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Effect Of Zinc- Humate And Zinc-Fulvate Complexes On Rice Drymatter And Availability Of Zinc

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ABSTRACT
The present study was carried out in college of Agriculture, Rajendranagar. The humic and fulvic acids were extracted, isolated and purified from manures. The efficacy of Zn-organo complexes in supplying zinc was evaluated in a greenhouse experiment with rice as the test crop along with $ZnSO_4$ at 2.5 ppm and 5.0 ppm levels of applied zinc. The highest Zn concentration and Dry matter production was observed when Zn was applied as $ZnSO_4$ at the level of 2.5 mg Zn kg^{-1} soil. Among the different treatments, the highest zinc content and uptake was recorded in the treatment T_4 - RDF + application of $ZnSO_4$ @ 2.5ppm (26.18 µg g^{-1} and 479.38 µ g pot^{-1}) followed by T_6 - RDF + application of Zn-fulvate @ 2.5ppm (22.23 µg g^{-1} and 380.32 µ g pot^{-1}) and the lowest zinc content and uptake was recorded with application of T_1-RDF alone (10.81 µg g^{-1} and 75.94 µ g pot^{-1}) same trend will be followed in case of dry matter production. Among various levels of zinc, application of 2.5 ppm was found to be optimum and resulted in higher yield and zinc uptake.
Key words: *Humic acid (HA), Fulvic acid (FA), Zn-humate (Zn-HA) and Zn-fulvate (Zn-FA)*

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INTRODUCTION

Humic substances are considered as the most important constituents of soil. They form the largest fraction of soil organic matter (SOM) and play pivotal role in improving soil productivity. They occur in soils, sediments and water as a product of the chemical and biological transformation of animal and plant residues. They are colloid-sized, polymeric substances having dark colours. On account of their wide range of molecular sizes and properties, humic substances are usually fractionated to obtain materials with similar properties. The three fractions of humic substances are: i) fulvic acid (FA), (ii) humic acid (HA) and (iii) humin. Humic acids (HAs) and fulvic acids (FAs) are the most natural widespread complexing agents.

Humic acid (HA) and fulvic acid (FA) components of humus are extracted from organic manures using the classical fractionation procedures based on their solubility characteristics (Kononova, 1966).

One of the most prominent features of humic substances is its complex formation through the functional groups, like carboxylic (–COOH), phenolic (–OH), amino (-NH3), and carbonyl (C=O) (Datta *et al.* 2001) with polyvalent metal ions, *viz.* Cu^{2+}, Zn^{2+}, Mn^{2+}, Co^{2+}, Fe^{2+} *etc*. with different stability constants *i.e.* solubility in water, depending on the nature of metal ions and organic ligands. Zinc (Zn), one of the seventeenth essential plant nutrients, is deficient in most of the Indian soils. Invention of high yielding varieties and intensive cultivation of crops coupled with imbalanced fertilizer application have resulted in deficiencies of micronutrients particularly that of zinc and there is a need to focus research on carrier of zinc in order to enhance yields through balanced fertilization. Moreover, its availability to plant is interfered by different factors like high soil pH, adsorption by the negatively charged clay colloids, presence of other competitive cations like Fe^{3+}, Cu^{2+}, Ca^{2+} etc., anions like PO_4^{3-}, SO_4^{2-} etc. In complexed or chelated form, the cationic zinc becomes masked and protected from the above negative impact of soil factors that would render it unavailable for plant uptake and a higher activity of Zn in soil solution is maintained. Higher activity of Zn in soil solution enhance the plant uptake of Zn through mass action and diffusion, the two most important mechanisms for transferring plant nutrients specially which are present in minute quantity, from soil solution to the root surface. The present study was undertaken to prepare and study the efficacy of different organo-zinc complexes *vis-a-vis* zinc sulphate (inorganic) on (micronutrient) Zn and uptake by rice (*Oryza sativa L.*) plants and to determine the optimum level of zinc from different sources.

A green house experiment was conducted in pot culture study to evaluate the availability of Zn-complexes of humic acid and fulvic acid in rice (MTU-1010) grown as test crop under pot culture conditions. $ZnSO_4$ and Zn-chelate were used to compare the effects of these complexes on parameters *viz.,* dry matter yield, zinc content and uptake.

MATERIAL AND METHODS

About 1 kg well-decomposed FYM and vermicompost sample was collected. Tyurin's method devised for extraction of humic substances from soil, as described by Kononova (1966) was followed for the separation of humic substances from FYM and vermicompost. Farmyard manure and vermicompost after acid treatment (0.1 *N* HCl) was extracted with 50 mL of 0.1 *N* NaOH repeated several times for complete extraction of humic fractions. Then the content was allowed to settle down. The dark colored supernatant of humic substance was siphoned off into a clean beaker and then centrifuged at 2000 rpm for 30 min for phase separation. The alkali treatment was repeated till the FYM and vermicompost yielded humic fraction. Based on differences in solubility behavior of different humic fractions, the extracted humic fractions were separated into humic and fulvic acids (Kononova 1966) by adjusting the pH of the supernatant to 2-3. This solution was stirred and heated on sand bath at 80 ^{0}C for 30 min. Humic acid fraction coagulated and settled at the bottom. The lighter colored supernatant *i.e.* fulvic acid was then siphoned off in a clean glass beaker without disturbing the coagulated humic acid fraction. The humic acid (HA) fraction was redissolved in 0.1 *N* NaOH and centrifuged four times to make it free from clay. The process of precipitation and centrifugation were repeated to attain partial purification of HA fraction (Stevenson, 1994). Then it was reprecipitated with 1 *N* H_2SO_4. The precipitated HA was taken in 1 L plastic beaker and treated with HF + HCl (0.5 ml 48% HF + 0.5 ml conc. HCl + 99 ml distilled water) and dialyzed thoroughly against double-distilled water until the outer solution showed negative reaction for Cl^- ion (Schnitzer and Gupta, 1965). The dialyzed fraction was evaporated under low temperature and finally dried. The dried sample were weighed and stored for further analysis. The acid soluble coloured phase, *i.e.* fulvic acid (FA) fraction was purified by adsorbing on an activated charcoal column. Then the column was repeatedly washed with 1 *N* H_2SO_4. The purified fulvic acid was eluted with 1 *N* NH_4OH. The NH_4OH extract was passed through a column. Thus obtained fulvic acid was dried at 40^{0}c and used for further analysis. In this and other calculations, the value of molecular weight used was 1000 for humic acid and 700 for fulvic acid. It was arrived based on elemental composition and functional group analysis data (Nagamadhuri, 1996, Reddy, 1997 and Sailaja, 1999).

The zinc-humate and zinc-fulvate complexes were prepared by using x-value *i.e*., the number of moles of HA/FA required to complex with one mole of Zn, from the stability constant data obtained at pH 7.0 and 30°C temperature. The value of "x" was found to be approximately 1.5 for both humic acid and fulvic acid. Amount of $ZnSO_4$ corresponding to 2.5 and 5.0 ppm zinc were weighed in beakers containing the required amount of HA/FA for complexation. The contents in the beaker were diluted with distilled water and pH of the solution was adjusted to 7.0.

Details of potculture experiment

A zinc deficient alfisol was used for conducting the pot culture experiment. The soil had a pH of 7.7. It was medium in organic carbon, low in available N (188 kg ha^{-1}), high in available P_2O_5 (57.8 kg ha^{-1}) and K_2O (286.5 kg ha^{-1}) and deficient in DTPA extractable zinc (0.56 µg g^{-1} soil). Five kilograms of soil was filled in earthen pots. In the present experiment, rice (var. MTU 1010) was used as test crop. The rice seedlings of 30 days old were transplanted in five hills @ two seedlings $hill^{-1}$ in each pot. The soil was kept under submergence from flowering till harvest. A uniform fertilizer dose @ 120 kg N + 60 kg P_2O_5 + 40 kg K_2O ha^{-1} was applied to each pot. Nitrogen was applied through urea in two equal splits *viz*., as basal and as top dressing at maximum tillering stage (35 DAT). Entire dose of phosphorus as single super phosphate and potassium through murate of potash were applied as basal. Humic acid, fulvic acid, Zn-humate, Zn-fulvate and Zn-chelate were applied as per the treatments to all the pots. 11 treatment combinations of different sources and levels of zinc with organic and inorganics imposed to rice crop. The treatments were T_1 - control, T_2 - humic acid, T_3 - fulvic acid, T_4- $ZnSO_4$ @ 2.5 mg Zn kg^{-1} soil, T_5 - Zn-chelate @ 2.5 mg Zn kg^{-1} soil, T_6 - Zn-fulvate @ 2.5 mg Zn kg^{-1} soil, T_7 - Zn-humate @ 2.5 mg Zn kg^{-1} soil, T_8 - $ZnSO_4$ @ 5.0 mg Zn kg^{-1} soil, T_9 - Zn- chelate @ 5.0 mg Zn kg^{-1} soil, T_{10} - Zn- fulvate @ 5.0 mg Zn kg^{-1} soil, T_{11} - Zn- humate @ 5.0 mg Zn kg^{-1} soil.

ANALYSIS

Plant samples collected at harvest were shade dried for 3- 4 days, oven dried at 65°C to a constant weight and the dry matter production (g pot^{-1}) was recorded.

Zinc content (µg g^{-1})

The zinc content in plant samples was determined by (Lindsay and Norvell, 1978) digesting with triacid mixture (Conc. HNO_3, H_2SO_4 and $HClO_4$, in the ratio of 9:4:1) and using atomic absorption spectrophotometer and expressed as µg g^{-1}.

Zinc uptake (mg pot^{-1})

The dry matter production (kg ha^{-1}) was used to compute nutrient uptake at harvest stage.

Zn uptake (µg pot^{-1}) = Zinc content (µg g^{-1}) x Dry matter production (g pot^{-1})

RESULTS AND DISCUSSIONS

Dry matter production

The data regarding total dry matter production as affected by Zn-sources and levels of Zn are presented in table 1 and furnished in figure 1.

Among all treatment combinations zinc application significantly increased the dry matter production. Highest dry matter production was recorded in the treatment (T_4) RDF + application of $ZnSO_4$ @ 2.5 ppm (18.31 g pot^{-1}) followed by (T_6) RDF + application of Zn-fulvate @ 2.5 ppm (17.11 g pot^{-1}) and (T_8) RDF + application of $ZnSO_4$ @ 5 ppm (15.13 g pot^{-1}) and the lowest dry matter production was recorded with application of RDF alone (7.02 g pot^{-1}). Among the various levels of zinc of application of 2.5 ppm increased the dry matter production significantly over control while the increase was very small at 5.0 ppm level. The relative effectiveness of Zinc sources in increasing dry matter production followed the order: $ZnSO_4$ > Zn-fulvate > Zn-humate > Zn- chelate.

The results obtained on dry matter production of rice (Table 1 and fig. 1) indicated that $ZnSO_4$ produced higher than that of different zinc complexes. Significantly highest dry matter yield (18.31 g pot^{-1}) was obtained in the T_4 - RDF + application of $ZnSO_4$ @ 2.5 ppm followed by T_6 - RDF + application of Zn-fulvate @ 2.5 ppm (17.11 g pot^{-1}). This could be due to the increased solubility of Zn from $ZnSO_4$ than that from zinc complexes and made readily available to crop plants. While the lowest dry matter content was recorded with the application of RDF alone (7.02 g pot^{-1}). Among levels of zinc, application of 2.5 ppm of zinc was found to be optimum for rice through these sources.

Zinc content and uptake

The total Zn uptake by rice was calculated by multiplying the Zn concentration with total dry matter production. The data regarding total Zn uptake by rice are presented in table 1 and furnished in figure 2.

Among all different treatments indicated that the treatmental effects on zinc content and uptake were statistically significant. Highest zinc content and uptake was recorded in the treatment T_4 - RDF + application of $ZnSO_4$ @ 2.5 ppm (26.18 µg g^{-1} and 479.38 µg pot^{-1}) followed by T_6 - RDF + application of Zn-fulvate @ 2.5 ppm (22.23 µg g^{-1} and 380.32 µg pot^{-1}) and which was closed followed in T_8 - RDF + application of $ZnSO_4$ @ 5 ppm (22.03 µg g^{-1} and 333.26 µg pot^{-1}) and the lowest zinc content and uptake was recorded with application of RDF alone (10.81 µg g^{-1} and 75.94 µ g pot^{-1}). Among various levels of zinc application 2.5 ppm resulted in highest zinc concentration and it differed significantly from 5.0 ppm.

Table 1. Effect of sources and levels of zinc on dry matter production, zinc content and zinc uptake by rice

TREATMENTS	Dry matter production (g pot^{-1})	Zinc content (µg g^{-1})	Zinc uptake (µg pot^{-1})
T1-Control	7.02	10.81	75.94
T2-Humic acid	11.22	19.24	215.86
T3-Fulvic acid	12.15	20.81	252.82
T4-$ZnSO_4$ (2.5ppm)	18.31	26.18	479.38
T5-Zn-humate (2.5ppm)	12.74	13.52	172.25
T6-Zn-fulvate (2.5ppm)	17.11	22.23	380.32
T7-Zn-chelate (2.5ppm)	11.54	16.69	192.59
T8-$ZnSO_4$ (5ppm)	15.13	22.03	333.26
T9-Zn-humate (5ppm)	11.12	15.82	175.91
T10-Zn-fulvate (5ppm)	12.19	20.17	245.86
T11-Zn-chelate (5ppm)	8.53	14.28	121.82
S. E m ±	0.25	0.131	5.416
C.D. (0.05)	0.737	0.387	15.987

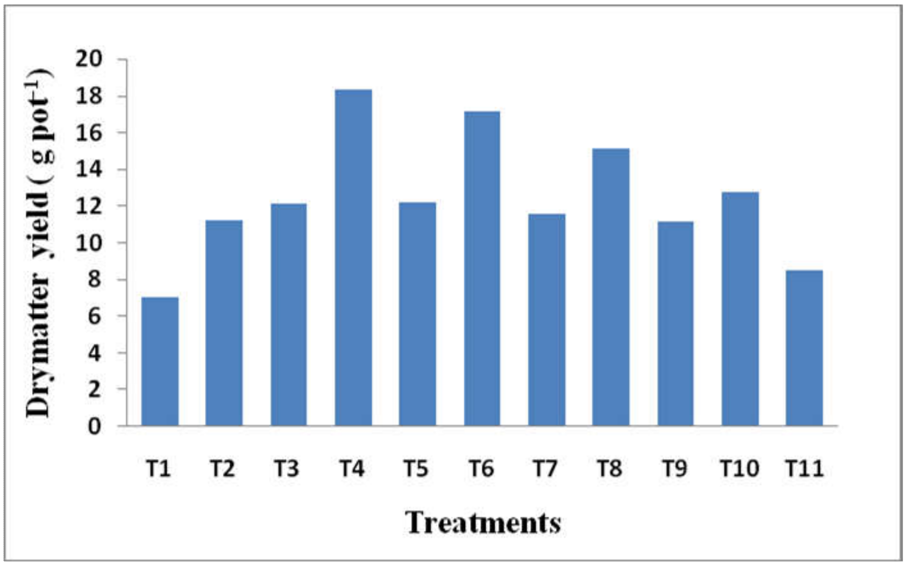

Figure 1. Effect of sources and levels of zinc on dry matter production (g pot^{-1})

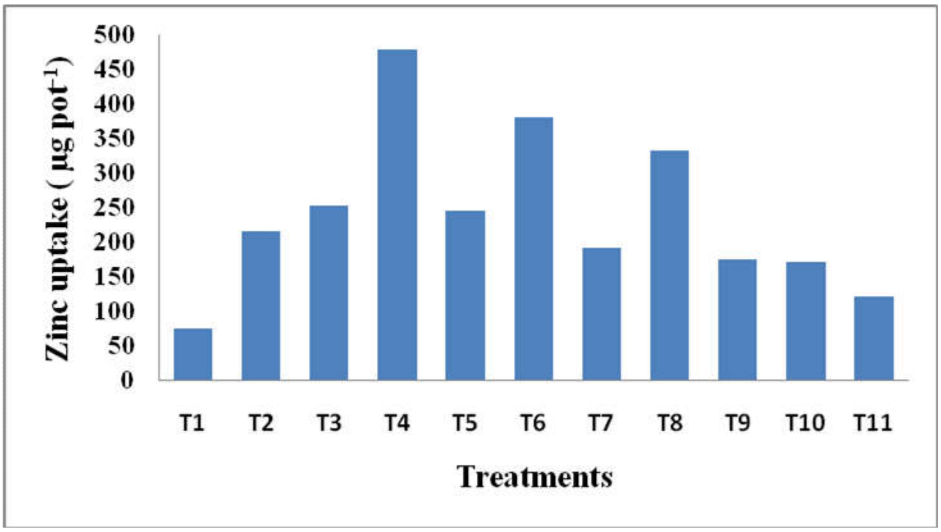

Figure 2. Effect of sources and levels of zinc on zinc uptake (µg pot^{-1}) by rice.

In the present study, application of zinc at 2.5 ppm gave maximum dry matter production (18.31 g pot^{-1}), zinc content (26.18 µg g^{-1}) and uptake (479.38 µg pot^{-1}) by rice. The percentage increase in dry matter production over control was 48.4, 91.1, 166.2 and 197.31 due to application of zinc chelate, zinc humate, zinc fulvate and $ZnSO_4$, respectively at 2.5 ppm level. Similarly Srilatha (2001) concluded that application of 2.5 ppm of zinc was optimum for rice under green house conditions.
Application of fulvic and humic acid alone also recorded higher dry matter production (12.15 and 11.22 g pot^{-1} respectively) than control (7.02 g pot^{-1}). Some of the polyphenols serve as respiratory catalysts with high chlorophyll and stimulate nutrient uptake and growth rates (Sailaja, 1999, Sangeetha and Singaram, 2007 and Thakur *et al.*, 2013). Similarly available zinc content (4.10 µg pot^{-1}) was also more in post harvest soil samples in the treatments consisting of $ZnSO_4$ application over other treatments.
Among various sources of zinc, the relative effectiveness of zinc sources in increasing dry matter production, zinc content and uptake followed the order: $ZnSO_4$> Zn-fulvate > Zn-humate > Zn-chelate. These results are in conformity with those of Srilatha (2001).

CONCLUSIONS
The optimum Zn concentration and highest dry matter was produced when Zn was applied at the level of 2.5 mg kg^{-1} soil through $ZnSO_4$. The efficacy of different zinc sources to increase dry matter production and Zn concentration in plants followed the order $ZnSO_4$> Zn-fulvate > Zn-humate > Zn-chelate. The study

clearly demonstrated the higher efficacy of inorganic source of Zn in plant growth and zinc uptake by rice plant than all the organic sources, especially in zinc deficient soil.

REFERENCES

1. Kononova, M.M. (1966) *Soil Organic Matter,* (Second Edition) Pergamon Press Ltd., New York. 365 p.
2. Lindsay, W.C and Norwell, A. 1978. Development of DTPA soil test for zinc, iron, manganese and copper. *Soil Science Society of America Journal*. 42: 421-428.
3. Nagamadhuri, K.V. 1996. Status and changes in organic matter under natural vegetation and continuous cultivation in soils of ANGRAU, Rajendranagar, Hyderabad. *M.Sc(Ag.) thesis* submitted to ANGRAU, Hyderabad.
4. Reddy, K.S. 1997. Status and nature of organic matter and its interaction with metal ion in certain alfisols and vertisols of Acharya NG Ranga Agricultural University, Rajendranagar Campus Hyderabad. *M.Sc.(Ag.) thesis* submitted to Acharya NG Ranga Agricultural University, Rajendranagar, Hyderabad.
5. Sailaja, M. 1999. Interactions between metal ions and humic fractions extraxcted from an Alfisol and Vertisol in relation to their plant availability, Rajendranagar, Hyderabad. *M.Sc.(Ag.) thesis* submitted to ANGRAU, Hyderabad.
6. Sangeetha, M and Singaram, P. 2007. Effect of lignite humic acid and inorganic fertilizers on growth and yield of onion. *Asian Journal of Soil Science*. 2 (1): 108-110.
7. Schnitzer, M and Gupta, U.C. 1965. Determination of acidity in soil organic matter. *Soil Science Society of America Proceedings*. 29: 274-277.
8. Srilatha, M. 2001. Characterization of humic substances and their interactions with metal ions in relation to their availability to rice. *M.Sc.(Ag.) thesis* submitted to Acharya N.G. Ranga Agricultural University, Rajendranagar, Hyderabad.
9. Stevenson, F.J. 1994. Humus chemistry: genesis, composition and reactions. John Wiley and Sons, New York.
10. Thakur, H., Rekha, K.B., Babu S.N.S and Padmaja, G. 2013. Effect of humic substances on growth and yield of sunflower (*helianthus annuus* L.). *Journal of Research. ANGRAU.* 41 (4): 106-108.

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