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Impact of silicon in enhancing seed germination and seed growth parameters of rice (*Oryza sativa* L.)

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Abstract
Cadmium pollution in soil has been observed all across the world and a high concentration of cadmium has been found to affect the seed germination, seedling emergence and the plant growth and development. The present study deals with application of potassium silicate in overcoming cadmium stress in rice. In this study design, three different concentrations *viz.* 25,40 and 50 µmolL^{-1} (T_1, T_2 and T_3 respectively) of cadmium, T_4(50 µM$CdCl_2$ +0.3mM potassium silicate L^{-1}) and T_5(50 µM$CdCl_2$ +0.5 mM potassium silicate L^{-1}) were used to investigate the effect of cadmium and potassium silicate on various seed germination parameters, such as %age of germination (GP), coefficient of velocity of germination (CVG), germination rate index (GRI), germination index (GI), mean germination rate (MGR) and seedling growth parameter of rice (*Oryza sativa* L.). Potassium silicate application has significantly increased the seed germination and seedling growth parameters of *Oryza sativa* L. Maximum seed germination (95%) was observed in T_5 treatment. Increase in seed germination percentage and other growth characteristics followed the order: T_5> T_4> control >T_1>T_2>T_3. Cadmium significantly affected the number of roots and significant rise was observed in silicon supplementation as comparison to control and as parallel to T_3 treatment. Mean Days for Germination (MDG) and peak value were also observed highest in T_5 treatment in comparison to other treatments. The results of the present investigation clearly indicate that potassium silicate is effective in mitigating the toxic effects posed by cadmium stress.

Keywords: Seed germination parameters, Cadmium stress, *Oryza sativa*, Potassium silicate

Introduction
Cadmium, a non-essential metal, occurring naturally making up about 0.1 ppm of Earth's crust. Various anthropogenic activities such as mining, textile industries, photocopying, landfill operations, intensive agriculture, overuse of synthetic fertilizers and pesticides [are making soil polluted of cadmium [1]. Due to high mobility, even small concentration of cadmium is toxic and effects can appear on plants. Cadmium can easily penetrate the roots by causing excessive destruction to these and translocated to above ground tissues, create osmotic pressure imbalance, affect stomatal opening, photosynthesis and transpiration resulting in chlorosis, necrosis, leaf roll and stunting growth in plants [2].
Rice (*Oryza sativa* L.) belonging to family Poaceae is considered as the chief and staple source of carbohydrate in majority of the developing nations. India is considered as the second largest producer of rice after China, as the country produces 175.58 million tonnes of rice annually. Approximately 44-million-hectare area is under rice production in India, signifying the importance of rice crop in Indian agriculture. The country has exported 44,14,562 MT of basmati rice worth of Rs. 32.8crores and 75,99,596 MT of worth Rs. 21.18crores to the world during the year of 2018-19 [3]. In India, top five states that grow rice are West Bengal, Uttar Pradesh, Andhra Pradesh, Punjab and Tamil Nadu.
To overcome the adverse effects of Cd toxicity, various strategies such as phytoremediation, microbial remediation etc. have been adopted, however the environment friendly approach is to supplement the soil with quasi-essential elements. Various studies have demonstrated about silicon's beneficial effects on plant growth and development and as a wonderful element to mitigate the effects of biotic and abiotic stresses as well as heavy metal toxicity [4][5][6]. Therefore, present study has been conducted to explore the role of Si to alleviate Cd toxicity in terms of seed germination and seedling growth parameters of rice (*Oryza sativa* L.; variety Karan Bhog 521) under cadmium stress conditions as soil of Haryana, India, was found moderately contaminated with cadmium and long-term use of phosphorous fertilizer may further elevate the level of Cd in agricultural and surface soils [7].

Material and Methods

Geographical position of the study site

The present study was done during July 2021 to August 2021 in the Department of Botany, Baba Mastnath University, Ashtal bohar, Rohtak, Haryana, India. The village Ashtal bohar falls in Rohtak district situated in Haryana state with an area of 1668 sq. km, between 28°40'30" N to 29°05'35"N latitude and 76°13'22"E to 76° 51'20"E longitude. The climate of area is arid to semi-arid with hot summers. Soil of district is loamy in nature. Major *rabi* and *Kharif* crops are grown in the district and rice, *bajra* and paddy are the major *kharif* crops.

Collection of the Rice seeds

The certified and healthy seeds of rice (*Oryza sativa* L.) variety Karan Bhog 521 were collected from "The Haryana State Seed Certification Agency", Rohtak, India.

Experimental Design

Preparation of different concentrations of treatments

Potassium silicate K_2O_3Si (molecular weight: 154.279 gmol^{-1}) and cadmium in the form of cadmium chloride (Molecular weight: 183.32 g mol^{-1}) were purchased from LOBA Chemie private limited, Mumbai. Three different concentrations of cadmium chloride *viz*. 25 µmol (T_1), 40 µmol(T_2) and (T_3) 50 µmol L-1 and two concentrations of potassium silicate *viz*. 0.3mM(T_4) and 0.5mM L $^{-1}$(T_5) were prepared with distilled water and used for the treatment. Treatment index for the study consists of; Control(C), $CdCl_2$ 25 µmol (T_1), $CdCl_2$ 40 µmol(T_2) and (T_3) $CdCl_2$ 50 µmol L-1, {$CdCl_2$ (50 µmol) + K_2O_3Si (0.3mM)} (T_4) and {$CdCl_2$ (50 µmol) + K_2O_3Si (0.5mM)} (T_5).

Experiment in disposable tea containers

Before seed germination test seeds were thoroughly washed with tap water, surface sterilized with 10:1 distilled water/ bleach (commercial NaOCl) solution for 5 min and then washed 6-7 times with distilled water. Control and treated seeds were planted in ordinary disposable tea containers (10 seeds in each one) containing soil(100g) and treatments in three replicates per treatment and were placed in laboratory conditions with natural light, and were watered with distilled water whenever required. The number of germinating seeds were counted every day after sowing, till the fifteenth day. After the end of fifteenth day, germination parameters and growth parameters were recorded.

Determination of Seed Germination and Seedling Growth Parameters

Different growth parameters of rice (*Oryza sativa* L.; variety Karan Bhog 521) such as germination percentage, relative germination rate, germination index, shoot length, root length, seedling length, biomass and vigour index were determined in control and treatments.

Methods of Calculations of Germination Parameters

To asses different seed germination parameters following formulas were used [8, 9, 10, 11]:

1. Germination Percentage (G%) = Total seeds germinated /number of initial seeds x 100
2. Mean Germination Time (MGT) = ΣGx/ΣG; where G is the number of seeds germinated on day X.
3. Coefficient of velocity of germination (CVG) = N1+N2+.N3…...+ /100 x N1T1+...+NiTi; where N is the number of seeds germinated every day and T is the number of days from seeding corresponding to N
4. Mean germination rate (MGR) = CV/100 = 1/T; where CV is coefficient velocity and T is mean germination time.
5. Germination Rate Index (GRI) = G1/1+G2/2+ G3/3.... +Gi/i; where G1 is the percentage germination at day 1, G2 is at day 2 and G3 at day 3 and so on.
6. Germination index (GI) = (15xN1) +(14xN2) + ... +(1xN15); where N1, N2 ...N15 is the number of germinated seeds on the first, second and subsequent days until 15th day and the multipliers (e.g., 15, 14 ...etc.) are weights given to the days of the germination.
7. Germination (G) was calculated by the following formula: = Number of seeds germinated / Days of first count + + + …number of seeds germinated/Days of final count (seed day-1).
8. Mean Daily Germination (MDG) was calculated according to the following formula
   MDG = GP/D Where, GP is a final germination percent, D is day of maximum germination during period of experiment).
9. Peak value (PV): The maximum quotient derived from all of the cumulative full-seed germination percentages on any day/ number of days to reach that percentages.

Seedling length

The length of seedling was measured in centimetres with a measuring scale. Seeds were considered to be germinated with the emergence of plumule and radicle both.

Vigour index

Vigour index of the rice seedlings was estimated by the formula Vigour index (VI) = Total seedling length (mm) x germination percentage [12]

Biomass estimation

After 15 days of seed sowing, fresh weight of the rice seedling was measured. For measuring dry weight seedlings were oven dried at 65^{0}C for 72 hours and dry weight was estimated.

Relative water content

For measuring Relative Water Content, after measuring fresh weight (FW) seedlings were immediately floated on distilled water at 25°C in darkness for 12 hours, and then seedlings were dried in an oven at 80°C for 48 hours to determine the dry weight (DW). The RWC was calculated by using the modified Bars and Weatherly method (1962) [13].

RWC (%) = (FW-DW) / (TW-DW) × 100

Statistical analysis

Using SPSS software, data were statistically analysed using analysis of variance (ANOVA) in Excel. The treatment means were analysed by Duncan's multiple range test (DMRT) at $p < 0.05$.

Results

Percentage seed germination

All concentrations of cadmium significantly reduced the germination percentage; however, maximum decrease was observed with T_3 concentration (Fig.1). Maximum seed germination (95%) was observed T_5 treatments and it was 18.75% more than control. Both the concentrations of silicon significantly increased the seed germination %age by 200 and 216.67 % respectively parallel to T_3 treatment.

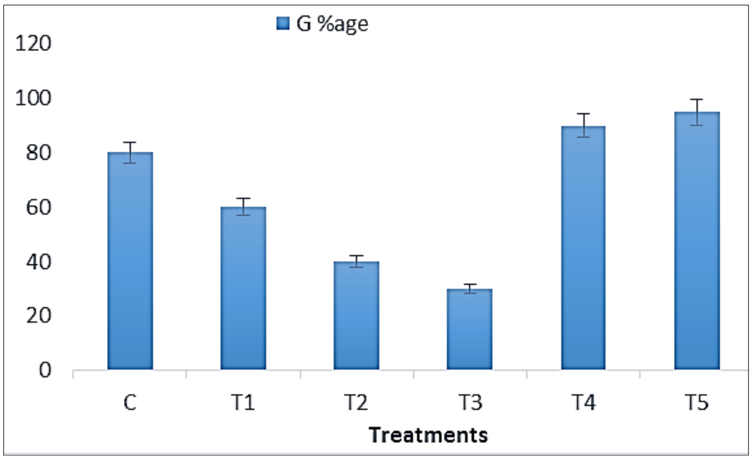


Fig 1: Effect of different concentrations of cadmium and potassium silicate on the seed germination % of *Oryza sativa* L.

Mean Germination Time and Mean Germination Rate

Significant increase in MGT was observed in all the concentrations of cadmium but maximum germination time was observed in T_3 which was 17.65% more as comparison to control (Fig 2.). Significantly less MGT was observed in both the concentrations of silicon which was 25.53 and 27% less than control. Both the concentrations of silicon significantly decreased the MGT by 35 and38 % respectively parallel to T_3 cadmium treatment. MGR was less in all the concentrations of cadmium but the minimum was in T3, which was 14.89% less as comparison to control and 54 and 61% rise was observed in T_4 and T_5 concentrations of silicon parallel to T_3 concentration.

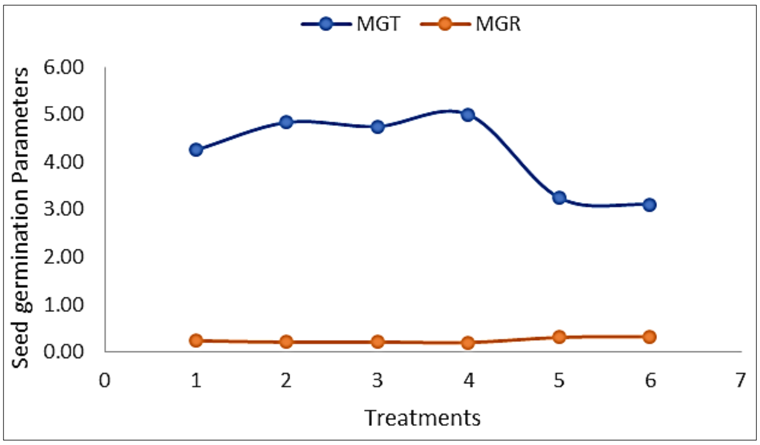


Fig 2: Effect of different concentrations of cadmium and potassium silicate on the MGT and MGR of *Oryza sativa* L.

Coefficient of velocity of germination (CVG) and Coefficient of variation of germination time (CV_t)

Significant decrease in CVG and CVt was observed in all the concentrations of cadmium but minimum was observed in T_3(Fig.3). Both the concentrations of silicon significantly increased the CVG by 53.8 and 61.29% and CV_t by 81.44 and 104.56 % in T_4 and T_5 respectively parallel to T_3 cadmium treatment.

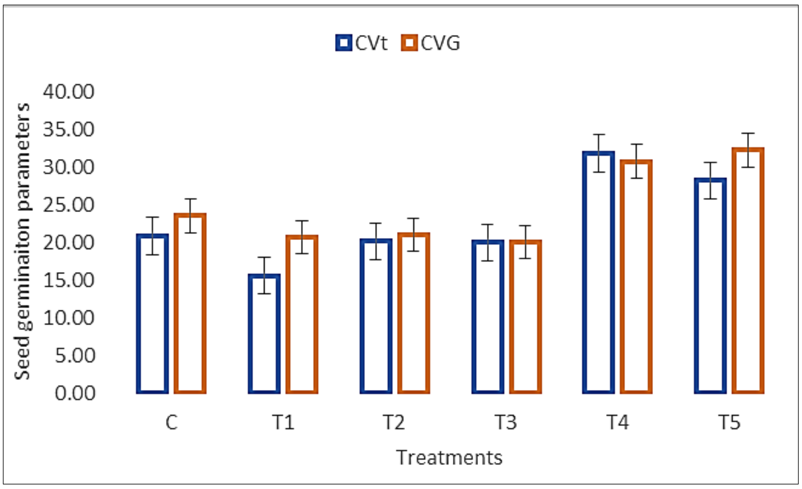
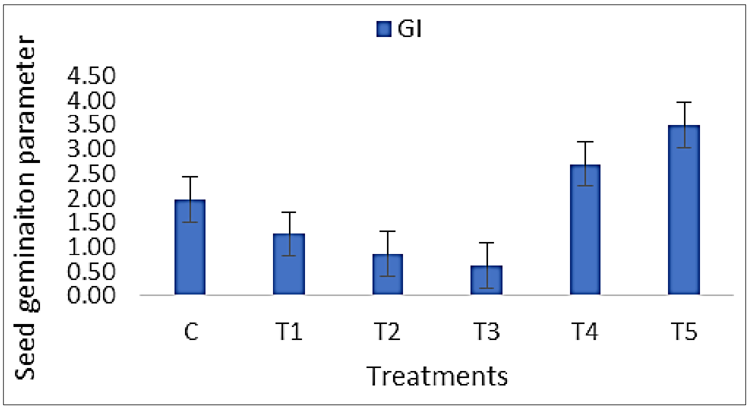


Fig 3: Effect of different concentrations of cadmium and potassium silicate on CVG and CVt of *Oryza sativa* L.

Germination Index and G value

The effect of concentrations of cadmium and Silicon on Germination Index and G value has been shown in fig.4 and 5 respectively. Significant decrease in GI and G value was observed in all the concentrations of cadmium but minimum was observed in T_3 which was 68.53 and 88.29 % respectively less than control. Significant increase in GI was observed in both the concentrations of silicon which was (37.66 (T_4) and 77.66 %(T_5) more as comparison to control. Both the concentrations of silicon significantly increased the GI by 337.48 and 464.52% respectively parallel to T_3 cadmium treatment. G value was observed maximum in T_5 which was 37.45 and 95.3% was more as compared to control and T_3 respectively.



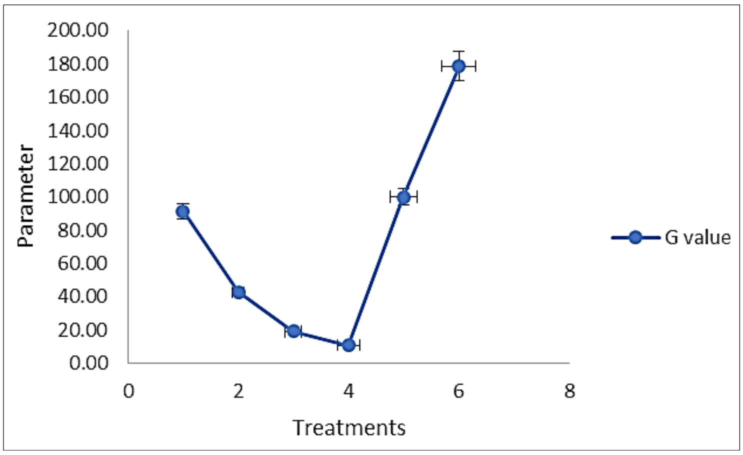


Fig 4 and 5: Effect of different concentrations of cadmium and potassium silicate on GI and G value of *Oryza sativa* L.

MDG and Peak Value

Mean Days for Germination (MDG) and Peak Value were also highest in T_5 treatment in comparison to other treatments and control (Fig.6). Minimum was observed in T_3 which was 68.75 and 62.52 % respectively less than control. Maximum rise was observed in T_5 which was 400 and 233% respectively more in contrast to T_3 treatment.

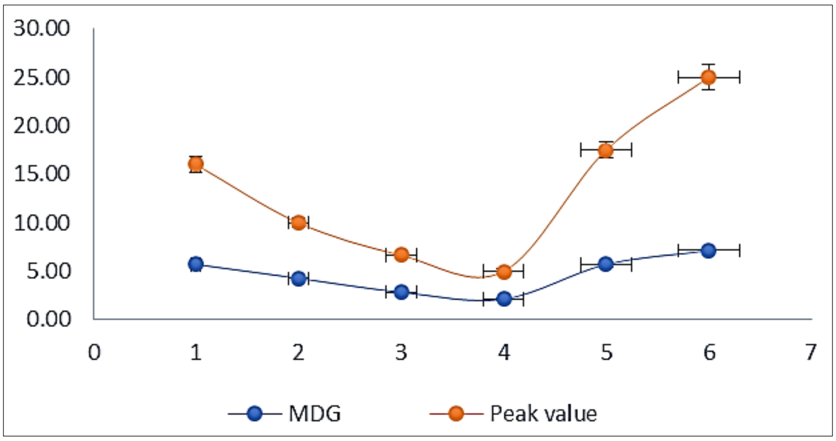


Fig 6: Effect of different concentrations of cadmium and potassium silicate on MDG and Peak Value of *Oryza sativa* L.

Shoot length, root length and number of roots

Cadmium significantly affected the length of shoot, root and seedling. Both the concentrations of silicon were found significantly effective in overcoming the stress caused by cadmium. Minimum number of roots were observed in T_2 and T_3 and maximum was in T_5. In T_5 treatment significant rise of 80 % was observed as comparison to control and 200% as parallel to T_3 treatment (Fig.7 and 8)

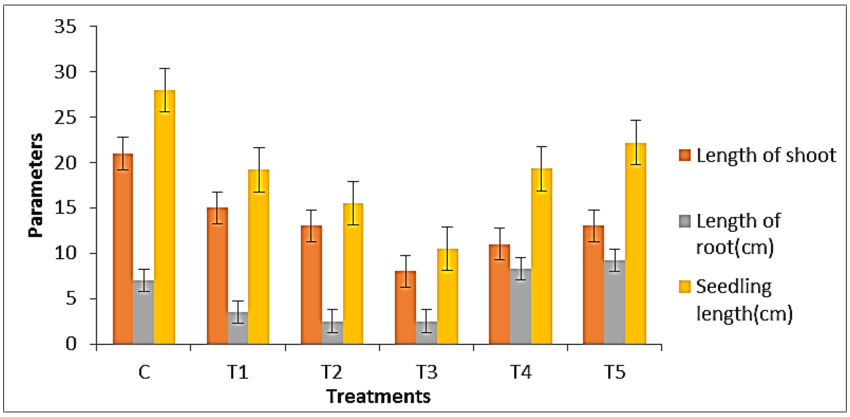


Fig 7: Effect of different concentrations of cadmium and potassium silicate on the length of shoot and root of *Oryza sativa* L.

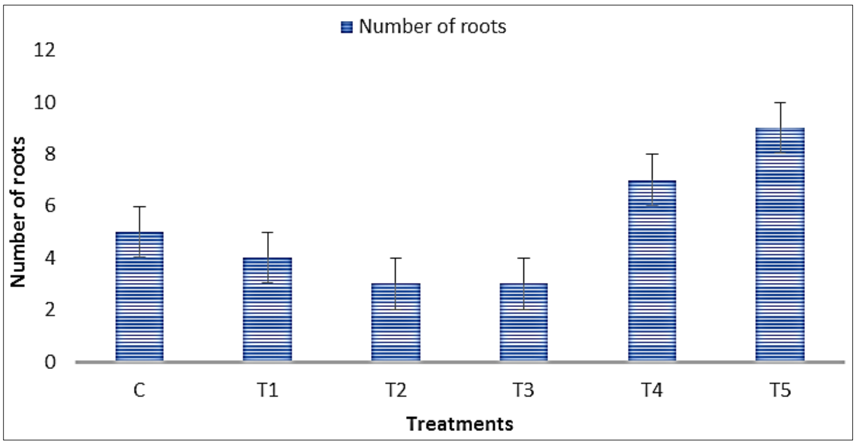
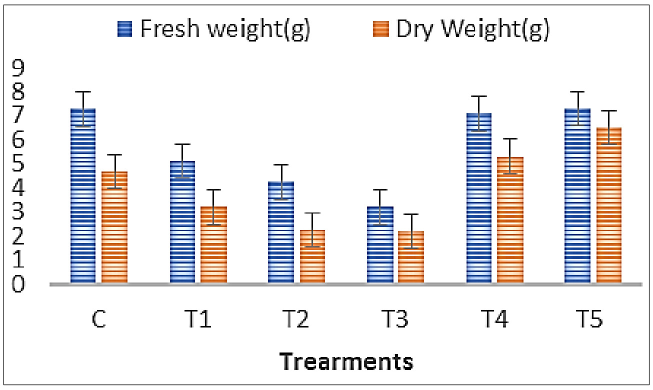


Fig 8: Effect of different concentrations of cadmium and potassium silicate on number of roots of *Oryza sativa* L.

Fresh weight, dry weight and Vigour index

The seedlings of rice showed significant increase in fresh and dry weight with different concentrations of potassium silicate in comparison to control. 7.29g fresh weight of rice seedlings was observed in control and it was further increased to 7.30g inT_5 treatments. Similarly highest dry weight (6.55g) was observed in T_5 treatment. Highest vigour index was observed in T_5 treatment in comparison to control. Increase in fresh weight and dry weight of 127.41 and 195.45 % was observed in parallel to T_3 treatment (Fig. 9and 10).



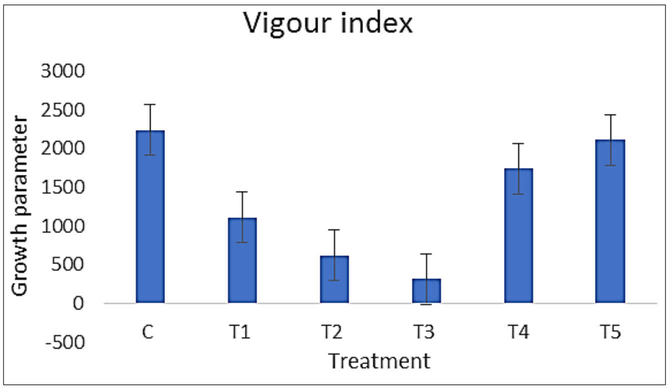


Fig 9 and 10: Effect of different concentrations of cadmium and potassium silicate on the fresh and dry weight and Vigour Index of *Oryza sativa* L.

Relative water content

Relative water content was also higher in both the concentrations of silicon and maximum 86.52% relative water content was observed in rice seedlings in T_5 treatment. Increase in relative water content was followed by the order: T_5> T_4> Control > T_3> T_2> T_1. (Fig.11)

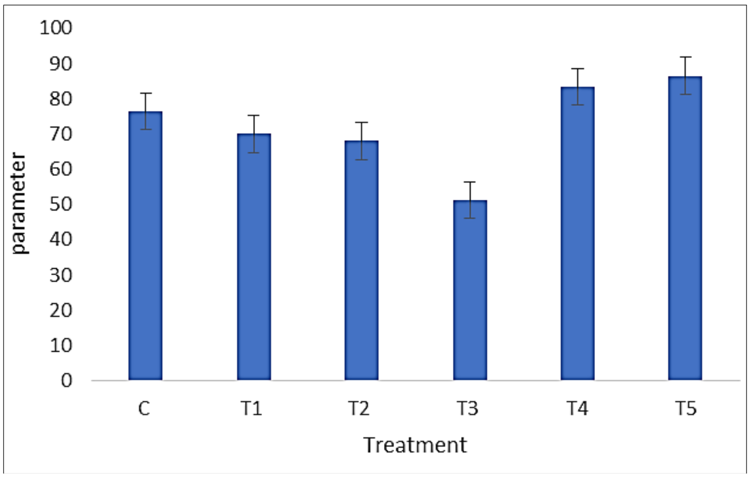


Fig 11: Effect of different concentrations of cadmium and potassium silicate on the Relative Water Content of *Oryza sativa* L.

Discussion

Seed germination and seedling emergence are the utmost significant activities in the life of plant. The results of our study depict the drastic effects imposed by cadmium on all the abovesaid parameters of rice. The decline in seed germination performance was observed at all the levels of cadmium and significantly least values *viz*. 62.5% decline at germination percentage, 14.89 % at MGR and 88.29 at G value were observed in T_3 concentration of cadmium. Similar findings have been dictated on the seed germination and seed growth parameter of lettuce, wheat, rice and citrus seedlings documenting sever effects of cadmium [14] [15]. In the present study silicon supplementation has been found significantly successful in alleviating the effects of metal stress by enhancing the performance of seed at all the germination levels from speeding up the germination process up to root length. Significant rise (216% in seed germination, 61% in MGR) were observed in T_5 silicon treatment in contrast with T_3 concentration of cadmium. Similar findings have been documented for increase in the growth of seedlings of wheat and rice on supplementation of silicon [16, 17]. Cadmium has also been found affecting the length of seedlings, number of roots, RWC, dry and fresh weight of seedlings too. Significant rise (80% in number of roots and 44.6% in seedling length) was observed in T_5 silicon treatment in contrast to cadmium T_3 treatment. It has been observed that supplementation of potassium silicate enhances fresh and dry weight and relative water content of rice and has been proven to be beneficial element for the healthy growth and development of many plant species [18].

Conclusion

In the present study silicon supplementation has been found effective in mitigating the adverse effects of cadmium stress and resulted in enhancement of the seed germination, seedling growth parameters of rice. and has significantly improved the seed germination and seedling growth parameters in comparison to control as well as cadmium stressed conditions. It may be due to the nutritional properties of potassium silicate which promotes the division, elongation and expansion of the cells, increased the absorption of water from the soil, increased the protease and α-amylase activity.; Hence, it can be recommended that in cadmium stressed soil, seeds of crop plants should be sown after the treatment with potassium silicate. Further detailed studies are required to explore the physiological, biochemical and molecular reason for the role of potassium silicate as fertilizer for the plants which suffer from heavy metal stresses during their growth and developmental stage.

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