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Investigation of Selcote Ultra Fertilizer Different Rates on Selenium Concentration in Alfalfa (*Medicago sativa* L.) in order to Satisfy Selenium Requirement

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RESEARCH ARTICLE

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ABSTRACT

BACKGROUND: Selenium is now recognized as essential trace element for ruminants.

OBJECTIVES: Studying the effect of different levels of selcote ultra fertilizer and farm yard manure on the concentration of selenium in alfalfa in order to achieve optimum concentration of selenium in this plant.

METHODS: The experiment was factorial based on randomized complete block design with three replications. Experimental factors consisted of Se levels (0, 5, 10, 20 and 40 g ha^{-1}) and farm yard manure (zero and 100 tons per hectare). Plant was harvested at the height of 20 cm in three turns and after preparation the plant samples, their selenium concentration was measured.

RESULT: The result of means comparison showed that the concentration of selenium during three harvests was affected by different levels of selenium fertilizer and farm yard manure. With increasing selenium rates, selenium concentration in the plant aerial parts in all three harvests raised significantly ($p<0.05$). Selenium concentration for the cattle in all treatments except for the control treatment in all three harvests was in the optimal range.

CONCLUSION: The treatment of 5 g ha^{-1} selenium could be used as a suitable treatment for providing cattle and human demands with selenium, because its application is more economical in comparison to treatments of 10, 20, and 40 g.ha^{-1}.

KEYWORDS: *Farm yard manure, Harvests, Optimum, Ruminant.*

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1. BACKGROUND

Selenium is one of the essential micro minerals of the bodies of living organisms. It is required for various body functions such as growth, reproduction, immune system and protection of tissue integrity and coordination in various parts of the body (Iqbal Khan *et al*., 2012). It is present in several selenoproteins that contribute to preventing oxidative cellular degradation (Zeng and Combs, 2008). Trace elements are components of antioxidant enzymes involved in antioxidant mechanisms. Selenium, as a selenocysteine, is a component of the active site of glutathione peroxidase (GPx). The main function of GPx is neutralization of hydrogen peroxide (H_2O_2) and organic peroxide (LOOH). Furthermore, selenium is a structural part of a large group of selenoproteins that are necessary for proper functioning of the body (Wolonciej *et al*., 2016). Furthermore, the essential or toxic effect of this element in humans depends on its chemical form (Reilly, 2006). Several means of administering selenium to deficient ruminants are available (Surai, 2006). Agronomic biofortification is defined as increasing the bioavailable concentrations of essential elements in edible portions of crop plants through the use of fertilizers. The potential for using selenium-containing fertilizers to increase forage selenium concentrations and, thus, dietary selenium intake has been demonstrated in Finland, New Zealand, and Australia where it has proven to be both effective and safe (Whelan *et al.,* 1994; Broadley *et al*., 2006). Plant species also differ in their ability to incorporate selenium from soil. Most forage plants are categorized as non-selenium accumulator plants (Hall, 2013). The Se requirements for animal and human nutrition can be met by ensuring adequate Se in crops frequently consumed in diet. Increasing the Se content of food crops such as wheat, cereals and vegetables could increase the Se intake of whole populations (Uttam *et al*., 2017). One of the strategies to eliminate difficulties that induced by selenium deficiency is applying selenium fertilizers. Selenium at high doses is toxic as it induces adverse cardio metabolic effects, associated with an increased risk of type-2 diabetes and hyperlipidemia (Lee and Jeong, 2012). The intake of selenium in human body is largely derived from plants (White, 2015). Therefore, when stressing the fortification of crop selenium nutrition, particular attention should be paid to how to take effective agronomic measures to balance the selenium concentration in crops grown in high-Se areas. The major source of Se in most human diets is provided by plants. The availability of Se to the plant is determined by soil properties and conditions. Thus Se can occur as inorganic (selenite and selenate) or organic forms. Selenate, which is more soluble than selenite, can pass directly into plant roots; in contrast the uptake mechanism for selenite is unclear (Lin, 2009). Selenate competes with sulphate transport in the root plasma membrane and it is much more abundant in leaves than selenite (Reilly, 2006). Inorganic Se absorbed by plants is metabolised in a variety of ways to organic Se compounds, the distinct molecular structures of which depend on the plant species (Gammelgaard and Jackson, 2011). Soils differ greatly in Se content, and in some geographical zone slow concentrations lead to a decrease in plant Se uptake (Moreno Rodriguez *et al*., 2005; Hawkesford and Zhao, 2007). In some countries, inorganic Se compounds are commonly used as additives in fertilizers to improve the nutritional quality of local food stuffs. This practice of Se fertilization has been applied mainly in

Finland and New Zealand (Eurola *et al*., 2001). A number of studies have addressed the effects of distinct forms of Se and cultivation conditions on edible plants. These studies mainly used selenite and selenate as sodium salts or barium salts (Rayman *et al*., 2008; Broadley *et al*., 2010). Selcote ultra is a slow release selenium granular fertilizer containing 1% of selenium that was used mainly for compensating selenium deficiency in forage crops. The selenium fertilizer composition included 90% sodium and barium selenate and 10% sodium and barium selenite. Results by Gupta *et al*. (1982) showed that through applying 2.24 kg selenium in each hectare of the soil in the form of selenate, the remaining effect of selenium lasted for 4-5 farming years for timothy and 2 years for barley, respectively. Utilizing high concentrations of selenium can lead to decrease in different products performance in given regions of the world. For instance, amounts exceeding 2.5 ppm selenium in soil, reduced wheat and sun flower growth in the soil with a pH of 7.9 in Harilna, India (Singh and Singh, 1978). Gissel-Nielsen (1981) grew some farming plants and vegetables in order to measure their difference in selenium uptake in a selenium-rich soil. The results revealed that there was up to 10 times difference in selenium uptake. Compared with the control treatment, foliar Se applications significant increased the grain Se concentration of wheat and maize by 0.02-0.31 and 0.07-1.09 mg kg^{-1}, respectively. Wheat and maize grain Se recoveries were 3.0-10.4 and 4.1-18.5%, respectively. However, Se concentrations in the grain of subsequent wheat and maize significantly decreased by 77.9 and 91.2%, respectively (Wang *et al*., 2017). Se fertilizer can be used to increase Se content of grape, especially for European and American species, with significant effect of increasing grape nutrition quality and an effective means of lowering heavy metals (Zhu *et al*., 2017).

2. OBJECTIVES

The current study is designed with the aim of studying the effect of different levels of selcote ultra fertilizer and farm yard manure on the concentration of selenium in alfalfa in order to achieve optimum concentration of selenium in this plant.

3. MATERIALS AND METHODS

3.1. Field and Treatments Information

The experiment was factorial based on randomized complete block design with three replications. Experimental factors consisted of Se levels (0, 5, 10, 20 and 40 g ha^{-1}) and farm yard manure (Zero and 100 tons per hectare). The soil sample was taken from four points of the farm in depth of zero to 30 cm, and the compound sample was provided after mixing the samples. Average selenium concentration in this soil was 0.4 mg kg^{-1} that was classified as selenium deficient soils. Some of the physical and chemical characteristics of the taken soil sample and farm yard manure were measured according to standard methods (Carter and Gregorich, 2006). Their results are shown in Tables 1 and 2.

3.2. Green house Management

After being air-dried the taken soil samples was passed through a 2 mm-sieve. 360 kg of the soil was divided into two equal parts and half of it was loaded with cattle manure equivalent to 100 tons per hectare and it was thoroughly mixed with soil. The prepared soils were moved to 30 pots with capacity of 6 kilograms. After having pots prepared, selcote ultra treatments were placed in center and depth of 10 cm of each pot.

Table 1. Some physical and chemical properties of soil

Index	Soil Texture	pH	ECe (dS m^{-1})	O.C (g kg^{-1})	$CaCO_3$ (%)	SO_4 (meq l^{-1})
Soil Sample	SiCL	6.08	2.4	1.6	32.75	0.04

Afterwards seeds of alfalfa equivalent to 30 kg per hectare were planted in depth of 3 cm and were irrigated immediately. The pots were transferred to the green house. Next irrigations were daily up to appearance of sprouts, then and during the growing phase they were irrigated every 4 days. Urea fertilizer was added to the pots during two steps; 22 ppm at the time of germination and 33 ppm when the vegetation was complete. Also 55 ppm of ammonium phosphate was added to all samples.

3.3. Measured Traits

Generally plants were harvested in three turns. The first harvest was when the plant approached the height of approximately 20 cm. Next harvests were done when the plant reached the height of 20 cm. In each harvest, the aerial parts of the plant were taken from 2 cm height from the soil surface. After getting prepared, the plant samples were moved to paper envelopes and they were dried and weighted in a ventilating oven for 72 hours at 65 ºC. The samples were then powdered by Wiley mill and their selenium concentration was measured by Atomic Absorption Spectrometry (Kopsell *et al*., 1997).

3.4. Statistical Analysis

Data obtained from each treatment were transferred to excel sheets. The diagrams were plotted using this software. Statistical analysis on data was performed using SAS software version 9.4 and Fisher LSD test at level 5%.

Table 2. Some characteristics of farm yard manure sample

Index	pH	EC (ds.m^{-1})	O.C (g kg^{-1})	N_t (g kg^{-1})	P (%)	K (%)
Farm yard manure	8.60	17	24.9	1.3	0.09	0.40

4. RESULTS AND DISCUSSION

4.1. The effect of selenium fertilizer on selenium concentration in alfalfa

The mean comparison of selenium concentration under the effect of different selenium fertilizer levels in three harvests is shown in Fig. 1. As can be observed, with increasing the use of selenium fertilizer, selenium concentration by alfalfa in three harvests displayed a decreasing trend and it was significant ($P<0.05$). This is also clearly evident in other researcher works that through increasing application rates of Se fertilizer resulted in increased alfalfa hay Se content for that cutting of alfalfa (0.07, 0.95, 1.55, 3.26 mg Se/kg dry matter for Se application rates of 0, 22.5, 45.0, or 89.9 g Se/ha, respectively). Feeding Se-fertilized alfalfa hay during the 7-wk preconditioning period increased the WB-Se concentrations ($P_{\text{Linear}}<0.001$) and body weights ($P_{\text{Linear}}=0.002$) depending upon the Se-application rate (Hall *et al*., 2013). Application of selenite and selenate increased the lentil grain yield by 10% and 4%, respectively, compared to the control. Seed Se concentration was significantly higher in lentils treated with selenate (1.4 mg kg^{-1}) compared to selenite (0.9 mg kg^{-1}) and the control (0.6 mg kg^{-1}) (Ekanayake *et al*., 2015). Through the first harvest to the third, there was a descending trend in plant selenium concentration (Fig. 1). It seems that, the reason for this decrease is due to reduction in fertilizer amount as a result of processes like concentration by the plant, leaching, absorption by microorganisms and their synthetic materials and finally evaporation of its organic forms. Also leaching in this case does not seem that much important since it is a slow releasing fertilizer.

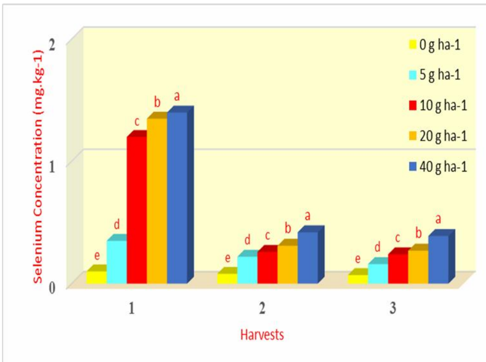

Fig. 1. Mean comparison of the different selenium fertilizer rates on selenium concentration at different harvest turns of alfalfa. (Bars having the same letter are not different at 5% probability level).

4.2. The effect of selenium fertilizer on the mean concentration of selenium in alfalfa

The mean comparison of selenium concentration by alfalfa under the effect of selcote ultra indicated in Table 3. As can be observed, with increasing application of selenium fertilizer, selenium concentration by the plant among control treatment and selenium fertilizer treated samples showed a significant increase ($p<0.05$). The treatment of applying 40 g selenium per hectare had the highest concentration and after that were treatments of 20 and 10 g selenium per hectare.

Table 3. Effect of selenium fertilizer levels on selenium concentration in alfalfa (mg per kg)

The amount of selcote ultra (g ha^{-1})	Mean of concentration
0	0.08^{e}
5	0.24^{d}
10	0.56^{c}
20	0.64^{b}
40	0.73^{a}

(Class having the same letter are not different at P = 0.05).

Leek was fertilized with sodium selenite (Na(2)SeO(3)) and sodium selenate (Na(2)SeO(4)) in a greenhouse to assess the impact of selenium (Se) fertilization on Se uptake by the crop and its speciation in the crop. The lowest Se uptake was observed when Na(2)SeO(3) was used as a fertilizer, which results in a higher risk for Se accumulation in the soil on a longer term. (Lavu *et al*., 2012). Selenium fertilization did not affect timothy DM yield, fiber concentration, or digestibility. Timothy Se concentration could not be successfully predicted by NIRS. A spring application of 10 g Se ha^{-1} as a slow-release fertilizer and its residual effect are sufficient to produce timothy with an adequate Se concentration (>0.1 mg kg^{-1} DM) to prevent deficiency diseases in livestock and allow diet formulation to meet optimal Se levels (Tremblay *et al*., 2015).

4.3. The effect of farm yard manure on selenium concentration in alfalfa during three harvests

Mean comparison of selenium concentration in alfalfa under the effect of different levels of farm yard manure in three harvests is presented in Fig. 2. As can be seen, increasing farm yard manure usage, selenium concentration in alfalfa showed a significant decrease in each harvest ($p<0.05$). Selenium concentration in first harvest of the control treatment was 0.84 $mg.kg^{-1}$ and in treatment of 100 mega grams per hectare was 0.25 $mg.kg^{-1}$ (Fig. 2).

In the second and third harvests also selenium concentration in the control treatments revealed a significant decrease in comparison to the treatment where farm yard manure was not used ($p<0.05$). The effect of organic material in selenium concentration, increased action of microorganisms and selenium concentration by them or conversion of selenium mineral forms to evaporating organic forms can be mentioned as factors that cause decreased selenium concentration in treatments where farm yard manure was used. Soil OM is an important component that retains Se in soils. The proportion of OM-bound Se can be affected by soil type in general or the composition and content of soil OM in specific (Johnson *et al*., 2000; Wang *et al*., 2016). Higher content of OM are usually found in peat soils. Tolu *et al*. (2014) reported that the influence of soil OM on Se mobility should be emphasized in these soils, while the mobility of Se is mainly controlled by Se adsorption onto oxy-hydroxides in volcanic soils, red earths, and other soils poor in OM. Results of Davies and Watkinson (2006) showed that with increasing organic material, selenium concentration decreased in plant.

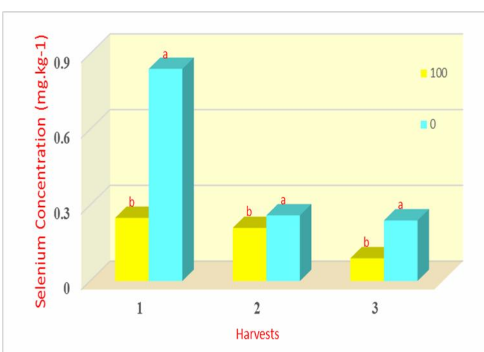

Fig. 2. Mean comparison of the different farm yard manure rates on selenium concentration in alfalfa at different harvest. (Bars within a manure class having the same letter are not different at 5% probability level).

High-Se silages [1.72 mg of Se.kg^{-1} of dry matter (DM)] were produced following a spring application of 2.5 kg.ha^{-1} of Selcote Ultra, whereas low-Se silages (0.05 mg of Se.kg^{-1} of DM) were produced in the Se-unfertilized portion of the same fields (Seboussi *et al.,* 2016).

5. CONCLUSION

Applying selenium fertilizer led to significantly selenium concentration rise in alfalfa ($p<0.05$). In next harvests, concentration in the studied plant showed a significant decrease. It seems that the reason for this decrease is decreased amounts of fertilizer as a result of processes like concentration by the plant, leaching, concentration by microorganisms and their synthetic materials and finally evaporation of the fertilizer organic forms. Applying 40 g ha^{-1} selenium in the form of selcote ultra led to increase selenium concentration in plant, but the obtained concentration did not approach the toxicity threshold for herd of cattle, hence its concentration up to the afore mentioned limit is recommended in this soil. Due to the impact of farm yard manure in decreasing the plant selenium concentration, use of selenium fertilizers is recommended in the soil with the organic fertilizers to supply selenium for animals and humans.

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FOOTNOTES

CONFLICT OF INTEREST: Authors declared no conflict of interest.

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