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Effect of Selenium Fortification on Vegetative and Reproductive Growth in Tomato (*Solanum Lycopersicum*)

KEYWORDS: Selenium, tomato, fortification.

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ABSTRACT *Nutritional research is the need of the hour, due to the prevailing food nutrition and sustainable agriculture practices. Among various micronutrients, selenium (Se) had been selected to be fortified in tomato plants, which is beneficial for mankind and the higher plants. It has positive effect on both vegetative and reproductive growth, when supplemented. The analysis of maturity and taste index along with the sensory traits prop up the Se fortification in tomatoes. Overall, foliar spray resulted in maximum selenium accumulation with enhanced plant growth and yield. Genetic characterization will enhance the percentage of organic Se accumulation in the fruit.*

INTRODUCTION

Vegetables are very the important source of nutritional consideration. Nutrients in the harvested products are not ready for the recommended dietary limit. Thus the cause behind nutrient depletion on crop cultivation should be concerted. Among the micronutrients, selenium (Se) had been selected; based on its role to improve the health. Being a powerful natural antioxidant, it is immune-stimulator, cardioprotective and anticarcinogenic (Golubkina, 2003). Se has long been recognized as an essential micronutrient, but the essentiality of Se to higher plants is still under debate (Terry, 2000 and Germ, 2007). The growth-promoting response of Se is mainly accompanied with the enhanced antioxidative capacity. Growth stimulating effect of Se had been frequently reported in many plants like lettuce (Xue, 2001), cabbage (Hajiboland, 2007) and potato (Seppanen, 2003).

Higher plants are thought not to require Se. But there are increasing sign that Se has beneficial functions. Agronomic biofortification of food crops with Se can improve their nutritive quality (Seppänen, 2010 and Stibilj, 2011). The window between its beneficial and deleterious effects is very narrow. Spraying leaves with Se increased the yield, due to better partitioning efficiency, as evidenced Djanaguiraman (2004). However, effect of Se on the time of flowering and other characteristics of reproductive growth was less investigated. Se ($SeO4^{2-}$) is taken up and assimilated through reduction pathway of sulfur ($SO4^{2-}$) (White, 2004).

Biofortification using selenium fertilizers must be performed under strict conditions, since over accumulation in the edible parts might be toxic for consumers. High Se concentration in the soil may not induce phytotoxic symptoms, but reduces the crop yield. The Se in plant is incorporated in the enzymes as selenocysteine, but there are many others organic forms of Se such as SeMet, MeSeCys (Ellis & Salt 2003). The less overt Se deficiency can affect human health in immunity, viral infection, reproduction (male fertility), thyroid function, asthma and inflammations (Rayman, 2002). Along with the prevention of cardiovascular disease (Stranges, 2006).

However, Se is not very abundant in soil nor in greenhouse production, most plants being cultivated using soilless substrates where Se availability is limited. Cultivation of plants enriched with selenium is an effective way of producing selenium rich foodstuffs thereby increase health benefits (Finley, 2001 and Lyons, 2005). This study aimed to investigate the effects of selenium on growth, selenium accumulation and some physiological characteristics in the tomato plants. Some physiological parameters during vegetative and reproductive growth were studied in concordant to the total selenium content in leaves, roots and fruits. Tomatoes are particularly interesting in this context, since selenium is found in their fruits mainly as selenium-containing proteins; that are known to be readily assimilable by man (Combs, 1997). However, results can differ significantly among different modes of fortification and the cultivars. Several concentrations were studied to identify an optimal Se fortification concentration with increased yield.

MATERIALS AND METHODS

Field Experiment

It was carried out in the greenhouse condition with 12h light and 12h dark period, at the temperature of 28-35°C at day and 20-28°C at night. Tomato seeds were purchased from the Super Agri Seeds Private Limited, India. The soil characteristics includes; pH of 7.7, nitrogen, potassium and phosphorous content as 84500, 500 and 20 kg/h respectively. Calcium carbonate content was in minimal evidence and EC value of 1.0 d/sm. The initial selenium content in the soil was 0.04 µg/g.

Fortification method includes; seed soaking, soil application and foliar spray. Each treatment includes five pots in triplicates. The 10 days old seedlings (two leaf stage) were transplanted to the plastic pots (7.5 × 15 cm) containing soil and sand mixture with fertilizers (cow dung, P and N in 3: 1: 1). After four weeks of seedlings growth, sodium selenate (Na_2SeO_4) was supplemented at four concentrations (0, 2, 4, 8 and 10 mg/L). Seed soaking involves 2 hours treatment, before sowing to the nursing bed for germination. In soil application and foliar spray method; sodium selenate was supplied, weekly once from 50 to 90 days growth stage directly to the soil and sprayed on the leaves respectively. The plants were maintained away from the rain water till their fruit yield for analysis, along with the control plants.

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Growth parameters
Plants were selected, based on their similar behavior and appearance. After sodium selenate treatment, the plants were uprooted for analysis of parameters like: relative water content (RWC), shoot length, number of leaflets per plant, number of branches per plant, root length and secondary root number. The physiological changes of selenium fortified tomato fruits were compared with the control for the number of flowers per plant, yield per plant and total yield, fruit biomass, soluble solid content (SSC), maturity and taste index, colour and brightness. The fruit samples were collected at their fully ripened stage and stored as pulp extract at -20°C for further analysis. Blanching was performed at 80°C for 10min before analysis.

Titratable acidity was determined using 0.1N NaOH to the endpoint of pH 8.2 and expressed as citric acidity percentage. In addition, the taste index and maturity were calculated using the equation proposed by Navez et al. (1999) starting from the Brix and the titratable acidity values as indicated in Herdandez Suarez et al. (2008):

Maturity = ◦Brix/acidity

Taste index = (◦Brix/20 ×acidity) + acidity

Total selenium content
The plant materials were rinsed with distilled water, dried at 70°C and 0.5 g was acid digested with perchloric acid and HCl reduction. Selenium content in leaves, root and fruits (at harvest, post-harvest and after blanching) were determined. The digests were analyzed by hydride generation atomic absorption spectrophotometry (HG-AAS) (Bharathidasan University, Tamil Nadu).

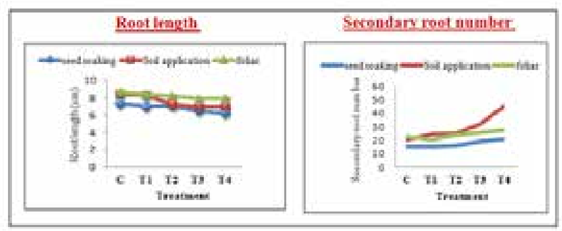
Tolerance index for selenium
The seeds were initially germinated on moist paper. The seedlings at two leaf stage were transferred to half strength liquid MS media with sodium selenate at respective concentrations for root length measurement. The Se tolerance index was calculated as root length grown in the presence of selenate divided by the root length on control medium and calculated to its percentage. The treatment was performed in triplicates.

Statistical Analysis
A minimum of three replicates was analyzed. Data were analyzed by the program Graphpad PRISM version 5.0 by one-way analysis of variance (ANOVA) and Duncan's multiple range tests to determine the significance of the difference among the samples, with a significance level of 0.05

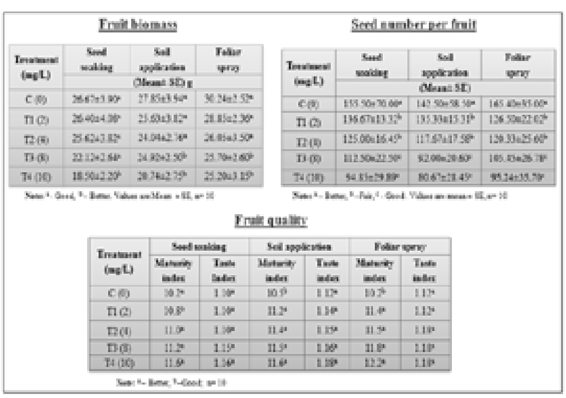
RESULTS AND DISCUSSION
Vegetative stage parameter
The functional foods are of increasing interest in the prevention and treatment of various diseases. Interest in Se metabolism derived primarily due to its nutrition and multiple health benefits. The data presented here provides new insight of selenium fortification in non-accumulator plants. For plants treated with Se, leaflet number and number of branches per plant increased significantly when fortified with selenium, under the concentrations studied. Similar reports were given in canola plants (Hajiboland, 2007). Regarding increase in the number of leaves upon Se treatment, a substantial augmentation in the photosynthesis is expected. However, application of higher Se concentrations reduced the relative water content in the leaves. Similar results were obtained in lettuce and tomato plants (Ahmed, 2010). It is reduced during any stress condition; as a consequence all nutrients are increased. Reduction in water content may be due to the increase in levels of sugars and antioxidants.

The restricted root elongation observed in higher concentration, might be due to root plasma membrane damage during selenium penetration into the root cells and disturbance in the mineral balance of the plant. Se accumulation increased the secondary root number with the increasing treatment concentrations (Figure 1). The Branching pattern offers lowest resistance and involves reduction in pathway length, which increased the secondary root number.

Figure 1: Characterization of root variation in selenium fortified tomato plant



Reproductive stage parameter
Selenium accelerated all stages of reproductive growth considerably in the concentrations studied. The length of inflorescence, fruit yield per plant and the total yield increased with increasing concentration. Foliar spray method showed the significant yield. The taste index >0.7 indicates the good quality fruits when compared to the control (Table 1). Similar reports were given by Pezzarossa (2014) and Golubkina (2003) in tomatoes. Timing of formation and total number of side branches were not influenced. The effect of Se on phytohormones balance and/or polyamine content could not be excluded. Polyamines implicated developmental processes including stimulation of cell division, embryogenesis, senescence, floral development and fruit ripening. Se enrichment not only could accelerate flowering and improve its yield, but also contributes to nutritional improvement. Similar yield increase was reported in lettuce and chicory plants (Malorgio, 2009).


Fruit biomass

Treatment (mg/L)	Seed soaking	Soil application	Foliar spray
	(Mean± SD) g		
C (0)	26.67±3.80^{a}	27.85±3.94^{a}	30.24±2.52^{a}
T1 (2)	26.40±4.30^{a}	23.63±3.12^{a}	28.83±2.30^{a}
T2 (4)	25.62±3.82^{a}	24.04±2.74^{a}	26.05±3.50^{a}
T3 (8)	22.12±2.64^{a}	24.92±2.50^{b}	25.70±2.60^{b}
T4 (10)	18.50±2.20^{b}	20.74±2.75^{b}	25.20±3.15^{b}

Note: ^{a}- Good, ^{b}- Better. Values are Mean ± SE, n= 10

Seed number per fruit

Treatment (mg/L)	Seed soaking	Soil application	Foliar spray
	(Mean± SE)		
C (0)	155.50±70.60^{a}	142.50±58.50^{a}	165.40±95.00^{a}
T1 (2)	136.67±13.32^{b}	135.30±15.31^{b}	126.50±22.02^{b}
T2 (4)	125.00±16.45^{b}	117.67±17.58^{a}	120.33±25.60^{a}
T3 (8)	112.50±22.50^{a}	92.00±20.60^{a}	105.83±26.70^{a}
T4 (10)	94.83±29.80^{a}	80.67±21.45^{a}	95.24±35.70^{a}

Note: ^{a}- Better, ^{b}- Fair, ^{c}- Good. Values are means± SE, n= 10

Fruit quality

Treatment (mg/L)	Seed soaking		Soil application		Foliar spray	
	Maturity index	Taste index	Maturity index	Taste index	Maturity index	Taste index
C (0)	10.7^{a}	1.10^{a}	10.9^{b}	1.12^{a}	10.7^{b}	1.12^{a}
T1 (2)	10.8^{b}	1.10^{a}	11.2^{a}	1.14^{a}	11.4^{a}	1.12^{a}
T2 (4)	11.0^{a}	1.10^{a}	11.4^{a}	1.15^{a}	11.5^{a}	1.10^{a}
T3 (8)	11.2^{a}	1.15^{a}	11.5^{a}	1.16^{a}	11.8^{a}	1.10^{a}
T4 (10)	11.6^{a}	1.16^{a}	11.6^{a}	1.18^{a}	12.2^{a}	1.10^{a}

Note: ^{a}- Better, ^{b}-Good; n= 10

Table 1: Selenium fortified tomato fruits analysis

Total selenium content
It increased in concordant to the increasing amounts of Se supplemented. The total Se content increased by 8, 22 and 35 percent by seed soaking, soil application and foliar spray method respectively (Figure 2). The total Se in T4 plant leaves were 32.25, 58 and 34.42μg/g DW in the same order. The peak value was reported as 26.52, 29.5

and 52.24µg/g DW in fruits developed by T4 concentration in the same order. A slight reduction was observed during post-harvest and blanching of the fruit samples. The rate of Se accumulation was 35.60 and 52.52% higher in the leaves and fruits respectively, when compared with the control plants. Among all, foliar spray method showed the significant selenium accumulation.

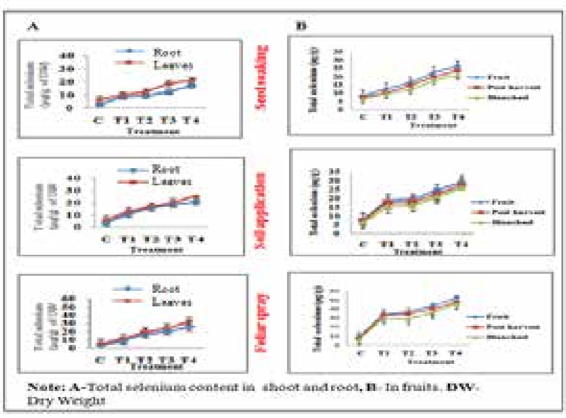


Figure 2: Total selenium content in selenium fortified tomato plant

Similar reports were observed in spinach, lettuce and chicory plants (Malorgio, 2009). Analogous Se accumulations were observed in the edible parts of cabbage, radish, garlic and onion on Se supplementation (Slekovec, 2005) and in tea plants (Hu, 2003). At lower concentrations, selenium stimulates the growth. While at high concentration, it acted as pro-oxidant, reducing the yields by inducing metabolic conflict. The growth provoking effect of Se may be related to its antioxidative role.

Index of tolerance for Se

The germination efficiency showed a significant decrease with increase in Se concentration. It was similar to reports in tomato, radish, lettuce and strawberry (Ahmed, 2010). The amount of Se left in the soil was not toxic, when new seeds were planted and several crops could be grown and supplemented in the same soil. Optimization in the field condition is desired, to improve the yield.

In conclusion, this work suggests that selenium fortification at the selected concentration by foliar spray method includes maximum Se accumulation with enhanced growth. Thus, the Se fortified tomatoes serves the adequate concentrations for providing the recommended dietary allowance for an adult. The success relied on the increase in total yield and the yield per plant. Se fortification may enhance the existing medicinal chemotherapeutic properties of tomatoes and will improve human health by fighting against multiple ailments.

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