EFFECT OF 2-PHOSPHONO BUTANE 1, 2, 4, TRICARBOXYLIC ACID ON GERMINATION AND SEEDLINGS OF VIGNA SINENSIS

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Abstract:

There is an urgent need to develop suitable soil amendments to resolve the problem. The attempts have been made to test 2-Phosphono Butane 1,2,4, Tricarboxylic Acid (Codex-551) as one of the chemical amendment for saline soil. The present work is based on the observation of seed germination *Vigna sinensis* (Cowpea) variety-Pusakomal in different concentrations of Codex-551. The work was carried out under laboratory conditions. The *Vigna sinensis* seedling growth were in 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm of Codex-551 concentrations and control consisted of soil suspension of saline-alkaline black cotton soil without any treatment. After 7 days in control the lowest (1.4 cm) root length and (1.10 cm) shoot length was observed while in 60 ppm, highest root length up to (3.2 cm) and shoot length up to (5.5 cm) was observed. After 15 days lowest root length (2.6 cm) and shoot length (3.9 cm) was observed in control while highest root length observed was (4.1 cm) and shoot length was (10.7 cm) in 60 ppm concentrations.

Key Words: Agriculture, Codex-551, salinity, soil abatement, and seedling growth.

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Introduction:

In India, about 6.73 million hectors are laying barren or produce very low and uneconomical yields of various crops due to excessive accumulation of salt (Sharma, et al. 2004; Zaka et al. 2005). The soluble source of Ca^{2+} is essential for reclamation of such soils. For this purpose, gypsum is the source of Ca^{2+} most commonly used to reclaim the sodic soil and improve soil water infiltration (Gupta et al, 1985). Seeds germination is usually the most critical stage in seedling establishment, determining successful crop production (Bhattacharjee, 2008). Salinity and drought are the major environmental constraints to crop productivity throughout the arid and Sami-arid regions of the world. Between 30 and 40% of the world irrigated agricultural lands are prone to salinity (Foollad and Yin, 1997; Garg, 2010). The United Nations Environment Program (UNEP) estimated that 20% of the total agricultural and 50% of the available cropland in the world is salt stressed or salt affected (Flowers and Yeo, 1995). Soil salinity imposes a serious environmental problem, affects vegetation cover and the availability of animal feed limiting the availability of grasses in arid and semiarid regions. Salt stress unfavorably affects the plant growth and reduces the productivity during all developmental stages of crops. It has been reported (El-Kharbotly *et al.*, 2003).

The high levels of sulfate and chloride can diminish the rate of nitrate absorption in plants. Specific ions such as sodium and chloride have toxic effect on plants. They reduce the growth or cause damage to cells and membranes. The nutritional deficiencies and toxicities of plants are characterized by necrosis that results in tip burning or marginal scorch, chlorosis which results in turning the leaves yellow in color, and abscission which results in premature dropping of leaves. Seed germination is the initial and the most crucial stage in the life cycle of plants (Grime and Campbell, 1991). Different abiotic factors such as temperature, soil salinity, photoperiod and soil moisture affect germination of halophytes (Noe and Zedler, 2000). However, the effect of soil salinity seems to dominate other factors in saline areas (Keiffer and Ungar, 1997). The present investigation was conducted to test the possibility to saline alkaline black cotton soil reclamation by Codex-551 with the case study of its impact on seedling growth of *Vigna sinensis*.

Material and Method:

Study area and soil sampling:

The soil sample was collected from Sharnapur, District Aurangabad. The upper layer (25cm) of saline soil sample was collected. The soil sample was collected in polythene bags. These saline soil samples were analyzed for physico-chemical parameters for the comparative study, the pH, electrical conductivity, sodium, total alkalinity etc. Garden soil was collected from Botanical garden of Dr. B. A. M. University, Aurangabad and characterized for same physico-chemical parameters using standard methods (Trivedy and Goel, 2000; Gupta, 2002). The *Vigna sinensis* seeds of variety- Pusakomal were procured from local market for the present study.

Nature of 2-Phosphono Butane 1,2,4, Tricarboxylic Acid (Codex-551):

The 2-Phosphono Butane 1,2,4, Tricarboxylic Acid (Codex-551) is the synonym of phosphono butane tri carboxylic acid and abbreviated as PBTC. Its chemical formula is $C_7H_{11}O_9P$ and molecular weight is 270. It is colorless or light pale yellow liquid having specific gravity 1.3^{+}- 0.01 at 25^{o}C. The pH of 1% solution is less than 2 due to active acidic nature. It has wide applications in cooling water treatment, boiler water treatment; Codex-551 was investigated as soil amendment for saline soil reclamation.

Experimental method:

The experimental work was carried out under laboratory conditions. The healthy seeds were selected, sterilized with 0.05 N $HgCl_2$ and seeds were germinated in Petri plats. Total of 20 seeds were kept for germination in each sterilized Petri plate with all sets in triplicate. At the bottom of each Petri plate wet filter paper were placed and 20 seeds of *Vigna sinensis* were arranged on it. The papers were replaced every two days to prevent accumulation of salts (Rehman et al. 1996). A set of control without any treatment was arranged for comparison with only saline soil suspension. Treatments were (6) control i.e., there was no addition any chemicals only saline soil suspension and sets 1 to 5 were treated with different ppm concentrations viz. 20ppm, 40ppm, 60ppm, 80ppm, 100ppm concentrations were prepared.

The seeds were considered to have germinated, when the emerging radical elongated to 1mm. Shoot and root length (cm), and seedling fresh and dry weight mg/plant were measured on the 7th and 15th day. Germination percentage of seeds was observed and recorded after 24 hours and 48 hours. Dry weights were measured after drying samples at 70°C for 48 hours in an oven (Bohm, 1979). Germination was recorded.

Results and Discussion:

The Physico-chemical parameters of saline alkaline-black cotton soil were studied. Salinity indicator parameters, electrical conductivity and exchangeable sodium values of soil were determined 4.19ds/m and 13.5 mg/kg respectively. Such saline soil reduces crop yields by upsetting the water and nutrient balance of plants (Mass and Hoffman, 1977: Mass 1987). All plant character decreasing with increasing level of soil salinity (Sharif and khan, 2009) has needed to treat the saline soil with suitable soil amendment.

Table 1: Effect of Codex-551 on seed germination of *Vigna sinensis*

Treatments Conc. of Codex-551	Mean ±SD After 24 hr.	Mean ±SD After 48 hr.
20 ppm	5±0.93	12.00±1.41
40 ppm	8.7±1.21	14.10±2.00
60 ppm	15.3±1.81	18.11±2.79
80 ppm	12.8±1.58	15.00±2.23
100 ppm	10.6±1.37	11.02±1.17
Control	1±0.44	06±0.92
Fertile soil	17±2.39	20+3.05

Codex-551 was used for the treatment of saline soil. Ten different concentrations at ranging from 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm were used for the treatment of saline soil. After the treatment of codex-551 with 20 ppm concentration to the saline soil, the growth in the average seed germination observed was 5±0.93 out of 20 seeds treated in each set after 24 hours. In the second set, the treatment with 40 ppm concentration, it was 8.7±1.21, the third set treated with 60 ppm concentration, the average seed germination was highest (15.3±1.81) was observed after 24 hours. In the fourth set treated with 80 ppm concentration, it was 12.8±1.58 and in the fifth set treated with 100 ppm concentration, it was 10.6±1.37

respectively. It observed that germination was decreasing after the treatment with higher concentration of codex-551 than 60 ppm concentration indicating the toxic effect or hindering effect on seed germination evidenced by reduced percentage of seed germination. The seed germination was not observed at all in saline-alkaline black cotton soil without any treatment which was treated as control ad in the fertile soil 17±2.39 seed germination was observed for the comparison. Similar results of various experiments performed using different amendments such as gypsum, pyrite, press mud, aluminum sulphate, and farmyard manure are reported (CSSRI, 1979: Tyagi and Minhas 1998; Tyagi, 1999), but Codex-551 supper or to all these.

The germination was increased after 48 hours and then 24 hours. After the treatment of codex-551 with 20 ppm concentration to the saline soil, the growth in the average seed germination observed was 12.00±1.41 with 20 seeds exposed to the each treatment in each set after 48 hours. In the second set, the treatment with 40 ppm concentration, the germination was 14.10±2.00. The third set treated with 60 ppm concentration, the average seed germination was highest (18.11±2.79) was observed after 48 hours. In the fourth set treated with 80 ppm concentration and higher concentrations it was decreased. The germination was 15.00±2.23. The germination was reduced to 11.02±1.17 in the fifth set treated with 100 ppm concentration, in the saline-alkaline black cotton soil without any treatment 06±0.92 seed germination was observed which was treated as control and in the fertile soil 20+3.05 seed germination was observed for the comparison. Sivapalan (2004) reported the improvement in seed germination, dry matter production and grain yield in soybeans grown in saline soil with polyacrylamides and gypsum treatments.

Table 2: Effect of Codex-551 on root, shoot length, fresh weight and dry weight of *Vigna sinensis* after 7 days

Treatments Conc. Of Codex-551	Root Length after 7 days in (cm) per plant	Shoot length 7 days (cm) per plant	Fresh weight after 7 days (mg) per plant	Dry weight after 7 days (mg) per plant
20 ppm	1.6	7.2	0.826	0.196
40 ppm	1.9	10.5	0.978	0.222
60 ppm	2.3	12.8	1.118	0.269
80 ppm	2.0	9.3	0.840	0.205
100 ppm	1.1	4.6	0.385	0.098
Control	0.6	1.7	0.112	0.030

Fertile Soil	3.2	15.11	1.345	0.328

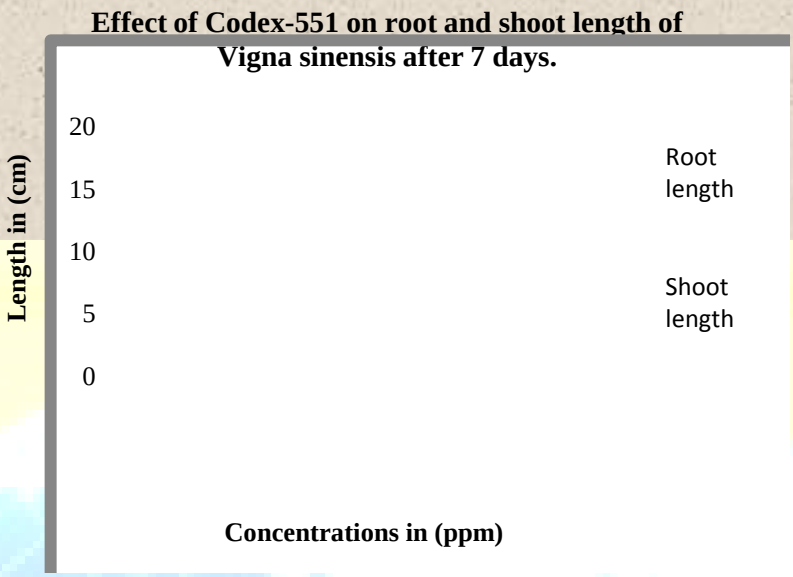

Fig. 1: Effect of Codex-551 on roots and shoot length of *Vigna sinensis* after 7 days

The treatment of codex-551 was 20 ppm concentration. The root length and shoot length observed were after 7 days 1.6 cm and 7.2 cm. In the second set treated with 40 ppm, the root length was 1.9cm and shoot length was 10.5 cm. The third set treated with 60 ppm concentration. The highest root length up to 2.3 cm and shoot length up to 12.8 cm was observed in after 7 days. Slight reduction was noticed in the fifth set treated with 80 ppm concentration. The root length was reduced to 2.0 cm and shoot length to 9.3 cm, fifth set treated with 100 ppm concentration also showed reduction in the root length (1.1 cm) and shoot length (4.6 cm). It is observed that the root length and shoot length were increasing with treatment concentration maximum at 60 ppm and were decreasing there after indicating the toxic effect. The lowest root length 1.1 cm and shoot length 1.5 cm was observed in saline soil without any treatment and in seventh set fertile soil the root length 3.2 cm and shoot length 15.11 cm was observed after 7 days.

The treatment of codex-551 was found beneficial at lower and moderate concentration and reflected through the increase in fresh weight and dry weight per plant. With its 20 ppm concentration, the total fresh weight of *Vigna sinensis* crop observed was 0.826gm and dry

weight was 0.196gm per plant 7 days. This increase in fresh weights were 0.978gm, 1.118gm, per plant in the sets treated with 40 ppm, 60 ppm concentrations of codex-551 after 7days and the dry weights were 0.222 gm, 0.269 gm per plant respectively. The results are similar to the fresh weight of forage cultivation on saline soil (Qadir, et al., 1996).

The fresh weights and dry weights in all sets treated were higher than the fresh weights and dry weights in control set but were found decreased at higher treatment concentrations of codex-551. In the set treated with 80 ppm conc. it was observed the total fresh weight of *Vigna sinensis* crop 0.840gm and dry weight 0.205gm per plant. The fresh weight per plant in sets treated with 100 ppm was 0.385gm and 0.098 gm. In saline soil (Control) without any treatment it was observed the lowest total fresh weight of wheat crop 0.112gm and dry weight 0.030gmand in fertile soil fresh weight was 1.345 and dry weight 0.328 was observed after 7 days respectively.

Table: **3** Effect of Codex-551 on root, shoot length, fresh weight and dry weight of *Vigna sinensis* after 15 days.

Treatments Conc. of Codex-551	Root length15 days (cm)	Shoot length15 days (cm)	Fresh weight after 15 days (mg)	Dry weight after 15 days (mg)
20 ppm	2.4	9.3	0.979	0.241
40 ppm	2.2	13.0	0.1145	0.285
60 ppm	3.5	16.5	1.303	0.324
80 ppm	2.0	10.11	0.1008	0.252
100 ppm	1.6	5.9	0.492	0.125
Control	0.11	2.1	0.186	0.048
Fertile soil	4.6	18.7	1.788	0.452

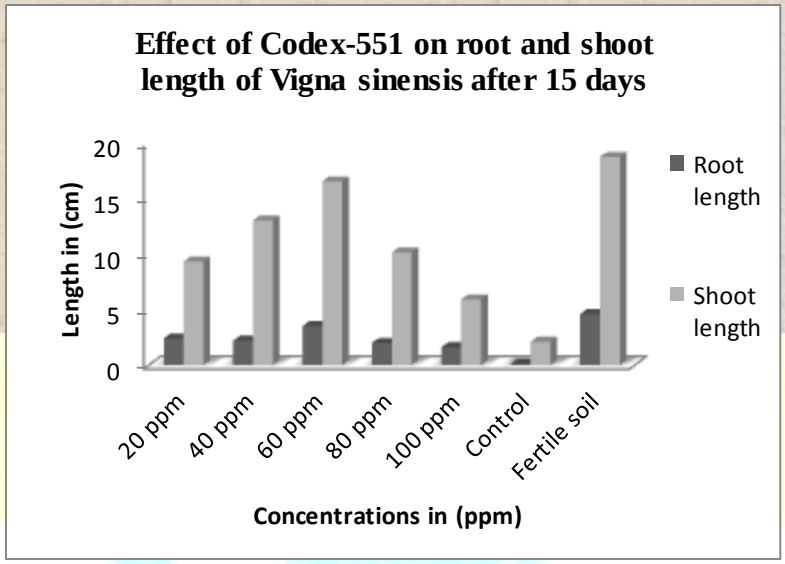

Fig.2: Effect of Codex-551 on roots and shoot length of *Vigna sinensis* after 15 days

The treatment of codex-551 with 20 ppm concentration the root length per plant was 2.4cm and shoot length was 9.3 cm per plant after 15 days. In the second set, the treatment with 40 ppm concentration, the root length per plant 2.2 cm and shoot length was 13 cm. The third set treated with 60 ppm concentration the root length highest was (3.5 cm) and shoot length was also highest (16.5 cm) after 15 days. In the fourth set treated with 80 ppm concentration the root length was slightly decreased to 2 cm and shoot length was 10.11 cm and fifth set treated with 100 ppm concentration the root length and shoot length were decreased 1.6 cm and 5.9 cm respectively. It observed that the overall root length and shoot length was decreasing after the treatment with 60 ppm concentration indicating the toxic effect or hindering effect evidenced by reduced the root and shoot length respectively. In saline soil (Control) without any treatment the lowest root length (0.11 cm) and shoot length (2.1 cm) was noticed and in fertile soil the root length 4.6 and shoot length per plant 18.7 cm was observed after 15 days. These results are supported by higher biomass production in soils treated with gypsum and polyacrylamides (Shainberg et al., 1990; Zahow and Amrhein, 1992; Cay et al., 2001; Deery et al., 2002)

The treatment of codex-551 with 20 ppm concentration the total fresh weight of wheat crop 0.979gm per plant and dry weight was 0.241 gm after 15 days which is lowest among the all treatments except control. In the second set was 40 ppm concentration. The total fresh weight of

wheat per plant was 0.1145gm and dry weight was 0.285 gm. The third set (60 ppm concentration) had highest total fresh weight (1.303gm per plant) and dry weight (0.324gm per plant) after 15 days respectively. In the fourth set treated with 80 pap concentration the total fresh weight per plant was decreased to 0.1008gm and dry weight to 0.252gm and in the fifth set treated with 100 ppm concentration the total fresh weight per plant was just 0.492gm and dry weight was 0.125 gm. The lowest total fresh weight per plant (0.186gm) and dry weight (0.048gm) was observed in saline soil (Control) without any treatment and in a fertile soil fresh weight 1.788 and dry weight 0.452 was observed after 15 days which clearly suggests that the Codex-551 reduces the toxicity in saline alkaline-black cotton soil. Similar, effective saline soil reclamation with coal powder and iron sulphate is reported by Raychev et al., (2001). The vegetation yield in terms of biomass was more than 30% higher than control with coal powder as soil amendment.

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