of nutritional plants, due to limited microelements and macroelements in various agricultural areas. Micronutrient elements as selenium are beneficial for human health at small quantities, but when taken in large quantities lead to toxicity. It seems that, plants that are rich in sulphur compounds such as cabbage and broccoli (brassicaceae), previously cruciferous family, accumulate high selenium concentration. Broccoli plants are good source of bioactive compounds (phytochemicals compounds), as vitamins, essential minerals glucosinolate, and phenolic compounds. Glucosinolates (GLs) classified as a secondary metabolites, mainly found in Brassicaceae, which may act against various types of disease as cancers. Exogenous application of selenium in broccoli stimulates the accumulation of glucosinolates concentration through promoting gene transcript involved in glucosinolate production. The main aim of this study is to determine the relationship between Se availability in soil and accumulation of glucosinolates compounds in broccoli sprouts (Immature inflorescence).

Keywords: *Selenium, Glucosinolate, micronutrient, Broccoli***.**

1. INTRODUCTION

Selenium (Se) is a vital element, which it is critical for living organisms health as a result of abilities to stimulate systems of antioxidant defence, but it has negative effects at excess amount (Wrobel *et al*., 2016). Recommended Dietary Allowance (RDA) of Se consumption is about for 55–75 microgram per day for adult human over 19 years. People will be faced malnutrition, when the level of daily consumed is low 40-µg, while toxicity symptom at consumed over 400 µg, (Johnson *et al*., 2003). Concentration of Se in soil, which it is estimate the uptake by plants. Therefore, consumption of agri food products- are the main source of selenium uptake. The total concentration of Se soils worldwide ranges between 0.01–2.0 mg kg−1 (Schiavon *et al.*, 2016).

Cancer one of the most diseases that cause human mortality. In 2020, International Agency for Research on Cancer (IARC) reported that 19.3 million new cancer cases and 10.0 million deaths, the global cancer burden is expected to be increase in 2040 compared to 2020 (Yuan *et al.*,2022). Bioactive compounds present in

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the cruciferous vegetables, which are high in phytochemical compounds (MeliM *et al*., 2022). Glucosinolate, a natural products found in brassicaceae family (Tian *et al*., 2018). Myrosinase enzyme which hydrolysis glucosinolates to produces bioactive volatile pungent compounds, that increase the benefits of this family (Yan and Chen. 2007).

1.1. Broccoli sprouts

Brassica oleracea var. Italica has a two growing seasons related to the brassica vegetables. According to purchasers, broccoli was registered in the top six for vegetables in 2022 (Kresin, 2022). Broccoli is seemed abound in vitamin (E and B6), 88.5% water, 2.7% available carbohydrates, 3.8% protein, 0.2% fat, 1.1% minerals, minor amounts of thiamine, riboflavin, folate (USDA-ARS, 2022). Broccoli consumption per capita increased with years from 1985 to 2021 (Davis *et al*., 2022). Le *et al*., (2020) reported that, edible sprouts of broccoli are known as fortified foods rich in phytonutrients, antioxidant, and antibacterial properties. Epidemiological studies have estimated that there are positively correlation between consumption of broccoli florets and decreased risk of many kinds of diseases (Bachiega *et al*., 2016). Broccoli sprout is a grown at cold temperature between 15 and 18°C, the seedling will develop after four to six weeks of planting and required more than two months to mature for harvest and harvested when immature flower diameters 18 cm length are bound together are still green. (Theodore *et al.*, 2022).

1.2. Bioactive Compounds in Broccoli

Bioactive compounds are phytochemicals found mainly in grains, fruits, and vegetables, which are capability of modulating metabolic processes and promotion of healthy (Santos *et al*., 2019). Broccoli is listed as an enriched food and good source of bioactive compounds. It also contains high content of organosulfur compound, is organic compounds that contain sulphur that inhibition different kinds of cancers (Chaudhary *et al*., 2014). In many studies, briefed that glucosinolates and related compounds are the primary set of phytochemicals presented in brassica plants, also analyzed of essential nutrients and phenolic compounds (Fig. 1). The young broccoli sprouts have higher quantity of glucosinolates levels 10-100 times than mature sprouts (Fahey *et al*., 1997).

1.3. Glucosinolates (GSLs)

They are set of natural plant metabolites derived from amino acids including abundant of nitrogen (N) and sulphur (S) presented mainly in cruciferous family such as cabbage, broccoli, and cauliflower (Ishida *et al.*, 2014). The glucosinolates are a large group of sulphur-containing glucosides found in brassica vegetables.. GSLs classified according to amino acid nature into three groups: aliphatic GSLs originated from methionine, aromatic GSLs that originated from phenylalanine, and indole GSLs that originated from tryptophan (Agerbirk and Olsen., 2012). Previous studies have identified over 120 distinct types of glucosinolates across various plant species. Among these, glucoraphanin stands out as one of the most prominent in broccoli sprouts. When hydrolyzed by the enzyme myrosinase, it produces sulforaphane, a compound recognized for its significant anti-carcinogenic potential (Fahey *et al.*, 2001). GLSs are hydrolysed by plant myrosinase enzymes, which present endogenously in myrosin cells (the special type of plant cell in vacuole of brassicaceae family), these enzyme helps breakdown GLSs when damage occurs in plant tissue due to wounding, insect or pathogen attack (Fahey *et al*., 2012). This reaction producing compounds as shown in Fig.2.

In broccoli, glucosinolate concentrations are based on the plant species and parts, environmental conditions stages of development, fertilization concentrations and plantation year (Bhandari

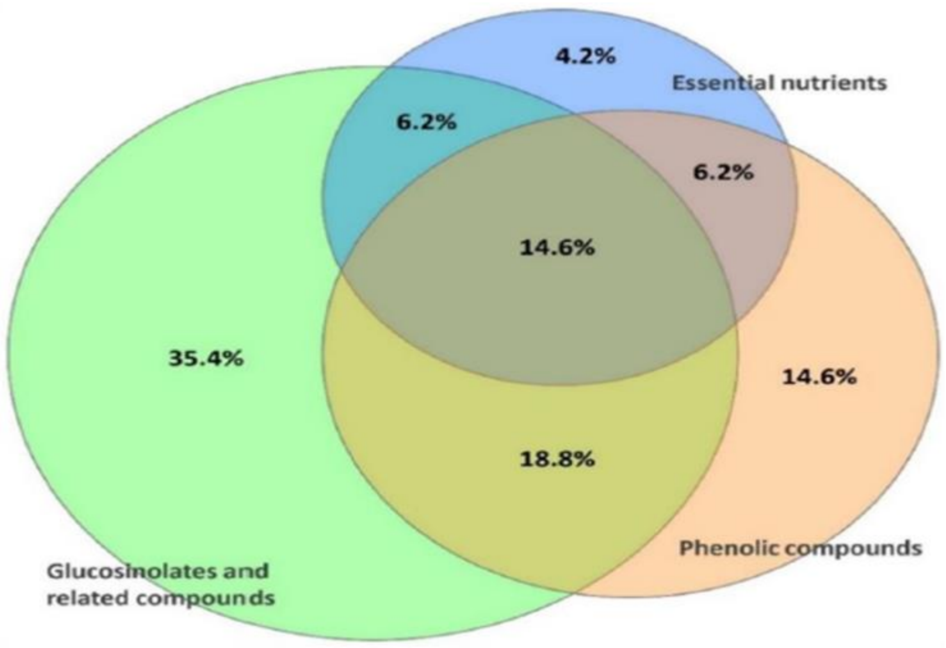

Fig. (1): Bioactive compounds analyzed in broccoli sprouts (Le *et al.,* 2020).

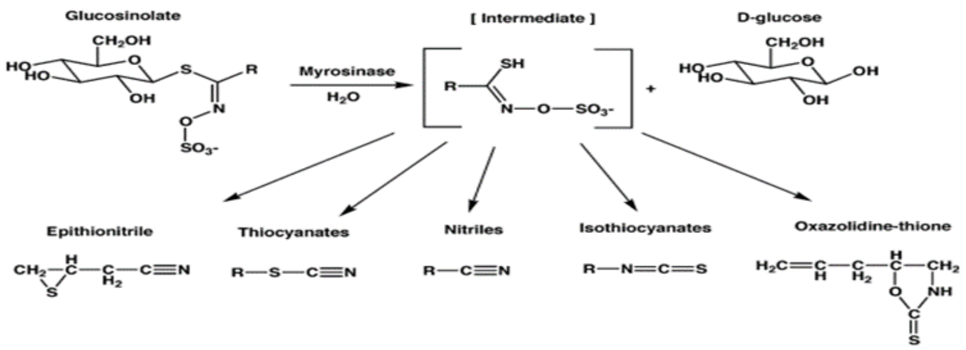

Fig. (2): Main products of glucosinolate hydrolysis (Rask *et al*., 2000)

*et a*l., 2018). The quantity of glucosinolate is influenced by different environmental elements (light, temperature, and soil type) (Cartea *et al*., 2008).

1.4. GLSs in Brassicacaea vegetables

Brassicaceae family is formerly called Cruciferous species, are widely grown all over the world. Brassicaceae vegetables are known for their pungent sulphurous aroma and tart taste. GSLs are the substances responsible for these flavor and odor (Chowdhury.,2022). Qualitative and quantitative GLs content are affected with many conditions: growing season, plant variety, cultivation conditions, and where GLs present in plant organs (Thomsen *et al.*, 2018). Under biotic and abiotic stress conditions, Brassica species activate their defense mechanisms, leading to the accumulation and production of organosulfur compounds such as glucosinolates (Sun *et al*., 2012).

1.5. Glucosinolates and human health

Afshin et al. (2019) reported that a low intake of fruits and vegetables, coupled with excessive sugar consumption, is a major risk factor for chronic diseases. In 2002, the World Health Organization (WHO) recommended that adults consume at least three portions of vegetables and two portions of fruit daily. Previous studies have also shown that the health benefits of vegetable crops vary; however, cruciferous vegetables are particularly valuable due to their rich content of the phytochemical glucosinolates (Aune *et al.,* 2017). Moreover, supplementation with glucosinolate-rich foods has been shown to reduce adipose tissue accumulation, alleviate liver disease, and improve diabetic conditions. However, excessive consumption of broccoli and other Brassica vegetables may lead to toxicity, as they can interfere with thyroid hormone function by enlarging the thyroid gland.

1.6. Selenium (Se)

Selenium is an essential trace element required by both animals and humans. It was discovered in 1816 by the Swedish chemist Jöns Jacob Berzelius. The name 'selenium' is derived from the Greek word Selene, meaning 'moon'. (Bodnar *et al.*,2012). It is available and occurring naturally element in the environment. At low concentration, has a useful impact on human and animals, which plays an important role in reducing threat of cancer, enhancing immunity of human, and alleviating heavy metals toxicity((Wrobel *et al.,* 2016). Recommended Dietary Allowance for selenium, recommended that adult male consumption 70 µg/day and females is 55 µg/ day of Se. When the level of daily consumed is less than forty micrograms people will be dietary deficiency, but more than 400 micrograms, people will be toxicity symptoms (Johnson *et al.,*2003). High concentration of Se promote oxidative stress in cells due to over generation of reactive oxygen species (ROS) and leads to protein tyrosine nitration (Gupta and Gupta., 2017). Whereas, selenium at low concentrations have more beneficial for a lots of plants through stimulating plants growth and antioxidant defense systems (Chauhan *et al*.,2019).

1.7. Se in Soil

The concentration of selenium in soil varies depending on soil type; sandy soils typically contain the lowest levels of selenium, whereas calcareous and organic soils tend to have the highest concentrations (1 El-Ramady *et al.,* 20147). Globally, the average selenium content in soil is approximately 0.44 mg kg^{-1}, typically ranging from 0.05 to 1.5 mg kg^{-1}. Higher selenium concentrations are often found in forest soils, calcareous soils, and the surface layers of volcanic soils (Garousi, 2017). The primary factors influencing the forms and behavior of selenium in soil include clay content, organic matter, soil pH, and the presence of hydroxides (El-Ramady *et al.*, 2014). Soil can be classified according to Se concentration as deficient soil when Se less than 0.125mg kg -1 , marginal soil (0.125 –

0.175mg kg -1), excessive soil, more than 3mg kg -1 (Kabata et al., 1992). Selenium in soil exists in four primary oxidation states: elemental selenium (Se^0), selenide (Se^{2-}), selenite (SeO_3^{2-}), and selenate (SeO_4^{2-}). The bioavailability of selenium is mainly influenced by its inorganic forms selenate and selenite that are more prevalent than organic forms in soil (Thiry *et al.*, 2012). According to Alfthan *et al.* (2014), the availability of selenium to plants and its mobility in soil are governed by several processes, including adsorption, hydrolysis, precipitation, and the formation of both organic and inorganic complexes.

1.8. Selenium uptake, accumulation in plant

Selenium uptake by plants is influenced by several factors, including developmental stage, plant species, selenium concentration and chemical form, as well as soil pH and salinity (Renkema *et al*.,2012). Plants primarily absorb selenium through their roots in the form of selenate (SeO_4^{2-}) or selenite (SeO_3^{2-}) (White *et al*., 2009). According to Jia *et a*l. (2018), low selenium levels have minimal effects on root morphology, whereas high concentrations can inhibit root elongation. Cappa *et al.* (2014) observed that selenium concentrations are generally higher in younger leaves compared to older ones during seedling growth. Fruits and vegetables typically contain low levels of selenium (Garousi, 2017). However, certain vegetables—such as broccoli, garlic, and onion—can accumulate selenium when grown in selenium-rich soils. Selenium adsorption is generally higher in uncultivated soils, whereas cultivated soils exhibit greater selenium mobility and availability due to the use of competing anions like phosphate in fertilizers (Lessa *et al*., 2016). Excessive application of sulfate and phosphate fertilizers can reduce selenium uptake by plants due to competition at the root level (Gupta and Gupta. 2017).

Based on selenium accumulation in plant tissues, plants are classified into three categories: non-accumulators, secondary accumulators, and hyperaccumulators (Saha *et al*., 2017). Secondary accumulators, including broccoli, accumulate between 100–1000 mg Se/kg dry weights; while non-accumulators, such as grasses, contain less than 100 mg Se/kg dry weight (Kushwaha *et al.*, 2022).

The process of selenium (Se) application through irrigation water, its uptake by plant roots, and subsequent translocation to the leaves. Within the leaves, Se undergoes metabolism via the sulfur assimilation pathway in plastids, eventually leading to its volatilization into the atmosphere (Pilon Smits *et al*., 2010). The inorganic form of selenium is first converted to selenite, a reaction requiring two enzymes: ATP sulfurase and APS reductase (Gupta and Gupta. 2017). Following this, selenite is further reduced to selenide by the enzyme sulfate reductase, a step that can also be facilitated by glutathione (Wallenberg *et al*., 2010). However, the metabolic mechanisms involving both organic and inorganic forms of selenium remain underexplored and require further research (Chao *et al*.,2022).

1.9. Selenium and plant

Worldwide, according to the FAO (2017), food production in soils with low mineral availability has contributed to nutritional deficiencies in humans. The application of selenium, either through foliar sprays or soil supplementation, has been shown to enhance antioxidant enzyme activity, which helps scavenge reactive oxygen species (ROS) (Reis *et al.*, 2017). Selenium can also mitigate the negative effects of various environmental stresses such as salinity (Habibi *et al*., 2017), water scarcity (Rady *et al.*, 2020), extreme temperatures—both high and low (Seliem *et al.*, 2020) and heavy metal toxicity (Shekari *et al*., 2019). Selenium enhances the integrity of cell membranes and decreases electrolyte leakage (Malik *et al*., 2012). It also promotes seed germination, supports plant growth and development, and improves the quality of crop yields (Zhou *et al.,* 2021). Through processes such as soil volatilization, internal translocation, and assimilation, selenium helps plants withstand adverse environmental conditions. Moreover, it offers protection against fungal pathogens, vertebrate herbivores, and other threats (Kushwaha *et al*., 2022).

1.10. Selenium and Glucosinolates in Broccoli Floret

The molecular mechanism by which selenium regulates glucosinolate (GSL) synthesis remains unclear (Wu *et al.*, 2022). Low doses of selenium supplementation have been reported to have minimal impact on glucosinolate accumulation (McKenzie *et al*., 2017). However, in broccoli, low selenium levels have been associated with reduced production of bioactive compounds (Dong *et al.*, 2001). Several studies suggest that the negative effects of selenium on glucosinolate content in broccoli sprouts may be linked to the direct influence of sulfur fertilizers on glucosinolate metabolism (Schiavon *et al*., 2016).

Treatment of broccoli with selenium has been shown to reduce the total phenolic acid content (Robbins *et al*., 2002). However, it simultaneously increases the total glucosinolate content (TGSL) and enhances antioxidant capacity (Barickman *et al.*, 2023). Significant changes occur in broccoli sprouts, including the synthesis of new compounds, interconversion of existing ones, a decrease in epithiospecifier proteins (ESP), and an increase in myrosinase enzyme activity (Pérez-Balibrea *et al*.,2011). Among the plant parts, immature broccoli inflorescences accumulate more selenium compared to roots, stems, and leaves (Sindelarova *et al.*, 2015). Foliar application of selenium is considered an effective method for enriching broccoli with this micronutrient. Kim and Juvik (2011) observed that broccoli cultivars with naturally higher glucosinolate levels showed a greater reduction in these compounds after selenium fertilization than cultivars with lower baseline glucosinolate concentrations. Conversely, Wu *et al.* (2022) reported that exogenous selenium treatment in broccoli can promote glucosinolate accumulation by upregulating the expression of genes involved in GSL biosynthesis.

Conclusion

The effect of exogenous selenium application on glucosinolate accumulation varies depending on the plant's developmental stage, the level of selenium fertilization, and the species. High concentrations of selenium tend to reduce glucosinolate accumulation. Broccoli is classified as a secondary accumulator, capable of accumulating between 50 to 100 mg Se kg^{-1}. Several studies have reported that selenium application increases both glucosinolate content and myrosinase enzyme activity in broccoli sprouts, while simultaneously decreasing phenolic compound levels. Additionally, selenium enhances the antioxidant capacity and total glucosinolate content (TGSL).

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تأثير الرش الخارجي للسيلينيوم على تراكم الجلوكوزينولات في البروكلي

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ملخص

ركزت العديد من الدراسات على تحسين جودة النباتات الغذائية، بسبب محدودية العناصر الغذائية الصغرى والكبرى في مختلف المناطق الزراعية. تُعتبر العناصر الغذائية الصغرى مثل السيلينيوم مفيدة لصحة الإنسان بكميات صغيرة، ولكن عند تناولها بكميات كبيرة تؤدي إلى التسمم. يبدو أن النباتات الغنية بالمركبات الكبريتية مثل عائلة الصليبيات (الملفوف والبروكلي) تتراكم بها تركيزات عالية من السيلينيوم. تُعتبر نباتات البروكلي مصدرًا جيدًا للمركبات النشطة حيويًا (المركبات الكيميائية النباتية)، مثل الفيتامينات والمعادن الأساسية والجلوكوزينولات، والمركبات الفينولية. تُصنف الجلوكوزينولات (GLs) على أنها مستقبلات ثانوية، تُوجد بشكل أساسي في العائلة الصليبية، وقد تعمل ضد أنواع مختلفة من الأمراض مثل السرطانات. يُحفز الرش الخارجي للسيلينيوم في البروكلي تراكم تركيز الجلوكوزينولات من خلال تعزيز النقل الجيني المعني بإنتاج الجلوكوزينولات. الهدف الرئيسي من هذه الدراسة هو تحديد العلاقة بين توفر السيلينيوم في التربة وتراكم مركبات الجلوكوزينولات في نموات (النورات الزهرية الغير ناضجه) في البروكلي.