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Effect of Calcium and Potassium Fertilizer on Potassium and Calcium Contents in Maize Saplings using XRF Technique

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ABSTRACT

A pot experiment was performed on maize (Zea mays indurata cv. 'Rattan') saplings with application of different amounts of calcium (Ca) and potassium (K) fertilizers in a closed environment. During growth, saplings were regularly monitored for their average length, colour and texture. The collected saplings were analysed for their K/Ca content using the EDXRF method of our laboratory. The outcomes indicate the suppression of Ca content with both fertilizers. The K content was found to be almost constant with K fertilization but was slightly reduced with Ca fertilization. The effect of fertilization on water uptake of the saplings was random in both cases. The K amount was comparatively high before the start of fertilization and was not greatly much affected by fertilization while the level of Ca was less. Further suppression of calcium with fertilizations supports the observed fact that saplings turned brown and collapsed on fertilizations. The fertilizations also affected the length of saplings.

Key words: fertilizers, macronutrients, plants, pot experiment and x-ray fluorescence.
***Abbreviations**: **EDXRF**, Energy Dispersive X-Ray Fluorescence; **XRF**, X-Ray Fluorescence*

INTRODUCTION

A plant's growth and survival depend on sixteen chemical elements. These elements are classified as non–mineral and mineral nutrients. Non-mineral nutrients (H, C and O) are found in air and water while the remaining thirteen mineral nutrients come from the soil. Mineral nutrients are further divided into two groups as macronutrients and micronutrients depending upon their intake by the plant, essential for its growth. Potassium and calcium are among the macronutrients needed for growth and development of plant. Potassium (K^+) is a non constitutive primary macronutrient in a plant system, second to nitrogen in plant tissue levels which ranges from 1-3% by weight. It plays an essential role in plant growth and metabolism [1]. It functions like an electrical charge conveyer in plant cells, activating over 60 enzymes. It plays a vital role in plant's tolerance for high/low temperatures, droughts, diseases and pest occurrences and balances the charge of anions [2]. One of the most essential functions of K^+ is osmoregulation which in turn helps in maintaining high cell turgor pressure that affects cell elongation for growth and regulates the opening and closing of stomatas, thereby, influencing the transpirational cooling and CO_2 uptake by photosynthesis [3]. Calcium is also an essential secondary plant macronutrient. It is a key element required in structural roles in cell walls and membranes. It plays a critical role in carbohydrate removal and neutralizes cell acids. Calcium also affects the membrane stability and respiratory rate of a tissue and its resistance to fungal infections [4]. Therefore, potassium and calcium together play a decisive role in growth and nutrition of plants. A similar experiment, performed earlier on rice saplings [5], was carried out on maize to investigate the effect of calcium and potassium fertilizers on contents of plants and growth of plant. The experiment was performed with different amounts of calcium and potassium fertilizers to the growing maize saplings in a closed environment. The saplings were analysed for their potassium and calcium contents. Since, all the pots had same soil, same seeds and the saplings were grown in same environment except fertilizations and therefore, the fertilizations were expected to affect the potassium and calcium amounts in the saplings while their substrates remained the same. The saplings were analysed using EDXRF set-up already established in the lab [5, 6]. X-ray fluorescence (XRF) is emission of x-rays from a target when it is bombarded with photons of suitable energy. In energy dispersive XRF (EDXRF) measurements, energies of the emitted x-rays characterize the elements present in the target and the corresponding intensity measurements provide the amounts of the elements. EDXRF set up includes a photon source of suitable energy, sample (to be studied) and a detector in a suitable geometry. Already, the XRF technique has been successfully employed for element analysis in plants' studies with uncertainties in the results within ±10% [7, 8, 9].

The details of experiment, observations and their interpretations are given in the following sections.

MATERIALS AND METHODS

Samples

Pot Experiment

In a spacious airy laboratory room of dimensions ~ 24 ft. X 21 ft. X 12 ft. with proper natural day light (day time luminance of 150-200 lx) and no artificial luminance at night, twelve pots containing a mixture of soil and sand in the ratio 1:1 were used to germinate 35-40 seeds of maize (*Zea mays indurata,* cv. 'Rattan') in each pot. The pots were watered with 200 ml water on alternate days. On 9th and 11th day after the germination of seeds, fertilizations with $CaSO_4$ (calcium fertilizer) [10] and with KCl (potassium fertilizer) [11] were done. The five pots numbered POT-1, POT-2, POT-3, POT-4, POT-5 were treated with K fertilizers as 50, 100, 150, 200, 250 mg of KCl per 200 ml water, whereas, the remaining five pots numbered POT-6, POT-7, POT-8, POT-9, POT-10 were treated with Ca fertilizers as 50, 100, 150, 200, 250 mg of $CaSO_4$ per 200 ml water (Fig. 1). The 11th pot was treated with both Ca and K fertilizers in equal amounts i.e. 150 mg each KCl and $CaSO_4$ in 200 ml water and 12th pot was left untreated. The fertilizations were done on two alternate days i.e. on 9th and 11th day after germination.

Fig. 1 Photograph of maize plants in pots during their growth

During the growth period, average length, colour and texture of saplings were regularly monitored. The observed average length, colour and texture of maize saplings at different times of growth are listed in Tables 1A-1B.

Sample Preparation

In order to analyze maize saplings for their elemental composition, the saplings were cut with stems above the soil surface. To determine water content in the maize saplings, they were weighed and then dried at room temperature for two days followed by drying in oven at 100-120°C for 5-6 hours for next two days. After cooling, the samples were again weighed to find the weight loss due to drying. To analyze maize materials, thick pellets [12] of samples were prepared. The preparation of sample targets involves;

- grounding of the dried maize saplings with an electric grinder and electric agate pestle mortar and
- pressing of accurately weighed fine powder of the material in a pellet die to obtain thick pellets of 2.5 cm diameter.

Table 1A Status of growth of maize saplings treated with different amounts of potassium fertilizer.

Time after planting (days)	Pot-1 Length (cm) C&T	Pot-2 Length (cm) C&T	Pot-3 Length (cm) C&T	Pot-4 Length (cm) C&T	Pot-5 Length (cm) C&T	Pot-11 Length (cm) C&T	Pot-12 Length (cm) C&T
3	4-5	3-5	3-5	4-5	5-6	4	4
	G&F	G&F	G&F	G&F	G&F	G&F	G&F
4	13.5	13	12.5	14	15.5	13	14
	G&F	G&F	G&F	G&F	G&F	G&F	G&F
5	20	18	17.5	20.5	19	18	23.5
	G&F	G&F	G&F	G&F	G&F	G&F	G&F
6	35	33.5	33	36	34.5	33	33.5
	G&F	G&F	G&F	G&F	G&F	G&F	G&F
7	42	38.5	39.5	37	36	36.5	37
	G&F	G&F	G&F	G&F	G&F	G&F	G&F
8	44	39	42	39	37	37	39
	G&F	G&F	G&F	G&F	G&F	G&F	G&F
9;amount of fertilizer (mg)/200 ml of water	KCl 50	KCl 100	KCl 150	KCl 200	KCl 250	KCl 150+ $CaSO_4$ 150	Untreated
10	Y/B	Y/B	Y/B	Y/B	Y/B	Y/B	Y/B
11; Fertilization done again as above	46	42	45.5	48.5	46.5	43.5	40.5
	FD/Y/B	FD/Y/B	FD/Y/B	FD/Y/B	FD/Y/B	FD/Y/B	FD/Y/B

G&F →Green & Fresh; Y/B →Yellow/Brown; FD/Y/B → Fall down/Yellow/Brown; C&T → Colour & Texture

Table 1B Status of growth of maize saplings treated with different amounts of calcium fertilizer

Time after planting (days)	Pot-6 Length (cm) C&T	Pot-7 Length (cm) C&T	Pot-8 Length (cm) C&T	Pot-9 Length (cm) C&T	Pot-10 Length (cm) C&T	Pot-11 Length (cm) C&T	Pot-12 Length (cm) C&T
3	5-6	4-5	5	5	4-5	4	4
	G&F	G&F	G&F	G&F	G&F	G&F	G&F
4	13.5	12	14.5	14.5	14	13	14
	G&F	G&F	G&F	G&F	G&F	G&F	G&F
5	21	16.5	19.5	20.5	19.5	18	23.5
	G&F	G&F	G&F	G&F	G&F	G&F	G&F
6	32	33.5	36	33	34.5	33	33.5
	G&F	G&F	G&F	G&F	G&F	G&F	G&F
7	39	40	40	40.5	36	36.5	37
	G&F	G&F	G&F	G&F	G&F	G&F	G&F
8	41	42	40	43	37	37	39
	G&F	G&F	G&F	G&F	G&F	G&F	G&F
9;amount of fertilizer (mg)/200 ml of water	$CaSO_4$ 50	$CaSO_4$ 100	$CaSO_4$ 150	$CaSO_4$ 200	$CaSO_4$ 250	KCl 150+ $CaSO_4$ 150	Untreated
10	Y/B	Y/B	Y/B	Y/B	Y/B	Y/B	Y/B
11; Fertilization done again as above	48.5	43.5	41	53.5	39	43.5	40.5
	FD/Y/B	FD/Y/B	FD/Y/B	FD/Y/B	FD/Y/B	FD/Y/B	FD/Y/B

G&F →Green & Fresh; Y/B →Yellow/Brown; FD/Y/B → Fall down/Yellow/Brown; C&T → Colour & Texture

The thick sample targets, so prepared, were kept in desiccators in order to prevent them from atmospheric humidity.

Set-up of Experiment for Potassium/Calcium Determinations in Saplings

To carry out determinations of potassium/calcium contents in maize saplings, the existing x-ray fluorescence (XRF) method [12] has been employed. The method involves selective production of analyte x-rays in thick targets of sample and its two references. The material of first reference is analyte itself or its chemical compound and that of second reference is the mixture of sample and first reference material in known ratio.

For identification of elements present in the maize plants, sample pellets were irradiated with 5.959 keV Mn K x-rays from ^{55}Fe radioactive source in a reflection geometrical set up of photon source, target and detector (Fig. 2). Si-PIN detector, AMPTEK model XR-100CR with 1 mil. Be window and of dimensions 25 mm^2/500 μm coupled to computer based ORTEC-MCA (model-465) was used to count the emitted fluorescent x-rays. The spectrometer energy resolution was ~200 eV at 5.959 keV Mn K x-rays.

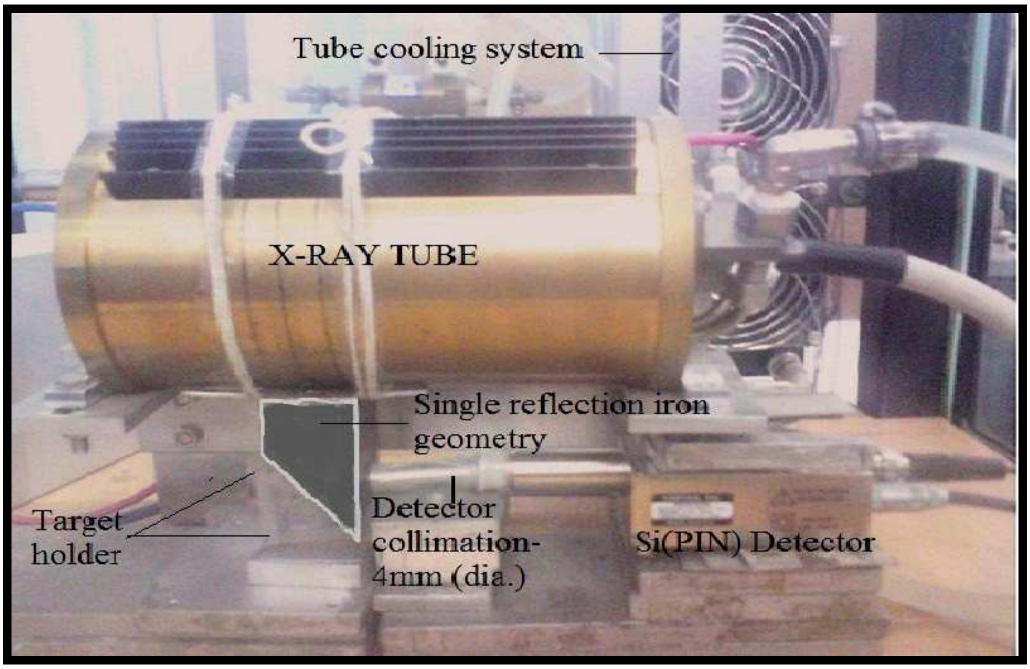

Fig. 2 Single reflection geometry set-up for photon irradiations

The scattering of 5.959 keV photons at 90° from sample pellets was recorded with equivalent borax targets and subtracted from the spectra of sample pellets. A typical net spectrum of maize saplings with identification of various peaks is shown in Fig. 3. In the spectrum, peaks are at channels which correspond to K x-ray energies of chlorine, potassium and calcium. These energies also correspond to L x-ray energies of the elements in the range $33 \le Z \le 64$. Irradiations of sample pellets with 59.57 keV γ-rays from ^{241}Am source ruled out the presence of elements $33 \le Z \le 64$ as in the recorded spectra no x-ray peaks were observed in the K energy region of elements $33 \le Z \le 64$. Thus, the peaks in the spectrum (Fig. 3) correspond to K x-ray energies of chlorine, potassium and calcium.

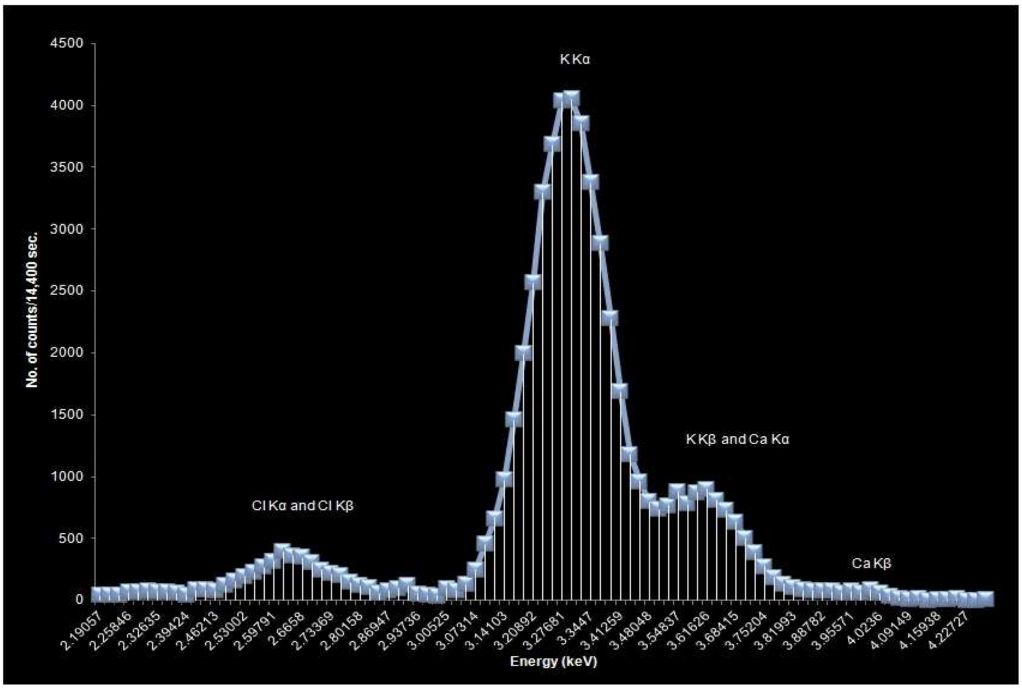

Fig. 3 Background subtracted spectrum of a maize sample irradiated with 5.959 keV photons from ^{55}Fe source

To determine potassium/calcium contents in different sample targets, calcium present in the samples was excited employing 5.959 keV Mn K x-rays from ^{55}Fe source in the geometrical set up, whereas, potassium was selectively excited by irradiating the samples with photons from the low power x-ray tube with Rh anode operated at 4 KV. The spectrum recording was done thrice for each target. Targets of $KNO_3/CaCO_3$ compounds were used as first reference material for respective potassium/calcium determination and second reference materials were known mixtures of samples and respective first reference materials. In all the three spectra of sample and its two reference materials, counts under the respective potassium/calcium photo peaks were collected for sufficient time to obtain statistical accuracy of counts < 1%. For maize saplings, the potassium/calcium content was determined using the relation [12]:

$$\alpha = \frac{M_a}{M_A}\alpha' \frac{\left[\frac{N_a^A}{N_a^{Sp}}\right] - 1}{\left\{\left[\frac{N_a^A}{N_a^S}\right] - \left[\frac{N_a^A}{N_a^{Sp}}\right]\right\}}$$

Where, M_A is mol. wt. of compound of analyte 'a' (first reference material).
M_a is at. wt. of analyte '*a*'.
S & Sp are two targets with different analyte contents in the same substrate i.e. sample and second reference material.
α′ is additional known amount of first reference added to the unit amount of S for Sp target material.
N_a^S, N_a^A & N_a^{Sp} are counts under the analyte '*a*' photo peak in the x-ray spectra of S, A & Sp respectively (A is $KNO_3/CaCO_3$).

Table 2 Determined values of potassium/calcium contents of maize samples from the pots treated with different amounts of calcium fertilizer

Treatment with fertilizer/ 200 ml of water	Weight of sample (g)		Fractional determined Ca/K amounts in maize saplings				Water content rel. to dry material
			Ca		K		
	Before drying	After drying	Dry sample (10^{-3})	Fresh saplings (10^{-3})	Dry sample (10^{-2})	Fresh saplings (10^{-2})	
Pot-12 (untreated)	2.630	2.226	5.0±0.6	4.2±0.6	11±1.4	9.3±1.2	0.182
Pot-6 (50 mg $CaSO_4$)	1.778	1.512	2.0±0.2	1.7±0.2	7.5±1.0	6.4±0.8	0.176
Pot-7 (100 mg $CaSO_4$)	2.024	1.696	2.3±0.3	2.0±0.2	6.2±0.8	5.2±0.7	0.194
Pot-8 (150 mg $CaSO_4$)	1.948	1.616	2.6±0.3	2.1±0.3	59.1±7.7*	49.0±6.4*	0.205
Pot-9 (200 mg $CaSO_4$)	2.559	2.166	4.0±0.5	3.3±0.4	9.8±1.3	8.2±1.1	0.200
Pot-10 (250 mg $CaSO_4$)	2.051	1.757	3.0±0.4	2.6±0.3	5.1±0.7	4.3±0.6	0.167
Pot-11 (150 mg KCl + 150 mg $CaSO_4$)	2.501	2.076	2.0±0.3	1.7±0.2	9.3±1.2	7.7±1.0	0.205

**exceptionally higher, can be due to mishandling of the sample target.*

Table 3 Determined values of potassium/calcium contents of maize samples from the pots treated with different amounts of potassium fertilizer

Treatment with fertilizer/ 200 ml of water	Weight of sample (g)		Fractional determined Ca/K amounts in maize saplings				Water content rel. to dry material
			Ca		K		
	Before drying	After drying	Dry sample (10^{-3})	Fresh saplings (10^{-3})	Dry sample (10^{-2})	Fresh saplings (10^{-2})	
Pot-12 (untreated)	2.630	2.226	5.0±0.6	4.2±0.6	11±1.4	9.3±1.2	0.182
Pot-1 (50 mg KCl)	1.225	1.032	7.2±0.9	6.1±0.8	9.9±1.3	8.3±1.1	0.187
Pot-2 (100 mg KCl)	2.394	1.982	3.5±0.5	2.9±0.4	9.2±1.2	7.6±1.0	0.207
Pot-3 (150 mg KCl)	1.250	1.069	4.1±0.5	3.5±0.4	10.6±1.4	9.1±1.2	0.169
Pot-4 (200 mg KCl)	2.279	1.909	1.6±0.2	1.3±0.2	7.9±1.0	6.6±0.9	0.194
Pot-5 (250 mg KCl)	2.181	1.804	2.4±0.3	2.0±0.2	10.1±1.3	8.3±1.1	0.209
Pot-11 (150 mg KCl + 150 mg $CaSO_4$)	2.501	2.076	2.0±0.3	1.7±0.2	9.3±1.2	7.7±1.0	0.205

The reliability of the present methodology has already been quoted [6] with nine synthetic samples having known fractions of potassium and calcium.

The measurements were performed on each sample 6-9 times at an interval of ~ hours-week and standard deviation as percentage of mean value was determined for each sample. The percentage standard deviations were ~ 5-13% in case of potassium and < 5% in case of calcium. It indicates that the measurements are reproducible. The comparatively large percentage deviations for potassium can be assigned to the low energy of potassium K x-rays. The determined amounts of potassium/calcium contents in maize saplings treated with different amounts of potassium and calcium fertilizers are listed in Tables 2-3.

RESULTS AND DISCUSSION

The monitored growth, colour and texture of saplings in different pots, observed at different time intervals (Tables 1A-1B), show that before fertilizations the average growth of all the saplings was almost 40 cm within 10% statistics. On the next day of first fertilization, colour of all saplings turned from green fresh to yellowish brown. After second fertilization, the saplings turned more brownish and eventually collapsed. Though, the duration of freshness and green colour was almost same in all the saplings but the growth and average length were affected with the application of potassium/calcium fertilizers (Fig. 4).

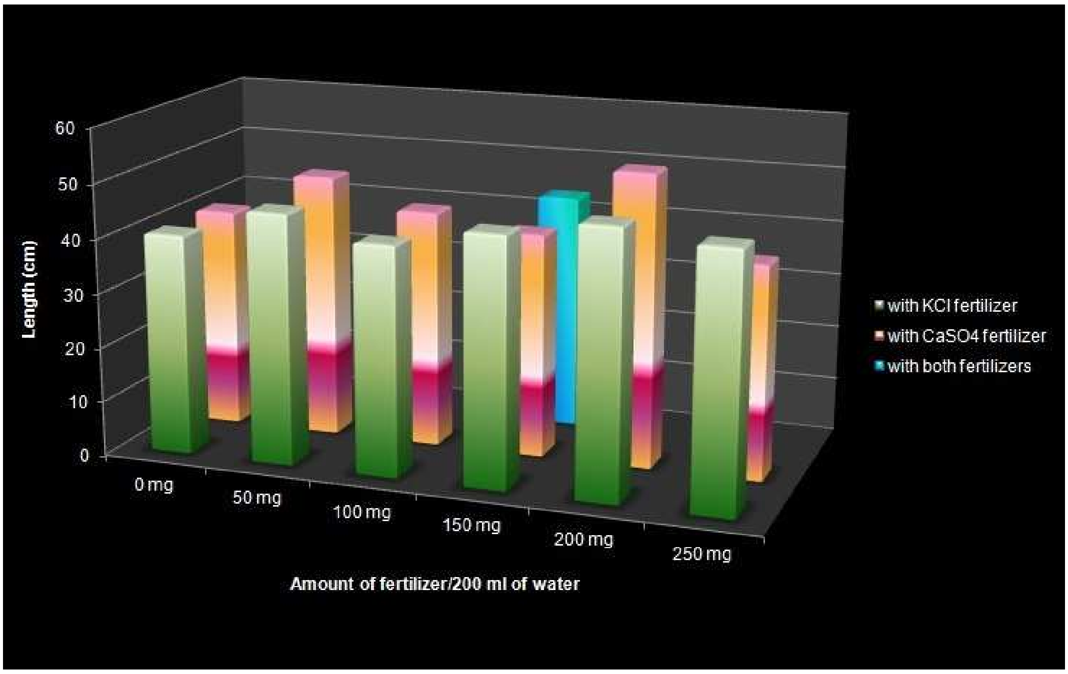

Fig. 4 Plot of lengths of maize saplings with different amounts of KCl/$CaSO_4$ fertilization

The lengths of saplings increased with fertilization except in the case of 250 mg $CaSO_4$ treatment [13]. The lengths were found maximum for 200 mg treatments with both calcium and potassium (Fig. 4).

The errors quoted in the measured values of potassium and calcium contents in dry weights of saplings were mainly due to counting statistics. The contents in fresh saplings before drying were evaluated by dividing the determined amounts in dry samples with the factors obtained from ratio of fresh to dry weights (listed in Tables 2-3). Correlation between the length, texture and colour of the plants (Tables 1A-1B) and potassium/calcium and water contents (listed in Tables 2-3) provides following interpretations;

- In saplings the calcium content got suppressed to almost half with the lowest applied calcium fertilizer and regained with the increasing amount of the fertilizer but remained less than that of untreated pot sample (Fig. 5). Whereas, with potassium fertilization, the calcium content enhanced at the lowest potassium treatment and suppressed with increasing amount of potassium fertilization. The suppressed contents were less than that of untreated pot sample (Fig. 6).
- Potassium in maize saplings was suppressed with applied calcium treatments. Whereas, it remained unaffected with potassium treatments within experimental uncertainties.
- With simultaneous potassium and calcium treatment in the saplings Ca reduced whereas potassium remained unaffected.
- The variation of water content in the saplings with fertilization (Fig. 7) showed no regular pattern.
- From Tables 2 and 3, the optimum calcium and potassium treatments for maize saplings are found to be 200 mg $CaSO_4$ and 150 mg KCl.

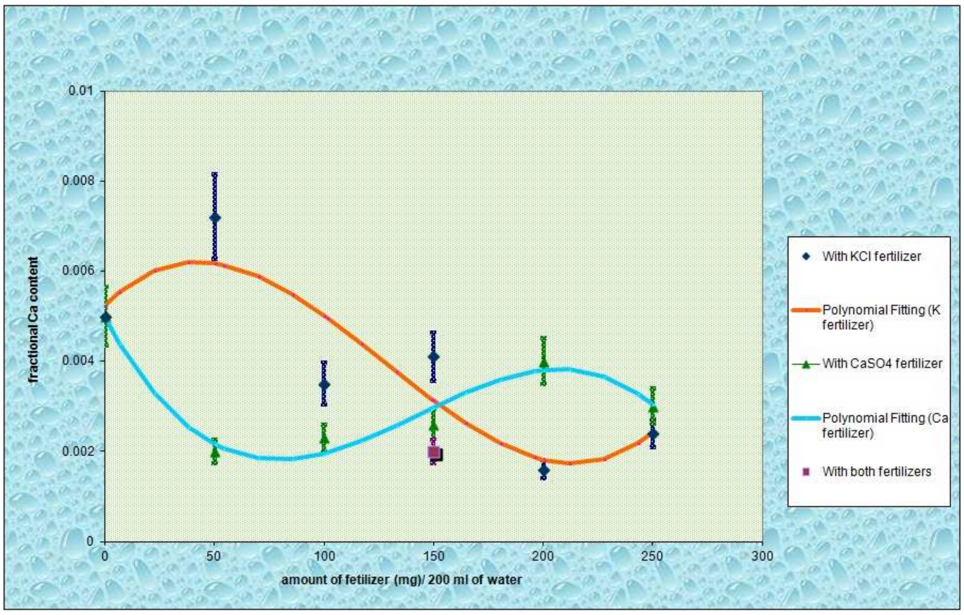

Fig. 5 Variation of Ca content in maize with different amounts of KCl and $CaSO_4$ fertilization

The suppression of calcium with calcium fertilization (Fig. 5) may be attributed to the calcium related disorder occurring in soils / substrates. Because of highly available calcium and its insufficient uptake by the maize plants due to the limited capability of fast growing saplings to regulate the calcium distribution internally in relation to the demand of growing leafy organs, the calcium uptake [14] is suppressed.

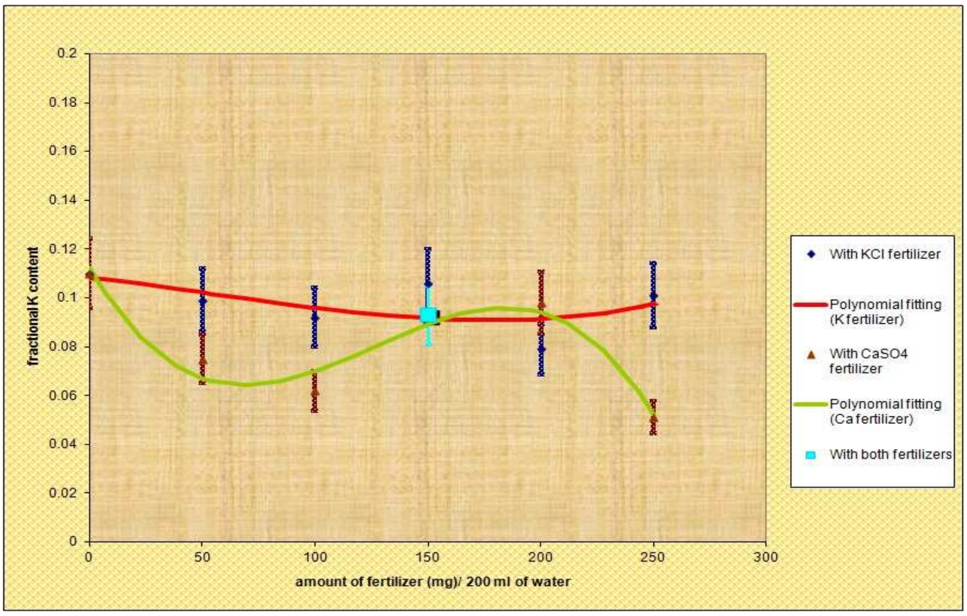

Fig. 6 Variation of K content in maize with different amounts of KCl and $CaSO_4$ fertilization

A sudden shoot in calcium contents of saplings with lowest potassium fertilization and then its subsequent decrease with increasing potassium fertilization (Fig. 5) may be attributed to the competition between the two nutrients, calcium and potassium, because of their similar sizes. With low potassium fertilization, the transportation of Ca^{2+} ions from soil to plant would have enhanced as potassium influences the uptake of other cationic species and interacts with almost all of the essential macronutrients, the secondary nutrients and the micronutrients [2, 15]; but with the increased amount of potassium fertilization, the rush of K^+ and Ca^{2+} ions again stressed the capability of growing maize saplings to regulate the calcium distribution internally [16, 17]. Hence, the application of potassium fertilizer reduces the ability of plant to absorb calcium.

Similar justifications can be applied for reduced potassium uptakes by the saplings on treatments with calcium fertilizers (Fig. 6). With potassium treatments, the potassium contents remained almost constant (Fig. 6), the fact can be assigned to the already present high contents of potassium, thereby, leading to no significant effect on the intake of potassium [18]. All the plots of nutrient amount vs. fertilizer contents are found to be governed by 3^{rd} order polynomial fits (Figs. 5-6). In case of pot 8 saplings, the potassium content is exceptionally higher than those of yields from other pots; therefore, for fitting purpose the value is kept out from the fitting data, this may be due to mishandling of the pot sample/target.

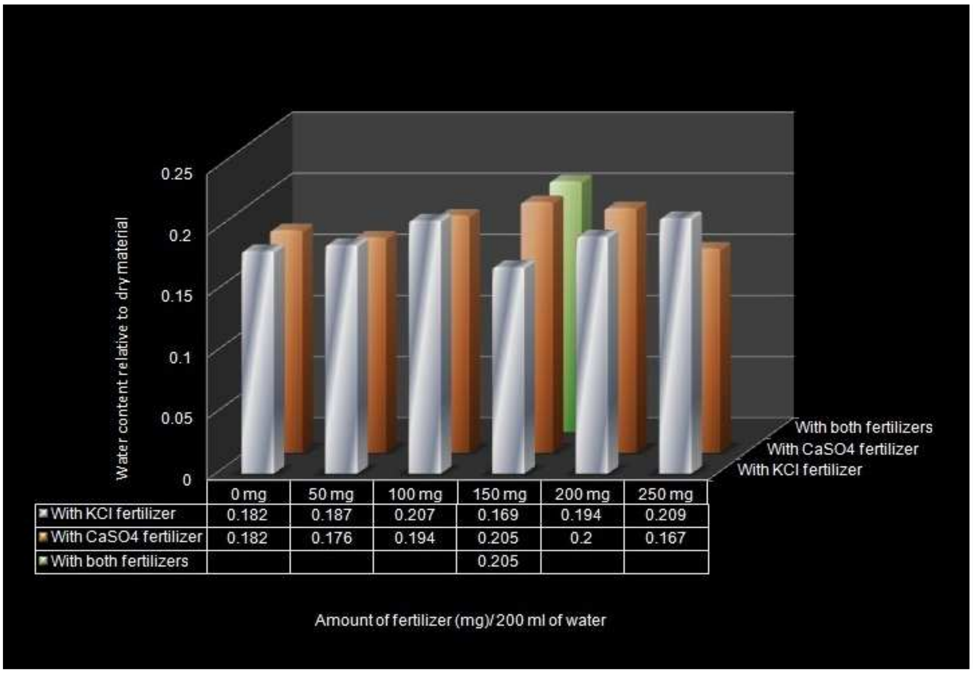

Fig. 7 Variation of water content with KCl and $CaSO_4$ treatments

From Fig. 7, it has been found that there is a random variation of absorbed water content in the saplings with fertilization, the fact already quoted by Jensen [19]. However, it is found to be less for optimum potassium fertilizer amount whereas the same is high for optimum amount of calcium fertilization.

The changes in colour of leaves from green to yellow and then to brown clearly shows the deficiency of calcium in all the treated and untreated saplings. The applied fertilizers encouraged the trend of suppression of calcium in the saplings. Calcium plays an important role in the senescence of leaf segments and its deficiency in plant leads to yellow colour of leaves at a faster rate (http://blog.fluidsensoronline.com/2010/01/calcium-deficiency-in-plants/).

CONCLUSION

In Ca/K fertilizations, calcium content (already present in lower amounts in maize) was suppressed with both the treatments and potassium content was suppressed with calcium fertilizations while it remained unaffected in case of treatment with potassium. The experimental observations and requisite explanations for them predict the deficiency of calcium and sufficient presence of potassium in all the treated and untreated maize saplings. The analytical findings lend support to the predictions as calcium amounts in maize saplings were almost lower than the potassium amounts by an order of 10.

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