https://doi.org/10.29321/MAJ.10.A04034

Copper Nutrition of Millets (Part I)

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Introduction: The establishment of essentiality of certain elements in minute quantities in the nutrition of plants led to an extensive study of micro-nutrients during the past few decade. As a consequence of recognising their importance, considerable attention was paid to their practical utility which promised great potentialities not only in the solution of problems of nutritional disorders in crops but in the matter of increased crop production. Crop failures that often occur in many regions may perhaps be attributed, at least in part, to a deficiency of micro-nutrients. For Indian crops and soil conditions particularly, information is lacking on micro-nutrients.

Among the micro-nutrient elements, Boron, Copper, Manganese, Zinc and Molybdenum are perhaps the most important. Very minute quantities are required to perform important functions in plant nutrition. Further, small amounts of them are enough to plants to restore themselves from specific abnormalities and impaired physiological functions caused by their deficiencies in the nutrient medium. Thus Bertrand (1905), Warrington (1923), Thumberg (1934) and Arnon and Stout (1939) proved that Manganese, Boron, Zinc and Molybdenum respectively play various essential roles in the plants' metabloic activities and their absence causes specific deficiency symptoms in plants.

In a similar way quite a few workers have reported on the copper status in plants. The development of Bordeaux Mixture in France with Copper Sulphate as the principal active component was of great help to combat the diseases that had invaded valuable vineyard. In Florida, its application helped not only to control devastation by insects and diseases but also acted as a stimulant to trunk crops. Soil dressings with copper sulphate were adopted in Holland to combat "Reclamation disease" and in U. S. A. to make muck soils productive and induce greater yields in silt loam. In synthetic fertilizer industry too, it is becoming increasingly common now-a-days to add Copper and such other micro-nutrients along with the main ingredients of Nitrogen, Potassium, and Phosphrus.

As early as 1914, Brenchley proved that although copper normally acted as a poison to higher plants, under special conditions and in very great dilutions, it showed stimulatary action. Harrion and Subramanya Aiyar (1917) recorded from pot culture studies, an increase of ten to thirty percent in grain yields of paddy as a result of irrigating with copper sulphate one in 20,000 (50 parts per million). McHargue (1925) found the range of copper to vary widely between traces and fortysix parts per million in various plants. Plants respond to suitable copper treatments (Millers, 1938), showing increase in vigour, yield, quality and control of chlorsis. A considerable increase in the growth of flax, tomatoes, and sunflower was affected by additions of small amount of Copper (Sommer 1931). Lipman and Mackinexy (1931) observed that flax and barley in water cultures failed to produce seed unless a small quantity of copper was present. Tokuoka and Morooka (1937), (1938) found 0·5 parts per million of copper rendered rice plant sterile and one part per million of copper was sufficient to kill them completely. Brenchley (1938) studied the toxic range of coper and its effect on barley roots. Besides general depression in growth, the roots were short and bunchy. Castillo (1940) working on upland rice in the Philippines found from pot cultures that three pounds of copper sulphate per acre foot of soil was optimum for increasing grain yields. It was noticed at Agricultural Research Station, Tindivanam that groundnut crop sprayed with Bordeaux Mixture as a prophylactic measure against "Tikka" leaf-spot diseases grew vigorously and gave better yields than unsprayed crop. But no systematic work has been made in India, especially on the micro-element nutrition of plants with particular reference to Copper. Hence an attempt in this paper is made to present the results of a systematic study on the role of copper in the nutrition of the major millets of Madras viz., Cholam, Ragi and Cumbu.

Material and methods: The main crops chosen for the study were Sorghum, *Sorghum vulgare* Co. 12, Ragi *(Eleusine coracana)* Co. 1, and Cumbu *(Pennisetum typhoides)* Co. 4. Physiological studies on the effect of varying doses of copper were made on germination, plant height, leaf/area, flowering, dry matter production and yields of earheads, straw and root. Chemical studies were made by determining the changes in the chemical composition at various stages of plant growth due to different concentrations of copper, and also the interrelations of various elements with copper. Reducing sugars were also determined at the active vegetative stages. In the case of germination twenty clean petri dishes containing 150 grams of pure washed sand were used. Calculated quantities of copper were applied in the form of copper sulphate solution so as to make 0, 10, 20, 50 and 100 parts per million in the medium, and the sand was allowed to dry. Hundred seeds were sown in each dish and the germination test was replicated four times. Seeds were kept at 30° C, for germination and counts were taken at the end of ten days.

Studies on growth and yield were conducted in glazed pots 10" x 10" containing 16 Kilograms of washed river sand sieved to one millimeter diameter. The sand was washed a number of times thoroughly with a mixture of hydrocholric acid (2%) and 0·1% Oxalic acid. Dilute copper sulphate solution containing calculated quantities were applied to washed sand so as to make the medium have different concentrations of copper viz., 0, 10, 20, 50, 100, 200 and 500 ppm. The layout of the experiment was replicated six times. 25, 15 and 25 seeds per pot were sown in the morning in the case of Cholam, Ragi and Cumbu respectively. After germination, plants were thinned to a suitable number. Suitable culture solutions were prepared and applied to the respective crops. Thus Cholam was raised using Jacobson's solution (1925) III R_2 S_1 Ragi with Kempton's solution (1943) and Cumbu with Arnon and Hoagland's solution (1940) respectively.

Jacobson's solution per litre (M/10 ml).

$K NO_3$ 54·0., $Mg SO_4$ 135·0., $Ca (H_2 PO_4)_2$ 0.63239 gms., $Fe SO_4$ 0·5% plus Tartaric acid 0·4% - 0·6., pH 6·3.

Kempton's solution per litre (M/10 ml).

$Ca (NO_3)_2$ 39·8., $K NO_3$ 29·96., $(NH_4)_2 SO_4$ 19·98., $Mg SO_4$ 19·03., $KH_2 PO_4$ 22·02., $Fe SO_4$ 0·5% plus Tartaric acid 0·4% - 0·6., pH 6·46.

Arnon and Hoagland's solution per litre (M/10 ml).

$K NO_3$ 10·0., $Ca (NO_3)_2$ 30·0., $NH_4 H_2 PO_4$ 20·0., $Fe SO_4$ 0·5% and Tartaric acid 0·4% - 0·6., pH 5·67.

Each experiment was laid in four sets so as to remove the plants for study at the four important physiological stages of growth viz., seedling, vegetative, flowering and harvest. Growth measurements were recorded by taking height at intervals of seven, fifteen, and twenty days for cumbu, cholam and ragi respectively. Data on leaf-areas were collected by deriving the product of the central length of the leaf third from the top, and the breadth at the middle of the leaf and expressed in 1/K square centimeter units where 'K' is the leaf factor. At the end of each of the four physiological stages fresh weight of plants were taken. Data on dry matter production (weight of whole plants) were also collected at these four stages for all the three crops and earhead yield data were collected at the final stage.

Solution culture studies were conducted to find out iron-copper relationship varying copper concentration in the nutrient medium, from 0 to 0·1., 0·5., 1·0, 2·0 and 5·0 ppm. The crop cumbu was taken up using Arnon and Hoagland's (1940) culture solution.

Chlorophyll estimations were carried out at vegetative stage on all the three crops receiving 0, 20 and 50 ppm Copper in sand cultures.

For the purpose of chemical analysis the leaves were sampled for estimation of Nitrogen, Phosphorus, Potassium, Calcium, Magnesium, Copper and Iron. For the estimation of micro-nutrients approved methods were employed as given in A. O. A. C. (1945). Special precautions were taken in the collection and preparation of plant samples for the determination of copper and iron. The plant material was scissored to two millimeter bits. Grinding the samples and use of copper ovens were avoided to prevent the chances of contamination of iron and copper. Samples were kept in paper packets in aluminimum trays. Analysis of copper and iron was carried out following the methods recommended by Piper (1951). Copper was determined by the use of triple acid digestion, followed by ammonium citrate and extraction with dithizone in carbon tetrachloride. Further digestion of copper dithizonate with perchloric and sulphuric acid was followed by neutralising with ammonia and after further reaction with sodium diethyldithio carbomate reagent copper was extracted with amylalcohol and compared with standards, (as low as ·001 mg copper). Similarly for iron after wet digestion and making upto 100 ml, the digest was dry filtered and using p.Hydroxy phenylglycine and dd′ dipyridyll, redcolour was developed using ammonia and ammonimum acetate. Standards were used for the determination.

Reducing sugars were estimated colorimetrically by the method of Somogyi (1939) as modified by Moyer and Holgate (1945) (1946). The method consisted of extraction of sugars by methyl alcohol and then using Somogyi's copper reagent and Nelson's arsenomolybdate reagent, blue colour was developed and estimated against standards by the use of Klett Summerson photoelectric colorimeter.

Results and discussion: The results of germination are furnished below:—

Germination:

		Control	10 ppm	20 ppm	50 ppm	100 ppm
Cholam	Mean Percentage	96·0	97·8	98·5	84·8	71·5
	% on Control	100·0	101·8	102·6″	88·3	81·0
Ragi	Mean Percentage	89·8	91·0	94·8	82·0	60·5
	% on Control	100·0	102·4	105·5″	92·4	67·4
Cumbu	Mean percentage	86·0	80·0	75·8	66·8	51·5
	% on Control	100·0	93·6	88·1	77·6	60·1

″ ″ — Significant at 1%

Germination is found to be improved at 10 and 20 ppm Copper to the extent of 1·8 and 2·6 percent in Cholam and 1·4 and 5·5 percent in Ragi respectively. At higher concentrations, germination is depressed. On the other hand, in the case of Cumbu 20 ppm copper is found to depress germination significantly.

These observations are in line with those reported by other workers. Evans (1948) found that copper at 1 to 2% depressed the germination of oat seeds soaked even for a short period of fiteen minutes. Similar observations were made by Mosheov (1938) on wheat *(Triticum durum)*. At lower levels of copper viz., 10 and 20 ppm the effect was stimulating. Poloshchuk and and Negovelov (1936) improved the germination in sesame seed by copper sulphate solution. Naundorf and Oliver (1949) reported that in presence of copper in low concentrations improved the germination of old wheat seeds. Thus, the optium concentrations for improving germination tend to be different for different seeds.

The data obtained on growth expressed as height and dry matter production during the different stages of the crops are presented below in a condensed form.

Growth: Plant height and dry matter production:

Condensed data on mean plant height (in Cm).

Crop	Days old	Control	10 ppm	20 ppm	50 ppm	100 ppm	200 ppm
Cholam	10	13·47	13·68**	12·70	9·53	6·75	...
,,	20	26·62	26·80**	25·05	19·75	13·36	...
,,	30	32·15**	31·88	30·33	28·68	24·55	...
,,	65	54·52**	50·72	50·83	31·23	18·92	...
,,	95	76·28	78·17**	75·92	71·52	45·88	...
,,	120	81·18	88·37	89·40**	86·75	65·88	...
Ragi	15*	7·18**	6·32	5·37	3·55	2.45	...
,,	30	31·60**	30·53	27·97	24·28	24·37	...
,,	56	58·45	53·60	48·48	43·28	32·77	...
,,	104	64·48	72·47**	70·20	60·83	58·28	...
Cumbu	23	62·30	63·12	64·22**	60·48	55·93	38·10
,,	30	71·07	76·70	79·25**	76·85	70·13	52·72
,,	42	107·11**	105·1	102·6	98·7	96·9	75·1
,,	51	117·6**	114·2	113·9	110·6	110·1	90·3
,,	60	117·9	123·3**	121·8	119·7	117·6	100·2

Significant at 1%

Condensed data on dry matter production (in grams).

Crop	Stage	Control	10 ppm	20 ppm	50 ppm	100 ppm	200 ppm
Cholam	Seedling	0·92	1·43"	1·15	0·80	0·80	...
,,	Vegetative	19·48"	17·08"	15·67	9·00	2·30	...
,,	Flowering	41·92	39·33	42·25	41·33	17·58	...
,,	Harvest	40·00	59·50	60·58"	49·33	34·75	...
Ragi	Seedling	9·75	6·50	6·33"	4·33	2·25	...
,,	Vegetative	25·83"	23·67	22·25	19·08	7·92	...
,,	Flowering	55·83	55·17	56·58	58·92"	24·25	...
,,	Harvest	54·67	56·92	52·00	57·08"	34·17	...

Crop	Stage	Control	10 ppm	20 ppm	50 ppm	100 ppm	200 ppm
Cumbu	Seedling	0·40	0·28	0·33	0·30	0·32	0·10
,,	Vegetative	7·48	7·82	9·78″	9·15	6·80	2·45
,,	Flowering	57·17	51·43	42·02	41·58	40·68	14·88
,,	Harvest	206·90	112·75	118·73	120·70	110·77	88·88
		Significant at 1%					

Yield : (Condensed data)

(Shoot/Root/Expressed as % on Control)

	Control	10 ppm	20 ppm	50 ppm	100 ppm	200 ppm
			Cholam Co. 12			
Shoot	100·0	152·4	158·4	132·9	96·5	...
Root	100·0	147·5	229·3	103·0	81·2	...
			Ragi Co. 1			
Shoot	100·0	108·1	102·1	106·7	62·7	...
Root	100·0	94·0	95·3	92·9	69·0	...
			Cumbu Co. 4			
Shoot	100·0	100·8	98·1	95·2′	76·4	71·4
Root	100·0	111·0	144·1	147·3	160·2	295·6

Ear head

	Cholam Co. 12		Ragi Co. 1		Cumbu Co. 4	
	Gram	% on Control	Gram	% on Control	Gram	% on Control
1. Control	2·92	100·0	24·00	100·0	5·93	100·0
2. 10 ppm Cu.	3·42	117·1	24·75	103·1	6·60	111·3
3. 20 ppm Cu.	4·25	145·5″	21·50	89·6	7·73	130·3″
4. 50 ppm Cu.	3·00	102·8	25·33	105·5″	7·67	129·3
5. 100 ppm Cu.	1·60	54·8	14·68	61·2	7·55	127·3
6. 200 ppm Cu.	...	...	...	...	4·48	75·5
Significant or not		Yes at 1 %		Yes at 1 %		Yes at 1 %
C. D.		1·63 Gm. (55·7%)		7·38 Gm. (30·8%)		2·51 Gm. (42·33%)
Ranking		3 2 4 1 5		4 2 1 3 5		3 4 5 2 1 6

Height : (Vide graph).

Cholam: Growth represented by height is found to be accelerated upto twenty days in ten and twenty parts per million of copper treatments and upto thirty days in fifty and hundred parts per million of copper and then a fall is noted till about 65 days Once again the growth is found to increase in treatments of ten to fifty parts per million copper. A steady growth without fluctuation is seen in hundred parts per million copper treatment.

Ragi: Here growth is found to be accelerated upto 30 days. Thereafter till the flowering stage growth is much depressed. However, there is an increased growth after flowering under treatments of 10, 20 and 50 ppm copper concentrations. Plants under 100 ppm. copper show steady increase in growth from seedling to harvest stage.

Cumbu: Growth is found to be accelerated upto vegetative stage with a subsequent retardation upto the flowering stage. Thereafter a rise is noted. The degree of stimulation in growth during the early stages is more pronounced in lower concentration as compared to higher concentration of copper.

It is thus seen in general, that in the case of Cholam and Cumbu there is a stimulation in height under low concentration of copper in the growing medium at the seedling and harvest stages while in the case of ragi, stimulation is discernible only at the harvest stage.

Copper is observed to influence growth by accelerating the seedling and post-flowering stages though it is normal to expect a modification in growth-rate during the central grand period of growth (Blackman) (1919) (Reed) (1920). The data gathered in the present study indicate, however, that it is not so, at least in regard to the effect of copper upon the growth of Cholam and Cumbu.

The observations on the stimulation of growth, particularly, between flowering and harvest stages are in line with that of Tokuoka, Matua and Syusin Gyo (1940) who found that the plant height was favoured by as much as fifty and twenty parts per million of copper in the pot experiment on wheat using soil.

Leaf area: Stimulation due to copper is seen reflected in all the three crops. Both Cholam and Cumbu show an increase in leaf area by 17·8 and 56·0 percent at the seedling stage due to 10 ppm. copper.

The differences get narrowed down at harvest stage. Ragi shows a difference from Cholam and Cumbu as was noticed in plant height as well. Only a 4% increase due to 10 ppm which becomes minus 2% at harvest stage.

Increased leaf area due to copper has also been observed by Lal and Subba Rao (1954) in barley, maize and paddy.

Flowering: The following extract represents the data on the time of flowering (in days) in the three crops.

Crop.	Control.	10 ppm Cu.	20 ppm Cu.	50 ppm Cu.	100 ppm Cu.	200 ppm Cu.
Cholam Co. 12	60·38	59·20	58·80	58·87	59·72	...
Ragi Co. 1	89·82	84·68	84·02	85·58	85·57	...
Cumbu Co. 4	42·90	42·35	43·23	43·82	41·17	49·08

Copper appears to induce flowering earliness by a small extent of one day in 10 and 20 parts per million copper, in cholam and ragi. Though statistically significant, it is too small to become agronomically important. Bailey and McHargue have noted a retardation of flowering to a great extent when copper was deficient in tomatoes but clear evidence is lacking on any definite effect of copper in hastening flower production in any crop.

Dry matter production: *Cholam:* Growths of cholam in terms of dry matter production is seem to be lowered under all the concentrations of copper between seedling and flowering stages. After the flowering stage, growth is found to be improved considerably.

Ragi: Growth in ragi as expressed in terms of dry matter production is found to increase steadily at all levels of copper concentrations. Suppression of growth seen at the early stages is found to disappear by the flowering stage.

Cumbu: Growth of cumbu is found to improve between seedling and vegetative stages. The intensity of acceleration in growth is due to 20 and 50 ppm copper concentrations. Till the flowering stage, growth goes down considerably and thereafter a rapid rise in growth is noticed.

Thus the three crops appear to have somewhat differently in their response to copper.

The optimum concentration in the growing medium for Cholam, ragi and cumbu are found to be 20, 50 and 50 ppm respectively.

Coming to final yield 10, 20 and 50 ppm Copper improved the shoot weight in Cholam. Roots and earhead production was also improved at 10 and 20 ppm. 100 ppm. Copper was found to be toxic affecting the yield of shoot, root and earhead.

In Ragi 10 and 50 ppm copper effected increase in shoot weight. The same levels increased the yield of earhead. The root yield was affected at all levels of copper.

Shoot weight was not found to be improved by copper application. But the root production was found to increase due to 20 and 50 and 100 ppm of copper. The optimum level for increasing the earhead yield was found to be 20 ppm. Toxic limit was noticed at 200 ppm on level.

Summing up, twenty parts per million concentration is found to be favourable in improving the yield of cholam and cumbu while fifty parts per million is beneficial to ragi.

From the results obtained there appers to be some slowing down in the drymatter yield between the seedling and flowering stages. This trend was noticeable with regard to variation in height also. Plant height and dry matter production are two important attributes of growth. Copper appears to have the effect of slowing down the rate of growing temporarily during the active period of plant growth. The exact relationship of copper with increased yields at the final stage presents a problem for future investigation.

The effect of copper in improving yields and dry matter production are in line with, Churchman, Russell and Manns (1936) who obtained an increase of 43·9% in tobacco and 17·9% in cotton by the use of mixed fertilizers containing fifty pounds of copper sulphate per ton of fertilizer. Others like Tokuoka, Matuo and Sinsen Dyo (1938) obtained increase in rice yield, Burger and Truogh (1948) in sweet corn and Forsee (1940) in Sorghum.

...*Continued.*