International Journal of Agricultural Sciences
Volume 17 | Issue 1 | January, 2021 | 42-47 ■ ISSN : 0973-130X
DOI:10.15740/HAS/IJAS/17.1/42-47
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RESEARCH PAPER

Effect of different sources of zinc on the activities of plant and soil enzymes

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Abstract : The present investigation was conducted to study the effect of zinc fertilization on major biomeric characteristics, yield and post-harvest soil nutrient status. The experiment was carried out at college of Agriculture, Vellayani during 2017 - 2019. The experiment was laid out in Randomized Block Design with eight treatments and three replications. The treatments were Absolute control (T_1), N, P, K as per POP- 75:45:25 kg ha^{-1} (T_2), N, P, K+ Soil application of Zn as $ZnSO_4$- 10 kg ha^{-1} (T_3), N, P, K+ Foliar application of Zn as 0.5 per cent $ZnSO_4$ (T_4), N, P, K+ Zn as Zn EDTA-18 kg ha^{-1} (T_5), N, P, K+ Zn solubilizer -5 per cent (T_6), N, P, K+ Zn Humate- 44 kg ha^{-1} (T_7), N, P, K+ K solubilizer 5 per cent (T_8). From the study, it was observed that the treatment T_7 with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) recorded the highest for enzymes such as carbonic anhydrase, peroxidase and catalase *viz.,* 910 EU g^{-1}, 48.17 activity min^{-1} g^{-1} and 27.06x10^3 units ml^{-1}, respectively. An incubation study was carried out to assess the trend os activity of the enzymes and the results revealed peroxidase and carbonic anhydrase showed an increasing trend in activitfy while peroxidase registered a decreasing trend. Treatment T_4 with the application of Soil + Zn as 1.5 ppm $ZnSO_4$ registered the highest value for peroxidase (11.98 activity min^{-1} g^{-1}) and carbonic anhydrase activity (385 EU g^{-1}) in the incubation study.

Key Words : Peroxidase, Carbonic anhydrase, Catalase, Zinc solubilizer

View Point Article : Aparna, B., Gladis, R., Gowripriya and Aswathy, U.A. (2021). Effect of different sources of zinc on the activities of plant and soil enzymes. *Internat. J. agric. Sci.*, **17** (1) : 42-47, **DOI:10.15740/HAS/IJAS/17.1/42-47**. Copyright@2021: Hind Agri-Horticultural Society.

Article History : Received : 17.08.2020**; Revised :** 11.11.2020**; Accepted :** 14.12.2020

INTRODUCTION

Zn plays an important role in regulating the nitrogen metabolism, cell multiplication, photosynthesis and auxin synthesis in plants and helps in the consumption of phosphorous and nitrogen during seed formation. Zn is essential for protecting the cells from oxidation damage and maintaining regeneration (Cakmak, 2000). Zn plays an important role in enzyme activation or catalysis, carbohydrate metabolism, chlorophyll production, energy dissipation, cytochrome and nucleotide synthesis. Sharma *et al*. (1995) explained the involvement of Zn in stomatal opening, as a component of carbonic anhydrase maintaining the bicarbonate in the guard cell and also in the K^+ uptake in to the guard cells.

Zn is required for functioning of more than 300 enzymes. It functions as a cofactor for 6 classes of

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enzymes like transferases, lyases, oxidoreductases, isomerase, hydrolases and ligases (Broadley *et al*., 2007). Zn is a structural part of enzymes such as alcohol dehydrogenase, superoxide dismutase, carbonic anhydrase and RNA polymerase. Zinc is the main structural part of many enzymes and is required for the plant enzymes formation; furthermore, many enzymatic reactions activated by zinc (Akay, 2011).

Extensive research has been done on the role of Zn fertilizers to correct Zn deficiencies in crops. The status of total zinc content in soils of Kerala ranged from 25-55 mg kg^{-1}. The Zn deficiency in Kerala was due to excessive quantity of phosphatic fertilizers applied and excessive levels of Fe, Mn and Al due to ion competition (Kumar *et al*., 2013). These fertilizers vary considerably in Zn content, chemical reactivity, cost and effectiveness for crops. Methods of Zn application varies, depending on the crop type, farming system and tools available. Therefore, it is important to know about Zn sources, their methods of production and application and their performance in soil, along with chemical evaluation and regulations affecting Zn fertilizers before applying them in the soil. Hence, the present study is carried out to assess the effect of different sources of zinc on the activity of plant enzymes in a zinc deficient soil.

MATERIAL AND METHODS

An experiment titled "Effect of zinc fertilization on major plant and soil enzymes in southern laterites" has been carried out in the Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vellayani during 2018- 2019. The study was envisioned to assess the effect of various sources and methods of application of zinc on the activities of major plant enzymes and soil enzymes, in laterite soils using tomato (*Solanum lycopersicum*) as a test crop.A soil test database has been developed and maintained by the Department of Soil Science and Agricultural Chemistry. From the database, laterite soil in Neyyatinkkara series with Zn deficiency was located. Then soil samples were collected and subjected to analysis of major and micro nutrients to confirm the deficiency of Zn. The soil of the experimental site was loamy, kaolinitic, isohyperthermic, typic haplustalf and belongs to vellayani series. Treatment combinations of the field experiment is depicted in Table A.

Table A : Treatment details

T_1	Absolute control
T_2	N, P, K as per POP (75:45:25 kg ha^{-1})
T_3	N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1})
T_4	N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$
T_5	N, P, K+ Zn as Zn EDTA (18 kg ha^{-1})
T_6	N, P, K+ Zn solubilizer (5 %)
T_7	N, P, K+ Zn Humate (44 kg ha^{-1})
T_8	N, P, K+ K solubilizer (5%)

A laboratory incubation study was conducted for a period of 3 months from 14-12-2018 to 8-03-2019. The main objective of the study was to assess the relationship between various doses of zinc and enzymes, at periodic intervals *viz.,* 0^{th}, 14^{th}, 28^{th}, 42^{nd}, 56^{th}, 70^{th} and 84^{th} day of incubation *viz.,* T_1: Soil aloneT_2: Soil + Zn as $ZnSO_4$ (0.5 ppm) T_3: Soil + Zn as $ZnSO_4$ (1.0 ppm) T_4: Soil + Zn as $ZnSO_4$ (1.5 ppm) T_5: Soil+ Zn as $ZnSO_4$ (2.0 ppm).

Carbonic anhydrase:

About 400 mg of leaf material was ground with 10 ml of ice cold 10 mM tris-Hcl buffer at a pH of 8.2 containing 5 mM 2-mercapto-ethanol. The mixture was centrifuged at 12,000 rpm for 10 min. The supernatant thus received was used for enzyme analysis. CA activity was assayed during fractionation by calorimetric method (Wilbur and Anderson, 1948). It was then expressed as enzyme unit per gram of fresh leaf weight (EU g^{-1}).

PEP carboxylase (PEPC):

The activity of PEPC was assayed by coupling with NAD malic dehydrogenase and monitoring NADH oxidation at 340 nm in a Shimadzu UV-Vis Spectrophotometer. The assay was performed at 30°C, the assay mixture (1 ml) contained 50 Mm TRIS- HCl, pH 7.3, 5 mM $MgCl_2$, 0.2 mM NADH, 2 mM MAD, 2.5 mM PEP, 10 mM $NaHCO_3$ and leaf extract. The extraction was done by taking 1 g of fresh leaves and washing it with deionised water. The leaf sample was ground into a fine paste in a chilled mortar and pestle by adding the extraction buffer was later filtered and centrifuged at 30000 x g for 30 min at 4°C. It was then expressed in ìmol mg^{-1} Chl h^{-1} (Parvathi *et al*., 2000).

Carboxy dismutase :

Five gram leaf sample was ground in 20 ml ice cold extraction medium (10 mM of $MgCl_2$, DTT, $MnCl_2$ and 1.3 g sodium salt of HEPES in 75 ml distilled water) and centrifuged at 20,000 g for 10 minutes. A reaction mixture

was prepared using 0.20 ml enzyme extract with 0.10 ml HEPES buffer, 0.05 ml RuBP, 0.05 ml $MgCl_2$ and 0.05 mL DTT and it was illuminated for 2 min. After illumination the reaction was initiated using 1 micro mole of $NaHCO_3$ and was incubated for 10 minutes after using 0.1 ml of 10 per cent acetic acid. Then it was filtered and counted. Along with this a blank was also prepared using 0.05 ml buffer instead of RUBP (Kung *et al*., 1980).

Catalase:

One gram plant tissue was grounded in chilled pestle and mortar using 0.0067 M phosphate buffer and the contents were centrifuged at 18000 g at 4°C for 15 min. The supernatant was used as enzyme source within 2-4 hours. 0.01ml of enzyme sample was mixed with 3 ml H_2O_2-PO_4 buffer and read against a control cuvette containing H_2O_2- free PO_4 buffer at 240 nm and the time was noted for a decrease in absorbance from 0.45 to 0.40. It was expressed in Units ml^{-1} extract (Luck, 1974).

Peroxidase:

One gram of leaf sample was homogenised in 5 ml of 0.01M sodium phosphate buffer (pH 6.5) and it is centrifuged at 5000 rpm for 15 min for 4°C. 50 µl of enzyme extract was taken and 1ml 0.05M pyrogallol was added and to initiate the reaction, one ml of H_2O_2 was added and the concentration was measured at 420 nm and was expressed in activity min^{-1} g^{-1} of sample (Srivatsava and Kumar, 1989).

RESULTS AND DISCUSSION

From the study it is observed that the treatments had significantly influenced the plant enzymes carbonic anhydrase, PEP carboxylase and Carboxy dismutase. In general, an increase in activity of these enzymes were noted in treatments applied with Zn sources. The highest value for carbonic anhydrase was noticed in the treatment T_7 (910.00 EU g^{-1}) with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) which was found to be stastically superior over all other treatments (Table 2). Zn is an essential constituent of carbonic anhydrase, the main function of these enzyme is to catalyses the reversible hydration of carbon dioxide to bicarbonate and hydrogen ions (Graham and Reed, 1971). This might have increased the photosynthetic CO_2 fixation along with this, Zn also have an important role in chlorophyll and carotenoid metabolism (Kumar *et al*., 1976). Thus, an increase in carbonic anhydrase along with photosynthetic pigments might have triggered an increase in activity of PEP carboxylase and carboxy dismutase thereby exhibiting a positive correlation between Zn and plant enzymes (Table 1). Similar results were also reported by Shotri *et al*. (1983). I the case of PEP carboxylase and carboxy dismutase the highest values of 131.33 µmol mg^{-1} 138 Units g^{-1} were recorded by the treatment T_4 with the application of N, P, K+ Foliar application of Zn as 0.5 per cent $ZnSO_4$, respectively.

From the study, it was observed that the treatments had imposed a significant effect on oxido- reductases such as dehydrogenase, peroxidase and catalase. These enzymes showed an increase in activity during the peak stage of fruiting. It was noticed that the treatment T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}) recorded the highest value of dehydrogenase. This might be due to the role of Zn in enhancing the activity of dehydrogenases like alcohol dehydrogenase, glutamic dehydrogenase, D-glyceraldehyde-3-phosphate dehydrogenase and malic dehydrogenase. The increased rate of Zn assimilation, N metabolism, photosynthesis, respiration and

Table 1 : Effect of treatments on plant enzymes

Treatments	Carbonic anhydrase (EU g^{-1}).	PEP carboxylase (µmol mg^{-1})	Carboxy dismutase (Units g^{-1})
T_1- Absolute control	362.50	52.00	100.00
T_2-N, P, K as per POP (75:45:25 kg ha^{-1})	268.67	68.67	103.50
T_3- N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1})	401.67	119.67	
T_4-N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$	665.67	131.33	227.00
T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1})	710.50	102.67	200.50
T_6- N, P, K+ Zn solubilizer (5 %)	575.83	73.00	110.50
T_7- N, P, K+ Zn Humate (44 kg ha^{-1})	910.00	91.67	129.00
T_8- N, P, K+ K solubilizer (5%)	404.67	78.00	107.00
C.D. (P=0.05)	21.83	8.94	16.13

carbohydrate metabolism might have also triggered an increase in dehydrogenase activity. Similar observations were recorded by Moore and Patrick (1988).

From the study, it was observed that the treatment T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) registered the highest value for peroxidase and catalase. Peroxidase and catalase constitute the H_2O_2 scavenging system in cells. The high peroxidase and catalase activity might be due to the exposure to Zn. This is in accordance with the findings of Jain *et al*. (2010). The excess of Zn might have triggered an intensive oxidative stress along with this, humic acid activated several biochemical processes which resulted in increased enzymatic activity. These metabolic changes led to an increase in the concentration of catalase and peroxidases. This findings corroborated with the results of Bertrand and Poirier (2005).

Incubation study:

In general there was an increasing trend with respect to carbonic anhydrase activity with the advancement of the incubation period. There was significant variation in carbonic anhydrase activity by the treatments during the 56th, 70th and 84th day of incubation.

From Table 3. it is noticed that the treatment, T_4 with the application of Soil + Zn as $ZnSO_4$ (1.5 ppm) and T_5 with the application of Soil+Zn as $ZnSO_4$ (2 ppm) recorded the highest mean value of 165 EU g^{-1} on the 0th day of incubation. On the 28th day treatment T_3: Soil + Zn as $ZnSO_4$ (1 ppm) registered the highest value of 220 EU g^{-1}. Treatment T_2 with the application of Soil + Zn as $ZnSO_4$ (0.5 ppm) and T_4 with the application of Soil+ Zn as $ZnSO_4$ (1.5 ppm) registered the highest value of 275 and 385 during the 70th and 84th day of incubation, respectively. Thus, in the case of carbonic anhydrase, a significant influence in activity was observed two month after the application of Zn. There is a general increase in Carbonic anhydrase activity which might be due to the limited production of oxidizing agents. It is also observed that treatment T_4: Soil + Zn as $ZnSO_4$ (1.5ppm) registered the highest value for carbonic anhydrase activity. This might be attributed by the production of limited oxidising agents and slightly acidic pH.

The treatment T_3 with the application of Soil + Zn as $ZnSO_4$ (1 ppm) registered the highest mean value of 13.53 activity min^{-1} g^{-1} on 0th day of incubation. On the 14th day there was an increase in peroxidase activity in

Table 2 : Effect of treatments on enzymes (Oxidoreductases)

Treatments	Dehydrogenase (µg TPF g-1 $24h^{-1}$)	Peroxidase (min^{-1} g^{-1})	Catalase (Units ml^{-1} extract.)
T_1- Absolute control	331.67	11.70	15134.8
T_2-N, P, K as per POP (75:45:25 kg ha^{-1})	247.99	22.17	13582.2
T_3- N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1})	365.26	34.60	13564.7
T_4-N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$	219.45	33.30	15134.4
T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1})	455.03	41.57	16972.4
T_6- N, P, K+ Zn solubilizer (5 %)	238.90	23.57	10461.8
T_7- N, P, K+ Zn Humate (44 kg ha^{-1})	338.33	48.17	27058.4
T_8- N, P, K+ K solubilizer (5%)	160.78	22.73	12334.4
C.D. (P=0.05)	22.693	0.599	300.488

Table 3 : Effect of treatments on carbonic anhydrase (incubation study)

Treatments	Carbonic anhydrase (EU g^{-1})						
Incubation period	0th day	14th day	28th day	42nd day	56th day	70th day	84th day
T_1: Soil alone	110	137.5	165	166.25	165	110	165
T_2: Soil + Zn as $ZnSO_4$ (0.5 ppm)	110	165	165	165	165	275	385
T_3: Soil + Zn as $ZnSO_4$ (1 ppm	110	165	220	220	220	247.5	330
T_4: Soil + Zn as $ZnSO_4$ (1.5 ppm)	165	165	165	220	330	275	385
T_5: Soil + Zn as $ZnSO_4$ (2 ppm	165	165	165	165	167.5	220	357.5
C.D. (P=0.05)	NS	NS	NS	NS	112.509	70.998	102.621

NS= Non-significant

all the treatments except T_3 with the application of Soil + Zn as $ZnSO_4$ (1 ppm). Treatment T_4 with the application of Soil + Zn as $ZnSO_4$ (1.5 ppm) recorded the highest mean value of 11.98 activity min^{-1} g^{-1} and was found to be on par with T_2: Soil + Zn as $ZnSO_4$ (0.5 ppm), T_3: Soil + Zn as $ZnSO_4$ (1 ppm) and T_5: Soil + Zn as $ZnSO_4$ (2 ppm). Treatment T_4 with the application of Soil + Zn as $ZnSO_4$ (1.5 ppm) recorded the highest mean value of 11.20, 7.97, 7.65 and 5.83 activity min^{-1} g^{-1} on the 42nd, 56th, 70th and 84th day of incubation, respectively. Treatment T_4: Soil + Zn as $ZnSO_4$ (1.5 ppm) reported the highest value for peroxidase activity. This might be due to the slightly acidic pH provided by 1.5 ppm $ZnSO_4$, as it becomes completely inactive at pH 2.5 and $\geq$ 8.5. Similar results were reported by Mizobutsil *et al.* (2010).

From the study it was observed that dehydrogenase activity was significantly influenced by Zn. On 0th day of incubation the highest mean value of 151.01 µg TPF g^{-1} 24 h^{-1} was registered by T_5 with the application of Soil + Zn as $ZnSO_4$ (2 ppm) and the lowest mean value of 120.87 µg TPF g^{-1} 24 h^{-1} registered by T_4 with the application of Soil + Zn as $ZnSO_4$ (1.5 ppm). The lowest mean value for all the treatments except T_1 (Soil alone) was noticed in the 14th day of incubation. The peak value of 248.18 µg TPF g^{-1} 24 h^{-1} was registered on the 84th day of incubation with T_5: Soil + Zn as $ZnSO_4$ (2 ppm). On the 28th, 42nd, 56th, 70th and 84th day of incubation treatment T_5 with the application of Soil + Zn as $ZnSO_4$ (2 ppm) registered the highest values of 147.89, 183.45, 177.21, 190.07 and the peak value of 248.18 µg TPF g^{-1} 24 h^{-1}, respectively. A general increase in the activity was observed. This might be due to the addition of FYM. FYM acted as a substrate which provided carbon and energy to the soil microbes resulting in higher dehydrogenase activity. This results corroborated with the findings of Walls- Thumma (2000).The highest mean value for dehydrogenase activity was given by T_5-Soil + Zn as $ZnSO_4$ (2 ppm). The addition of Zn sources might have resulted in the multiplication of microbial population and thereby an increase in dehydrogenase activity.

Conclusion:

From the study it was observed that Treatment T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}) registered the highest value for dehydrogenase while T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) recorded the highest value for peroxidase, catalase, carbonic anhydrase.With respect to the incubation study, the treatment T_4: Soil + Zn as $ZnSO_4$ (1.5 ppm) was found to be superior over all treatments for Peroxidase and Carbonic anhydrase whereas T_5: Soil + Zn as $ZnSO_4$ (2 ppm) was the best treatment for dehydrogenase. Thus, from the study it is inferred that Zn Humate can serve as an efficient nutrient source of nutrients which increases the nutrient uptake and increase the effectiveness of applied nutrients as well as nutrients in the labile pool.

Table 4 : Effect of treatments on peroxidase (incubation study)

Treatments	Peroxidase activity(min^{-1} g^{-1})						
Incubation period	0th day	14th day	28th day	42nd day	56th day	70th day	84th day
T_1: Soil alone	5.35	6.03	7.77	5.98	5.69	5.46	4.85
T_2: Soil + Zn as $ZnSO_4$ (0.5 ppm)	10.75	11.78	11.33	8.60	7.94	7.48	5.58
T_3: Soil + Zn as $ZnSO_4$ (1 ppm	13.53	11.43	9.43	9.03	5.98	6.03	5.44
T_4: Soil + Zn as $ZnSO_4$ (1.5 ppm)	8.28	11.98	7.79	11.20	7.97	7.65	5.83
T_5: Soil + Zn as $ZnSO_4$ (2 ppm)	9.45	11.13	7.95	6.02	7.77	6.50	5.35
C.D. (P=0.05)	1.071	1.194	1.262	1.348	1.262	0.891	0.371

Table 5 : Effect of treatments on dehydrogenase (incubation study)

Treatments	Dehydrogenase activity (µg TPF g^{-1} 24 h^{-1})						
Incubation period	0th day	14th day	28th day	42nd day	56th day	70th day	84th day
T_1: Soil alone	127.16	130.86	130.90	151.01	147.89	128.70	112.28
T_2: Soil + Zn as $ZnSO_4$ (0.5 ppm)	130.90	109.07	115.45	173.61	134.36	171.64	173.42
T_3: Soil + Zn as $ZnSO_4$ (1 ppm)	130.86	89.35	128.70	141.03	136.28	166.60	176.44
T_4: Soil + Zn as $ZnSO_4$ (1.5 ppm)	120.87	83.40	127.16	176.97	153.55	167.52	192.61
T_5: Soil + Zn as $ZnSO_4$ (2 ppm)	151.01	112.28	147.89	183.45	177.21	190.07	248.18
C.D. (P=0.05)	1.700	1.535	1.515	1.226	1.551	1.266	1.183

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