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RESPONSE OF *RICINUS COMMUNIS* L. TO CHELATED IRON & ZINC.

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ABSTRACT

Castor is cultivated for the seeds which yield a fast drying non-yellowing oil, used mainly in industries and pharmaceuticals. Nutrient deficiencies in plant have been corrected by using metal chelators. They are more effective than normal salt. The absorption of iron and zinchas been known to be greater with EDTA than when given alone. In the present study the various dosesof 20,50 & 100ppm of Fe-EDTA & Zn-EDTA induced female flower number, total number of fruits and oil content in *Ricinus Communis*L.Single spray of 50 ppm Fe-EDTA & Zn-EDTA at 6 to 8 leaf stage enhanced maximum increase ones control of female flower number (75% & 51.32%) and single and double spray of 50 ppm of Fe-EDTA and Zn-EDTA at the same stage enhanced maximum increase over control of fruit number (65.82% & 49.59%) and Double spray of 50 PPM Fe-EDTA & Zn-EDTA at same stage enhanced maximum increase over control of oil content (32.43% & 40.04%).

Keywords: Metal Chelator, EDTA, ppm, Maximum increase over control.

INTRODUCTION

Ricinus communis belongs to Euphorbiaceae family and is commonly known as castor oil plant. It is grown as annual Plant and its dwarf variety reaches up to 4-5m. The main stem is terminated by the First or primary raceme. The racemes usually bear pistillate flowers on the upper 30% and staminate flower in the lower 70% of the racemes. Castor oil plant is cultivated for the seeds which yield a fast drying, non-yellowing oil used mainly in industry and medicines. Although *Ricinus communis* is an economically important Plant, it is generally grown as a weed in poor soil. Therefore, it requires a sustained supply of soil nutrient to ensure maximum yield.Nutrient deficiencies in plant have been corrected by using metal chelator. Iron and Zinc is one of the most essential metal for cell metabolism. The absorption of Iron and Zinc has been known to be greater with EDTA than when given alone. They are more effective than normal salt of these nutrient. They are soluble in water and dissociate slightly. It is because of these facts they are ideally suited for the application to the crop deficient in particular micronutrient. The metal chelate complex diffuse to plant cell where the metal ion are absorbed leaving the chelating agent.

"Chelator is a compound with a metal atom joint to two or more electron donor groups of a single organic molecule. This give a ring configuration in which the metal is firmly held and the complex has charge and solubility properties alien to the metal itself."

The important metal chelators are:-

EDTA- Ethylene diamine tetra acetic acid.

DTPA- Diethylene triamine Penta acetic acid

EDDHA- Ethylene diamine dihydroxy phenyl acetic acid.

METHODS

The seeds of *Ricinus communis* Linn (Var. Chandraprabha) were obtained from Chandra shekhar Azad university of Agriculture and Technology Kanpur. The plants were raised in earthenwere pots (25 cm diameter) filled with garden soil and kept in garden of Christ church college, Kanpur. Plants were arranged in random block design having three blocks. Three replicates were picked up for collecting data. The seeds were shown in the first week of September and the observation were completed by the end of March. Preliminary experiment was conducted between September 2004 and March, 2005. The experiment were repeated for the following two years. The result presented here are of final observation. Metal chelator (EDTA) in combination with Fe-EDTA and Zn- EDTA were obtained from the market. Effect of metal chelator on seed germination and seeding growth were performed in the laboratory. For laboratory experiment seeds were soaked in 10, 20, 50, 100 and 200 ppm and the effect on seedling were studies using Garrads technique (1954). To study the effect on (A) vegetative and (B) Reproductive morphology, field experiments were conducted. For field experiment, which was aimed to assess the effect of metal chelator on growth and yield of *Ricinus communis* 20,50 and 100 ppm of Fe-EDTA and Zn-EDTA were applied either as single or double spray at four stages of plant development viz.

(i) Prior to germination of seeds i.e.- seed soaking stage 'A'
(ii) 3 to 4 leaf stage 'B'
(iii) 6 to 8 leaf stage 'C' and
(iv) 0 to 12 leaf stage 'D'.

The average number of days required to obtain stage B, C and D were 30, 60 and 90 days respectively from sowing seeds. Second spray if given was 10 days after the treatment.Control plant were water sprayed with a few drops of teepol. The final data were recorded 150 days after sowing where fruit setting started.The observation were recorded from 3 tagged plant. Fruit count were made 180 days after sowing.

Preparation of Solution:

For the Preparation of solution of metal chelator, one gram of each chelator was taken in an individual in 500 ml distilled water with constant stirring. The volume of solution was finally constituted to one litre. This was the 1000ppm stock solution of each chemical. The flask containing chemical were covered with muslin cloth to avoid any contamination for preparation of 10, 20,50, 100 and 200 ppm solution of each metal chelator 10, 20, 50, 100 and 200 ml of liquid from the stock solution was taken in a well cleaned measuring flask and constituted to 1000 ml.

Soaking of the seed in Solution :

After preparation of solution of Fe-EDTA and Zn-EDTA, seeds were soaked in desired solution for 12, 24,& 48 hr at 25^0+ c temp. Simultaneously a control in which seeds were soaked in distilled water for 48 hr. was also conducted. Result from the effect of chelator showed that soaking seed for 48 hr was most effective in inducing growth for field experiment. 48 hr, seed soaking in metal chelator were used. Preliminary experiment on seed germination were conducted by Garrad's technique in test tube.The seeds were placed in test tube between blotting paper and the wall of the tube.Two seeds of each treatment were placed for germination at 25^0 c. in three replications in complete Randomized design in a tray. Observations were made on six randomly selected, seedlings on the 10^{th} days.

Treatment: Based on preliminary experiments the dose of metal- chelator were 20, 50 and 100 ppm of Fe-EDTA and Zn-EDTA applied as different 4 stages. (A,B,C,D).

(a) A single spray of Fe-EDTA and Zn-EDTA

= t_2 t_3 t_4 t_6 t_7 t_8

Stage (B) (C) (D) (B) (C) (D) = 6x3 = 18

(b) Double spray of Fe-EDTA & Zn- EDTA

= t_9 t_{10} $t_{11}t_{12}$

(C)(D) (C) (D) = 4x3= 12

(c)Seed soaked in Fe- EDTA & Zn-EDTA

t_1 t_5 = 2x3 = 6

Total = 18 +12+6 = 36

36 Treatment + 1 Control = 37

37x3 = 111 Plant arranged in 3 blocks

In three blocks:- 2 samples were takes for each treatment.

111 x 2 = 222 (Total plants)

Parameters Taken for study :

(i) Female Flower Number:-

For this the female flowers on the first inflorescence were counted every alternate day, till fruit setting has begun. Both bloomed and unbloomed flowers were counted.

(ii) Fruit Number:

The total number of fruits of the first inflorescence was considered.

(iii) Oil Content:-

Oil extraction was done by soxhlet method and as modified by synder *et al.*(1991). The sample of seeds were taken and dried in oven. Seeds were then ground into small particles and then wrapped in blotting paper and put in the bent tube. A clean beaker was taken and weighed petroleum ether was taken in the beaker. It was then boiled at 40- 50^0 C.The petroleum ether was boiled and the vapour so formed entered into the conductor due to evaporation. Water was used as a coolent in the conductor. The vapours when formed completely settled down on the bent tube.The oil came out and settle in the beaker. This process took about 8-9 hours. At the end of the extraction process which lasted few hours, the flask containing the solvent and lipid was removed.

Calculation :

Wt. of filter paper (F.P.) = x

Wt. of F.P. +Seed = Y

Wt of sample = Y-X

Wt of FP + extracted seed = Z

Loss of wt = (Y-X) – Z =A

% of oil in seed = $\frac{A}{Y-X} X100$

The percentage of oil in the sample seed was thus calculated.

Statistical Analysis:

For determining the significance of differences caused bydifferent treatments data were subjected to statistical analysis and significant response at 5 percent level were computed whenever necessary.Critical deference (CD) was worked out for comparison of mean value for various treatments and their effect.

Standard error and critical difference value were calculated by the method of fisher and Yates (1937).L.S.D. compassion at 5% level were determined. Bar diagrams given at 5% level help to interrelate the treatment groups based on effect of treatment and doses.

Abbreviation Used: t_1;t_5= Single treatment of Fe-EDTA & Zn- EDTA respectively given at stage A. (seed soaking stage)

t_2; t_6 = Single treatment of Fe-EDTA & Zn-EDTA respectively given at stage B (3 to 4 leaf stage)

t_3;t_7 = Single treatment of Fe- EDTA & Zn-EDTA respectively given at stage C (6 to 8 leaf stage)

t_4;t_8 = Single treatment of Fe-EDTA & Zn-EDTA respectively given at stage D (10 to 12 leaf stage)

t_9;t_{11}= Double application of Fe- EDTA & Zn-EDTA respectively given at stage C (6 to 8 leaf stage) at interval of 10 days.

t_8; t_{12}= Double applications of Fe- EDTA & Zn-EDTA respectively given at stage D (10 to 12 leaf stage) at interval of 10 days.

d= dose; L.S.D. = Least significant difference; C.D. Critical difference;

A,B,C,D, = Various stages of treatment;

Observation :

Female Flower Number:

Table 1. Effect of various treatment (+), doses (d) of metal chelator and thus interaction on the number of female flowers on the first inflorescence of *Ricinus, communis* L.

Metal Chelators	Stage (Treatments)	Dose in ppm			Mean
		20 (d_1)	50 (d_2)	100 (d_3)	
Fe-EDTA	A(t_1)	21.77	25.87	24.73	24.12
	B(t_2)	25.90	28.57	26.80	27.09
	C(t_3)	28.97	34.70	30.90	31.52
	D(t_4)	26.87	31.83	28.83	29.18
Zn-EDTA	A(t_5)	21.63	24.63	23.20	23.16
	B(t_6)	22.7	27.83	24.83	25.12
	C(t_7)	25.87	30.87	27.93	28.22
	D(t_8)	25.90	28.83	26.33	27.02
Fe-EDTA × 2	C(t_9)	31.10	35.70	25.53	30.78
	D(t_{10})	24.57	13.50	11.03	16.37
Zn-EDTA × 2	C(t_{11})	25.43	30.80	22.90	26.38
	D(t_{12})	24.10	16.47	13.37	17.98
	Mean	25.40	27.47	23.87	
-					
	Control				20.40

C.D. (P = 0.05)	t	d	t × d	cont v/s treat
	0.84	0.42	1.47	1.47

Figure 1. Response of various treatments of metal Chelators (t) and Dose (d) to number of female flowers of *Ricinus communis* L.

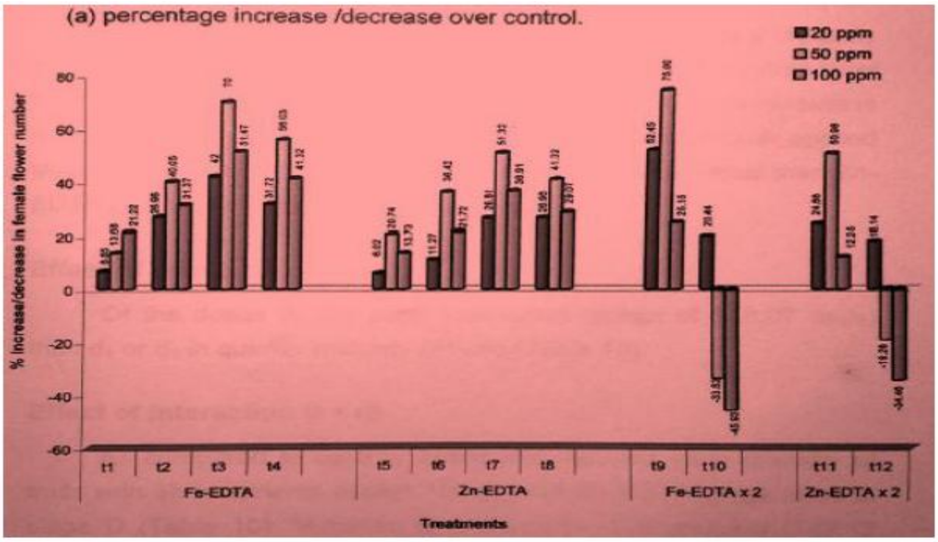


Fig 1 (a) Percentage increase / decrease over control

Fig-1 (a) Shows that an increased percentage of female flower number over control was observed with all treatment of metal cheater except 50 and 100 Pm applied twice at stage D. The increase was generally more with 50 ppm (d2) than 20 ppm (d1) or 100 ppm (d3) (75% over central).The percentage of increase was maximum with two doses of 50ppm of Fe-EDTA applied twice at stage c (tg x d2) followed by single dose of 50ppm of fe-EDTA (70.00%) (t3Xd2).The percentage of increase was also maximum (51.32%) with single dose of 50ppm of Zn-EDTA applied single at stage c (t7 xd2), followed by double dose of 50 ppm of Zn- EDTA applied twice at stage c (50.98) (t11xd2).

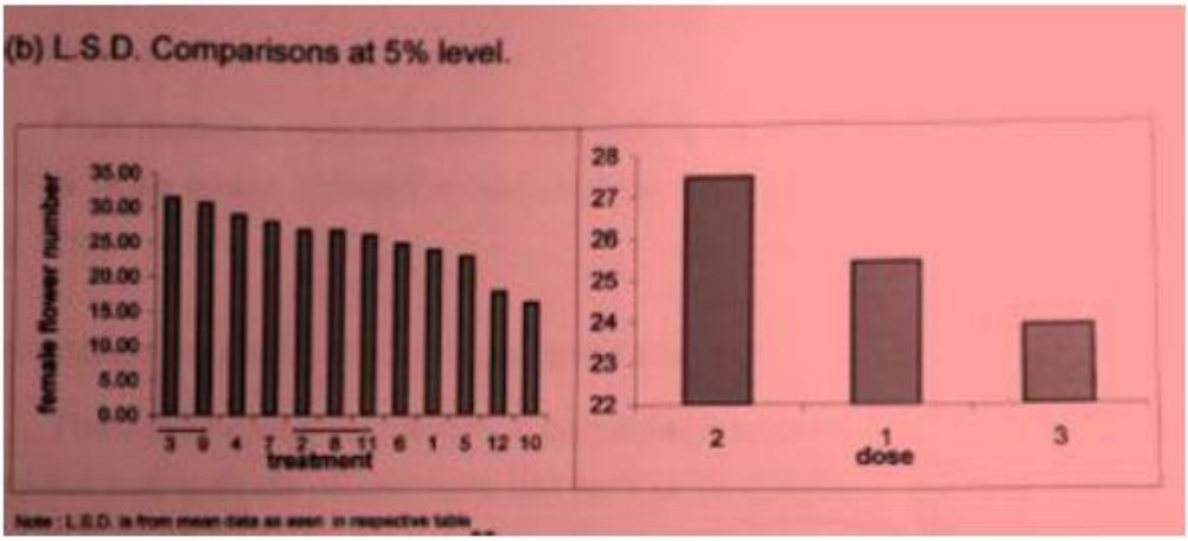


Fig -1 (b) – L.S.D. Comparison at 5% level:

Female flower number increased to its maximum with t3 followed by to and the effect of the two were not significantly different fig 1(1b)). The dose which was best to increase female flower number was d2.

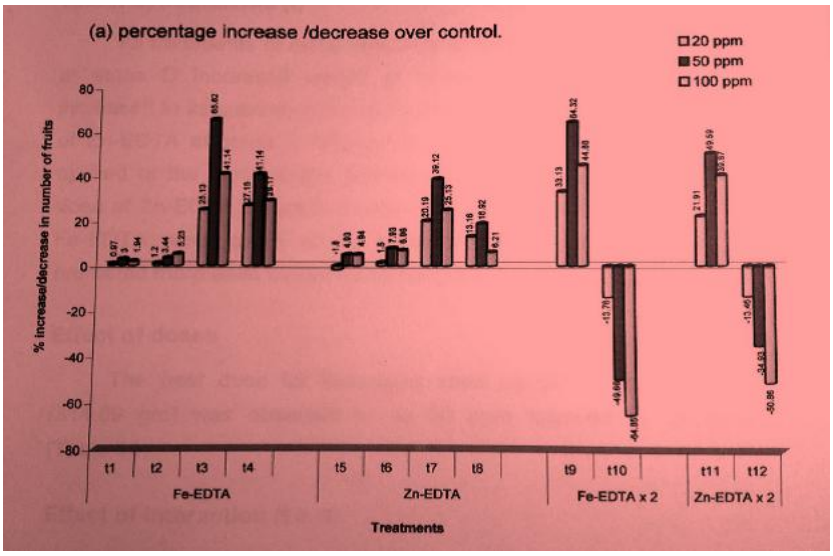
Fruit Number:

Table 2. Effect of various treatment of metal chelatars (t), different doses (d) and their interaction on number of fruits per plant in *Ricinus communis* L .

Metal Chelators	Stage (Treatments)	Dose in ppm			Mean
		20 (d_1)	50 (d_2)	100 (d_3)	
Fe-EDTA	A(t_1)	13.50	13.77	13.63	13.63
	B(t_2)	13.53	13.83	14.07	13.81
	C(t_3)	16.73	22.17	18.87	19.26
	D(t_4)	17.00	18.87	17.27	17.71
Zn-EDTA	A(t_5)	13.13	14.03	14.03	13.73
	B(t_6)	13.57	14.43	14.30	14.10
	C(t_7)	16.07	18.60	16.73	17.13
	D(t_8)	15.13	15.90	14.20	15.08
Fe-EDTA × 2	C(t_9)	17.80	21.97	19.37	19.71
	D(t_{10})	11.53	6.73	4.70	7.66
Zn-EDTA × 2	C(t_{11})	16.30	20.00	18.67	18.32
	D(t_{12})	11.57	8.70	6.57	8.94
	Mean	14.66	15.75	14.37	
-					
	Control				13.37

C.D. (P = 0.05) t 0.69 d 0.34 t × d 1.19 cont v/s treat 0.85

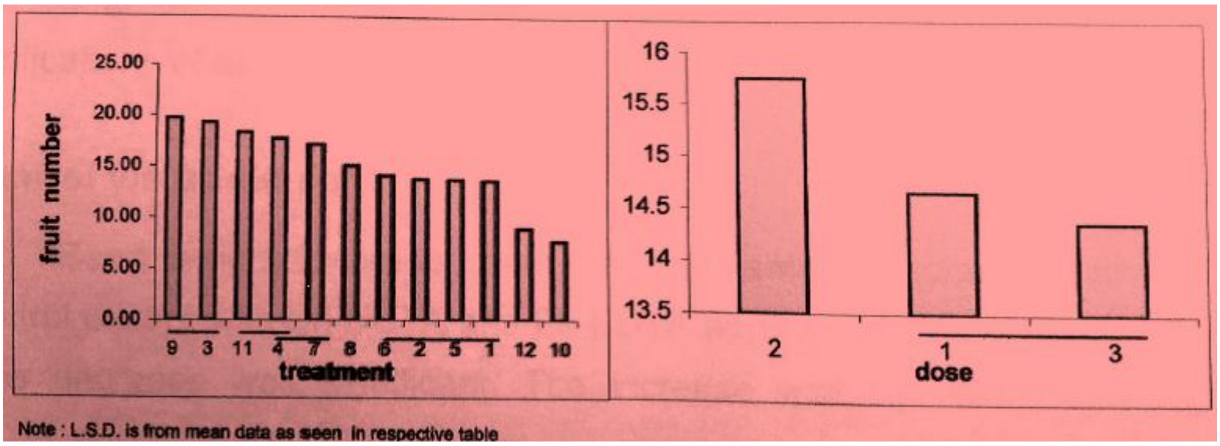
Fig 2a. Percentage increase / decrease over control.



As seen in figure 2(a) that total number of fruit per plant increased with all treatment except two applications given at stage D. The increase was more with 50 ppm than 20 or

100ppm. Treatment given at stage c brought greater increase in fruit number than at other stages.The number was most (65.82% and 64.32% over control) with 50 ppm of fe-EDTA applied as single or two sprays (t3xd2 and t9 x d2) respectively as compared to other treatment. The percentage increase was also maximum (49.59% over control) with double dose of 50ppm Zn-EDTA applied twice at stage c (t11 x d2).

Fig 2b. L.S.D Compassion at 5% level:



Fruit number increased to maximum with t_9 treatment followed by t_3. The dose which induced maximum fruit number was d_2.

Oil Content :

Table 3 a. Effect of various treatments (t), different dose (d) and metal chelators and their interaction on oil content of *Ricinus communis* L. seeds.

Metal Chelators	Stage (Treatments)	Dose in ppm			Mean
		20 (d_1)	50 (d_2)	100 (d_3)	
Fe-EDTA	C(t_3)	34.20	39.12	36.49	36.60
	D(t_4)	35.12	36.12	34.30	35.18
Zn-EDTA	C(t_7)	36.20	43.62	40.17	40.00
	D(t_8)	35.89	40.17	38.16	38.07
Fe-EDTA × 2	C(t_9)	37.60	45.25	30.23	37.69
	D(t_{10})	35.94	41.62	28.50	35.35
Zn-EDTA × 2	C(t_{11})	39.50	47.85	32.16	39.84
	D(t_{12})	36.15	38.64	29.14	34.64
	Mean	36.33	41.55	33.64	
	Control				34.17

C.D. (P = 0.05) t 1.418 d 0.87 t × d 2.46 cont v/s treat 1.84

Data is percentage of oil in seeds.

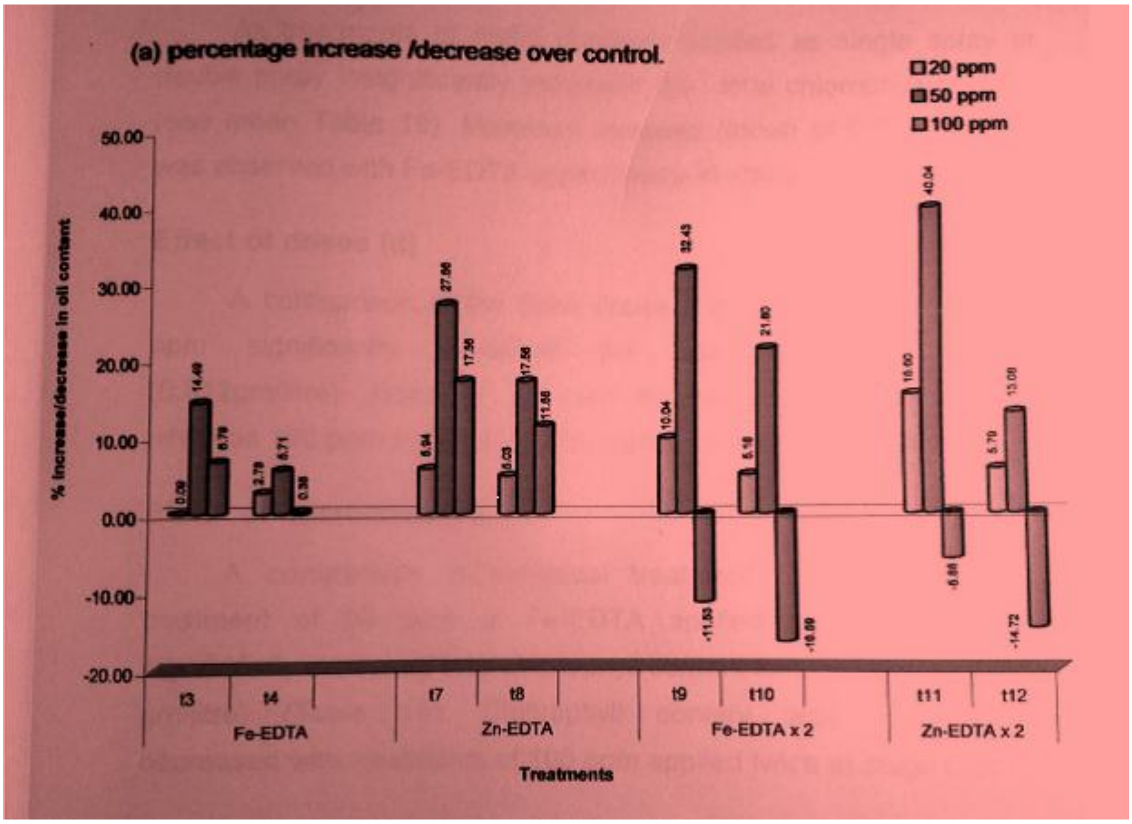
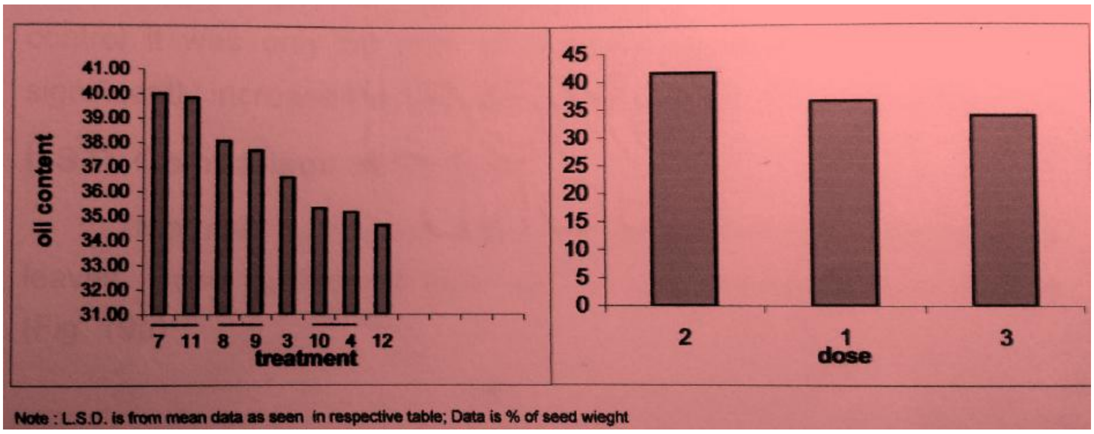


Fig 3(a) indicates that oil content increased with treatment and was better with 50 ppm than with 20 or 100 ppm. Treatment at stage C induced more oil content and was maximum (40.04% increase over control) with 50 ppm of Zn-EDTA applied twice at stage C. (t_{11} x d_2). The percentage increase was also maximum (32.43%) with double dose of 50 ppm of Fe-EDTA applied twice at stage C (t_9x d_2).

<u>Fig (3b). L.S.D. Comparison at 5 % level</u> :



The Percentage of oil in seeds was maximum with t_9 and t_{11}. The dose which induced maximum oil in seeds was d_2.

RESULT AND DISCUSSION

The effects of metal chelators on *Ricinus communis* showed interesting result during the present study. In the present study, the various doses of 20, 50 and 100 ppm of Fe-EDTA and Zn-EDTA induced female flower number, total number of fruits and oil content in *Ricinus communis* L. Iron and Zinc are two essential nutrients for crop production and reproduction. These nutrients are absorbed in the form of ions or chelates from the soil solution. Zinc influence the formation of growth hormone and is involved in auxin metabolism. It is important for reproduction and water uptake of plants. It is also an essential component in number of dehydrogenases, proteinases and peptidases and also influence the protein synthesis and affects electron transport reaction (cakmak *et. al* 1989)

Zinc deficient plants are deficient in growth regulators(Follett *et al.* 1981) due to depression in the rate of photosynthesis (Bottrll *et. al.* 1970). Iron is essential for chloroplast formation. It play an important role in electron Transport system in photosynthesis and respiration (ohki 1976). It is essential component of flavoprotein and also function in iron porphyrin proteins. Since application of one mineral in the soil can cause deficiency of another by interaction and also because excess fertilizer can cause eutrophication, application of chelates with the essential nutricent element, (metal ion) could be more effective and beneficial. They are more effective than the normal salt of these nutrients. Naturally occurring chelates like deoxymugineic acid has been found to be secreted by rice in response to Zn or Fe deficiency to take up Zn or Fe (Suzuki et al 2008).

The chelation of a compound is of great importance for cell structure and for the function of enzymes. The metals thereby serving as activator or as mediators in electron transfer. The addition of chelates forming compounds to a nutrient media therefore should affect plant growth and must be assumed to depend upon the chelation of metals. Chelating compounds have been used for curing mineral nutrient deficiencies in plant (Weinstein et al. 1956 a, b) and this is increasingly gaining importance (Szladits 1956,Kratzer 1986, Pandey & Pandey 2002).Recaldin and Health (1856) reported that 3 indole acetie acid act on growth in the same way as chelating agent by forming chelate compound. Burstrom (1963) is of the opinion that chelating compound such as EDTA exert growth action in low concentration similar to auxins. They observed increasing shoot and decreasing root growth as in the present investigation an action similar to auxin.Chelating agent can modify flowering response (Hillman-1959). In present investigation, the chelating compounds Fe-EDTA and Zn-EDTA were given the good response in increasing female flower number, fruit number and oil content.

Treatment	Parameters	% increase over control		Dose
		t3	t3	
t_3, t_9	Increased female flower number	70% (Fe-EDTA)	75.00 (Fe-EDTA)	
	Increased female flower number	65.82% (Fe-EDTA)	64.52 (Fe-EDTA)	d_2
t_9, t_{11}	Increased oil content	32.43 (Fe-EDTA)	40.04 (Zn-EDTA)	d_2

(1) Results, indicates that the lower concentration of 50 ppm (d_2 dose) of Fe-EDTA and Zn-EDTA applied once or twice has promoted the female flower number, fruit number and oil content.

(2) A comparison of the effect of Fe-EDTA and Zn-EDTA indicates that Fe-EDTA was more better in increasing female flower and fruit number than Zn-EDTA as indicated in chart.

(3) Zn-EDTA was better in increasing oil content than Fe-EDTA.

Finally,it is concluded that the metal chelators can help to promote the much-required productivity of castor oil with improved quality of the produce.The right treatment may specially help to correct the mineral deficiencies when the crop is grown on less fertile soil.

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