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Role of Nutrients in Management of Mango Sudden Death Disease in Punjab, Pakistan

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Abstract.- Nutrients play a major role in the tolerance by the strength and integrity of the cell walls which is first line of defense against fungal diseases like mango sudden death. This study aimed to evaluate the positive effects of nutrients in management of mango sudden death. Leaf and soil analysis were found significant among all the treatments which indicated nutrients status in soil as well in plant leaves. The progress of disease indicating symptoms, *i.e.* oozing**,** and canker**,** curling or drying of leaves and rotting sign on root and stem on different portions of tree appeared to be non significant but overall 18.61% reduction in disease severity was calculated after application of different nutrients. The maximum reduction in disease severity reached 30.69% with nutrients $ZnSO_4$+$CuSO_4$+NP+humic acid followed by $ZnSO_4$+$FeSO_4$+NP+humic acid and $CuSO_4$+NP+humic acid as 19.51% and 19.37%, respectively. Only the application of nitrogen and phosphorus without micronutrients was found to reduce the minimum disease severity of 8.39% followed by 9.35% when combined all nutrients *i.e.* Zn SO_4+ $FeSO_4$+$CuSO_4$+NP+humic acid. The results indicate that balanced use of micronutrients *i.e.* zinc, copper and iron in combination with humic acid as soil conditioner have significant effects in reduction of severity on infected plants. Therefore, nutrients application is highly encouraged by improving tree vigor against the management of sudden death of mango.

Key Words: Mango, sudden death disease, macro and micronutrients, leaf and soil analysis.

INTRODUCTION

Mango (*Mangifera indica* L Family *Anacardiaceae*) is the second major fruit crop in Pakistan and falls in third rank for production of mangoes in the world (12.25 million tonnes) (Anonymous, 2007). In mango Pakistan earns 9,012 million rupees foreign exchange per year through export to foreign countries *i.e.* UK, France, Germany, Dubai, Switzerland, Saudi Arabia, Malaysia, Holland, Italy and Singapore (Anonymous, 2008). The main mango growing districts in the Punjab province are Multan, Bahawalpur, Muzaffargarh and Rahim Yar Khan. In the Province of Sindh, it is mainly grown in Mir Pur Khas, Hyderabad and Thatta, while in Khyber Pakhtun Khwa it is grown in Peshawar and Mardan. The climate of Sindh becomes warm about one month earlier than the Punjab which has given the province the privilege to harvest early varieties of mango (Anonymous, 2007).

The present situation of mango orchards needs special attention, since about all mango orchards are suffering from Mango Sudden Death Disease or quick decline of mango trees. Recently, incidence of this menace was found 20% and more than 60% in Punjab and Sindh Provinces of Pakistan respectively and 60% in Al Batinah region of Oman (Al-Adawi *et al*., 2006; Saeed *et al*., 2006). Initial symptoms of mango decline are *i.e.* gummosis from the stem bark and branch decline on affected trees, while other contributing symptoms *i.e.* vascular discoloration beneath the bark leading to tree death within six months of first symptom appeared (Al-Adawi *et al*., 2006; Masood *et al*., 2010a). The infected plants show abundant gum secretion from branches, stem, and main trunk. Initially the gum appears as a small droplet. However, as the disease progresses, it increases and covers most of the branch and trunk. Under severe conditions, the outer wood of a branch cracks and splits and exudes a yellow to brown, gum-like substance (Malik *et al*., 2004; Saeed *et al*., 2007). It was also observed that majority of diseased or stressed mango trees either nutrients or water illustrate the presence of tiny holes made by the bark beetle, *Hypocryphalus mangifer* (Wood, 1982; Masood *et al*., 2009, 2011).

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Although, bark beetle, *H. mangiferae* was formerly reported as an indigenous wood borer in mango as secondary pest (Mohyuddin and Mahmood, 1993) but now due to its role in disease transmission as a vector it has gained the status of primary pest, *i.e.* due to transmission of *Ceratocystis fimbriata* and *Lasiodiplodia theobromae*, the causal organisms of mango sudden death syndrome (Masood *et al*., 2010b, 2011).

There are different opinions from plant disease specialists, but it is a complicated case that emerged mostly due to the combined attack of anthracnose, stem blight or die back, root rot, tip die back, leaf blight, bacterial leaf spot and malformation diseases, which are responsible for death of trees. High temperature, high relative humidity, high soil temperature and drought contribute to spread of this disease on weak plants (Das Gupta and Zacchariah, 1945; Ploetz *et al*., 1996). Besides these abiotic factors, there are in general poor orchard management practices comprising lack of balanced fertilizers use, improper irrigation, intercropping with unsuitable crops and deep plowing resulting in root injuries (Malik *et al*., 2004; Saeed *et al*., 2007).

Research to date suggests that mango decline is caused by deficiencies of manganese and iron (Crane *et al*., 2006). These deficiencies may predispose trees to infection by fungal pathogens (*Botryosphaeria ribis* and *Physalospora* sp.), which attack shoots, or by root feeding nematodes (*Hemicriconemoides mangiferae*) (Minnatullah and Jah, 2002; Hasna, 2007)**.** Leaf symptoms include interveinal chlorosis, stunting, terminal and marginal necrosis, and retention of dead leaves that gradually drop. Dieback of young stems and limbs is common and even tree death may occur. Increased applications of iron, manganese, and zinc micronutrients have been observed to reduce or ameliorate this problem (Minnatullah and Jah, 2002; Heyman, 2008).

Generally nutrients affect plant disease. Some nutrients have direct and greater impact on plant diseases than others (Hewitt, 1951; Heyman, 2008). That is why, Fertilizers are therefore, recommended to optimize nutrient uptake to ensure normal growth and yield. In most situations, this level of nutrients would also be sufficient to enable the crop to maximize disease resistance.

In this study, the main objectives were: a) to conduct soil and leaf analysis of mango trees before and after nutrients application; b) to compare the development of disease symptoms on each treatment, and c) to evaluate the disease severity and its reduction over untreated control.

MATERIALS AND METHODS

Study site

This study was carried out at a private farmer field "Aziz Mango Orchard" situated at a distance of 5 Km from Bahauddin Zakariya University in North West on Bosan Road, Multan. All agronomics practices were kept uniform except from the application of micronutrients. Rotavator was used to remove weeds from orchard. Two rings were formed around all mango trees trunk under experiments. First ring was formed two feet away from trunk and second ring was formed where the tree canopy finished. All the fertilizers and micronutrients were applied in between these two rings. After spreading fertilizers irrigation was applied in this ring. Foliar application of (Carbandaz m) Topsin-m@ 2g/liter of water as fungicide and insecticide (synthetic pyrethariods) Bifenthrin @ 80 ml/100 liter of water was applied. Leaf and soil analysis was also performed for each treatment.

Nutrients application

Twenty four plants of Chounsa variety of almost same age (30-40 years) were selected. Micronutrients were applied in combination with nitrogen (urea), phosphorous (triple super phosphate) and humic acid @ 150 g/tree in each of eight treatments. This experiment included eight different treatments with three replications under randomize complete block design (RCBD). In all treatments, split dose of fertilizer was given through soil at the time of harvesting and before flowering. The treatments comprised T_1= $ZnSO_4$ (305g), T_2= $FeSO_4$ (526g), T_3= $CuSO_4$ (200g), T_4= $ZnSO_4$ + $FeSO_4$ (305+526) g, T_5= $FeSO_4$ + $CuSO_4$ (526+200) g, T_6= $ZnSO_4$ + $CuSO_4$ (305+200) g, T_7= $ZnSO_4$ + $FeSO_4$ + $CuSO_4$ (305+200+526) g, T_8= only NP (1Kg) as control. Micronutrients *i.e.* zinc was applied in the form of $ZnSO_4$ (zinc sulfate 33%),.

copper was used in the form of $CuSO_4$ (copper sulfate 24%) and iron in the form of $FeSO_4$ (ferrous sulfate 19%). Nitrogen and phosphorus was applied in the form of urea and triple super phosphate (TSP). Potassium was not applied because soil analysis indicated appropriate quantity in soil.

Assessment of MSDS

For each treatment, the symptoms of disease were observed at the five tree parts *i.e.* roots, collar, stem, limbs and leaves that show the following symptoms: oozing, canker formation, blackening, bark splitting, rotting, drying of twigs/branches, curling/drying, severe shedding before the drying of the whole tree and attachment of leaves after the drying of the whole tree. All the symptoms were recorded with a fifteen days interval from 20 August, 2008 to August, 2009 *i.e.* after 1^{st} harvesting of fruits to second. According to Masood *et al*. (2010a), the MSDS disease severities of different symptoms were described according to a allotted scale of 0-7 *i.e.* 1= 1-10 % severity, 2= 11-20 %, 3= 21-30 %, 4= 31-40 %, 5= 41-50 %, 6= 51-60% and 7= more than 60% area infected with MSDS.

Disease severity was measured according to following formulae.

Disease severity = (Infected tissue area/total tissue area) X100

$$\text{Mean severity (\%)} = \frac{\text{Sum of numerical ratings on the trees}}{\text{Total number of trees observed}} \times \frac{100}{7}$$

Methodology for leaf analysis

Mature leaves were collected from selected trees in all four directions. Total 60 leaves were plucked from each treatment before and after one month of application; samples were brought to laboratory, where washing was done with water. After washing leaves, they were oven dried at 65°C temperatures for 48 hours. Oven dried leaves were then grinded to powder. The 100 ml beakers were taken and labeled according to trees from where sample were taken. One gram powdered leaf was added in each beaker, to which then twenty ml of nitric acid and per chloric acid (2:1) solution were added A beaker, containing 20 ml solution without powder of leaves was used as a control. All beakers were placed on hot plate at 200°C for two hours in open ventilated place. Temperature was increased gradually. Firstly, red fumes emerged followed by white fumes. After digestion of samples, the volume of each sample was made upto 50 ml by adding distilled water. The digested samples were analyzed for Zn, Cu, Fe and Mn on Atomic Absorption Spectrophotometer.

Soil analysis

Soil samples were taken at four depths *viz.*, 15, 30, 45 and 60 cm with the help of agar around selected trees and five points were randomly selected. These soil samples were analyzed for E.C., pH, organic matter, available phosphorus and potassium.

Statistical analysis

The comparison of nutrients after leaf analysis and disease symptoms at different tree portions were statistically analyzed on the basis of analysis of variances (ANOVA) and their significant means were separated by least significant differences test (LSD at $P \leq 0.05$) by using computer based software (XLSTAT, 2008).

RESULTS AND DISCUSSION

Leaf analysis was found significant among all the treatments which indicated the nutrient's status in different treated mango trees (Fig. 1, Table I). The progress of disease indicating symptoms appeared to be non significant but overall 18.61% reduction in disease severity was recorded after the application of different nutrients compared with severity on treatment trees before application (Table II, Fig. 2). The maximum reduction in disease severity was 30.69% by nutrients combination $ZnSO_4$+$CuSO_4$+NP+humic acid followed by $ZnSO_4$ + $FeSO_4$+NP+humic acid and $CuSO_4$+NP+humic acid as 19.51% and 19.37%, respectively (Fig.1). The minimum reduction (8.39%) in disease severity was found due to nitrogen and phosphorus followed by nutrients combination of $ZnSO_4$+$FeSO_4$+ $CuSO_4$ + NP+humic acid (9.35%). In T_6 before application of micronutrients and NP the level of zinc was

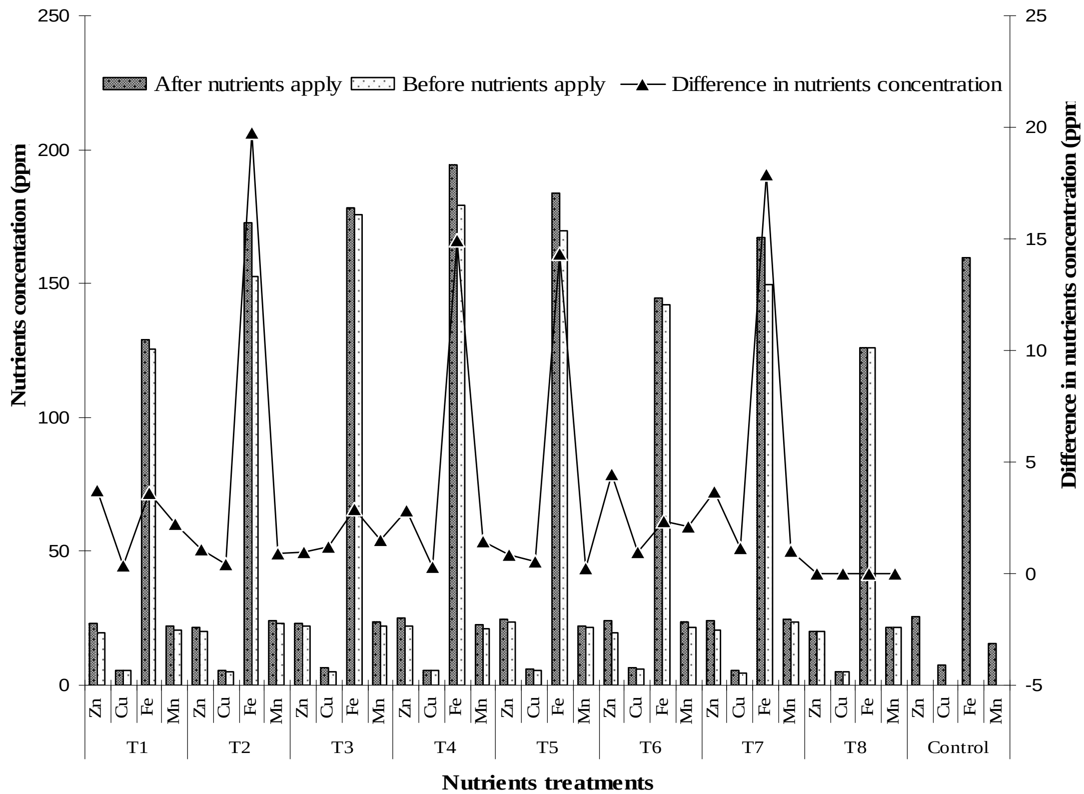

Fig. 1. Leaf analysis of different nutrients after and before nutrients application and difference in nutrients concentrations
T_1, $ZnSO_4$ (305g) + NP (1Kg) + humic acid (150g); T_2, $FeSO_4$ (526g) +NP (1kg) +humic acid (150g); T_3, $CuSO_4$ (200g) +NP (1Kg) + humic acid (150g); T_4, $ZnSO_4$ + $FeSO_4$ (305+526g) + NP (1Kg) + humic acid (150g); T_5, $FeSO_4$ + $CuSO_4$ (526+200g) + NP (1Kg) + humic acid (150g); T_6, $ZnSO_4$ + $CuSO_4$ (305+200g) +NP (1Kg) + humic acid (150g); T_7, $ZnSO_4$ + $FeSO_4$ + $CuSO_4$ (305+200+526g) +NPK (1Kg) + humic acid (150g); T_8, NP (1Kg); Control, laboratory control.

Table I.- Mean difference of nutrients (Zn, Cu, Fe, Mn) on all treated mango trees. Mean values sharing similar letters show non-significant differences (P<0.05) by using LSD test.

Nutrients treatments*	Zinc (Zn)	Copper (Cu)	Ferrous (Fe)	Maganese (Mn)
T_1	3.75 ab	0.35 b	3.60 c	1.40 ab
T_2	1.07 c	0.43 b	19.77 a	0.90 bc
T_3	0.97 c	1.23 a	2.92 c	1.48 ab
T_4	2.82 b	0.33 bc	14.95 ab	1.47 ab
T_5	0.87 c	1.23 a	14.35 b	1.40 ab
T_6	4.43 a	0.98 a	2.33 c	2.08 a
T_7	3.65 ab	1.13 a	17.90 ab	1.02 bc
T_8	0.62 c	0.08 c	0.57 c	0.12 c
ANOVA values (df;f;p)	7; 9.94; <0.0001	7; 30.92; <0.0001	7; 22.34; <0.0001	7;2.77; 0.043

*For details of nutrient treatment, see Figure 1.

16.10, 21.35 and 21.35 ppm and level of copper before application was 6.05, 5.75 and 5.55 in each replication, whereas after application the level of zinc was increased to 21.80, 25.25 and 25.05 ppm and that of copper was increased to 7.10, 6.95 and 6.25 in each replication (Fig. 2). The increasing level of zinc and copper and maximum reduction of disease severity showed that combined application of zinc and copper was more effective than combined application of zinc and iron. These results were similar to those of Minnatullah and Jah (2002) on cereal.

Although there is reduction in disease severity after treatment with iron combined with zinc and copper (*i.e.* T_4 and T_3) they were not so effective as the combination of zinc and copper. The

Table II.- **Evaluation of disease symptoms severity (%) on different portions of mango trees after nutrients application. Mean values sharing similar letters show non-significant differences (P<0.05) by using LSD test.**

Nutrients treatments*	Oozing (collar)	Blackening (Roots)	Oozing (stem)	Bark splitting (stem)	Oozing (limb)	Drying of twigs (limbs)	Bark splitting (limbs)	Curling (leaves)
T_1	0.00 a	5.00 a	13.33 a	0.00 b	5.00 b	0.00 b	0.00 b	53.33 a
T_2	0.00 a	5.00 a	19.17 a	0.00 b	7.50 b	0.00 b	0.00 b	38.33 a
T_3	0.00 a	0.00 a	10.00 a	0.00 b	7.50 b	0.00 b	0.00 b	75.00 a
T_4	5.00 a	0.00 a	7.50 a	0.00 b	2.50 b	0.00 b	25.83 a	60.00 a
T_5	0.00 a	0.00 a	2.50 a	0.00 b	2.50 b	0.00 b	0.00 b	31.67 a
T_6	2.50 a	0.00 a	2.50 a	20.83 a	18.33 ab	2.50ab	23.33 a	68.33 a
T_7	0.00 a	0.00 a	0.00 a	5.83 b	32.50 a	5.00 a	0.00 b	45.00 a
T_8	0.00 a	0.00 a	2.50 a	0.00 b	5.00 b	-5.00 c	-12.50b	34.17 a
ANOVA values (df; f; p)	7;0.89; 0.539	7;0.85; 0.56	7;0.92; 0.519	7; 2.8; 0.043	7; 1.94; 0.12	7; 3.4; 0.021	7; 5.15; 0.003	7; 0.52; 0.8o5

*For details of comparison of various treatments see legend of Fig. 1.

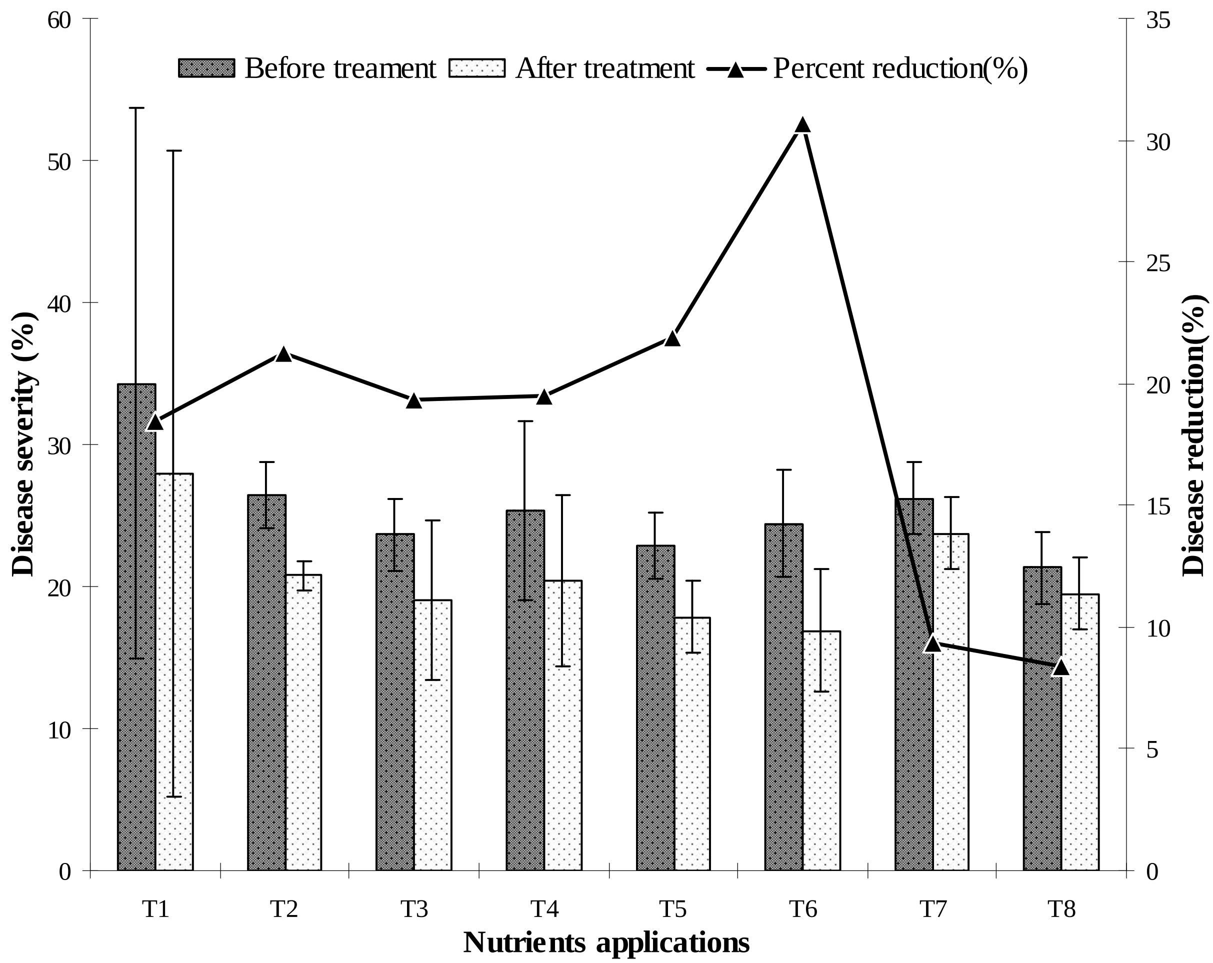

Fig. 2. Disease severity (%) and its reduction (%) after nutrients application on cv.chounsa
For details of comparison of various treatments see legend of Fig. 1.

Table III.- Soil analysis before nutrient applications at four different sampling sites to represent the whole experimental area at Aziz Farm.

Sr. No.	Treatment*	Depth (λ)	E.C dS/m	pH	Organic matter (%)	Available phosphorus (ppm)	Available potassium (ppm)	Saturation (%)	Texture
S1	R_1T_3	0-15	1.14	8.6	0.88	12.00	180	40	Loam
S2	R_1T_3	0-30	1.93	8.7	0.87	12.05	185	42	Loam
S3	R_1T_3	0-45	2.02	8.6	0.85	11.50	190	43	Loam
S4	R_1T_3	0-60	2.20	8.6	0.80	11.00	185	42	Loam
S1	R_1T_5	0-15	3.26	8.3	1.06	17.55	200	38	Loam
S2	R_1T_5	0-30	2.33	8.4	1.00	17.75	185	40	Loam
S3	R_1T_5	0-45	1.67	8.7	1.00	18.05	185	38	Loam
S4	R_1T_5	0-60	2.84	8.5	0.98	16.85	180	38	Loam
S1	R_2T_3	0-15	2.02	8.7	1.08	13.00	195	32	Loam
S2	R_2T_3	0-30	15.30	8.8	1.00	13.10	195	34	Loam
S3	R_2T_3	0-45	2.02	8.8	0.98	12.50	200	30	S. Loam
S4	R_2T_3	0-60	1.54	8.8	0.97	11.40	185	30	S. Loam
S1	R_2T_5	0-15	3.15	8.0	0.23	7.00	200	32	Loam
S2	R_2T_5	0-30	2.65	8.3	0.22	6.50	185	30	S. Loam
S3	R_2T_5	0-45	2.49	8.0	0.21	6.00	185	30	S. Loam
S4	R_2T_5	0-60	2.68	7.4	0.20	6.00	190	32	Loam

*For details of comparison of various treatments see legend of Fig. 1.

Table IV.- Soil analysis after nutrient applications at four different sampling sites to represent the fertility status of experimental area at Aziz Farm.

Sr. No.	Treatment*	Depth (λ)	E.C dS/m	pH	Organic matter (%)	Available phosphorus (ppm)	Available potassium (ppm)	Saturation (%)	Texture
S1	R_1T_3	0-15	2.84	8.0	0.97	18.65	195	36	Loam
S2	R_1T_3	0-30	1.95	8.2	1.06	15.00	190	38	Loam
S3	R_1T_3	0-45	6.92	7.9	0.90	16.40	190	36	Loam
S4	R_1T_3	0-60	2.45	7.8	1.00	16.05	180	40	Loam
S1	R_1T_5	0-15	1.70	7.5	1.10	21.40	200	39	Loam
S2	R_1T_5	0-30	2.43	7.7	0.99	20.95	195	42	Loam
S3	R_1T_5	0-45	1.84	8.0	0.91	21.25	200	42	Loam
S4	R_1T_5	0-60	2.28	8.2	0.93	20.00	190	40	Loam
S1	R_2T_3	0-15	1.87	8.1	0.90	18.35	185	40	Loam
S2	R_2T_3	0-30	2.57	8.3	0.98	18.05	195	36	Loam
S3	R_2T_3	0-45	3.22	8.2	0.91	16.95	190	36	S. Loam
S4	R_2T_4	0-60	2.56	8.0	0.92	15.95	200	37	S. Loam
S1	R_2T_5	0-15	3.10	7.3	0.23	10.45	195	32	Loam
S2	R_2T_5	0-30	2.60	7.6	0.22	10.25	190	31	S. Loam
S3	R_2T_5	0-45	2.44	7.5	0.21	10.35	195	31	S. Loam
S4	R_2T_5	0-60	2.69	7.0	0.20	9.85	200	32	Loam

*For details of comparison of various treatments see legend of Fig. 1.

minimum reduction of severity occurred where without application of micronutrients was found in range of 8.39 and 9.35 percent on T8 (only NP) followed by T7 all nutrients ($ZnSO_4$+ $FeSO_4$+ $CuSO_4$+NPK+humic acid) respectively as given in Figure 2. In treatment T_1 ($ZnSO_4$ only), the reduction in disease severity was 18.45%. The level of zinc in T_1 before application of $ZnSO_4$ was 20.25, 22.30 and 15.10 but after application the zinc level increased to 24.55, 24.95 and 20.10. This indicates that increase in zinc level reduces the disease severity which was again confirmed in findings by Minnatullah and Jah (2002). In T_2 ($FeSO_4$ group), the reduction of disease severity was 21.25%. The level of Fe before application was 151.25, 171.60 and 135.55, but after application the Fe level was increased to 172.35, 190.05 and 155.30. It meant that increase of Fe level also reduced disease severity. Another important outcome from the leaf and soil analysis result was that the humic acid increased the ability of plant to absorb different micronutrients from soil (Fig. 1, Table IV).

The present study was aimed at developing the most effective disease management through micronutrients application in mango orchards. Results indicated that the maximum reduction in disease severity was 30.69% recorded in treatment T_6 where $ZnSO_4$+$CuSO_4$+NP+humic acid were applied. Zinc is essential to the integrity and stability of plant membranes and has been beneficial in the control of various fungal diseases (Spectrum Analytic Inc., 2002) *i.e.* root rot, mold, wilt, powdery mildew, foot rot, head scab, leaf spot and take all. Copper has also been beneficial in the control of following diseases such as mildew, leaf spot, leaf rust, ergot, blast, and wilt and take all. Minnatullah and Jah (2002) found that spraying of zinc sulfate resulted in the lowest disease severity and highest grain yield, followed by $CuSO_4$. Our results are also compatible with the findings of Reuveni *et al*. (1997), Ehret *et al. (*2002), Kalim *et al*. (2003), Vanitha *et al.* (2005) and Heyman (2008) who stated that nutrition has great impact on disease management. In the present study, maximum reduction in disease severity was recorded by the application of nutrients *i.e.* zinc, copper, iron with combination of humic acid (Fig.1), whereas, minimum reduction (8.39%) in disease severity was found due to nitrogen and phosphorus only. This may be possible that humic acid plays a significant role to increase the ability of plant to uptake different micronutrients from soil as demonstrated in our findings. However, our results are contradictory to Camilla *et al.* (2007) who stated that foliar fungicide comprising phosphate/ phosphonates, copper, manganese and zinc did not significantly affect disease severity.

This study indicates that with application of macro- and micronutrients the overall nutrient status of soil is improved and there is no negative interaction. Copper and zinc have been reported to act synergestically in boosting the yield of rice (Brar and Sekhon 1978; Hulagur, 1975; Gangwar, 1988). Our experiment also indicate that the maximum reduction in disease severity was recorded in treatment T_6 where $ZnSO_4$+$CuSO_4$+ NP+humic acid were applied. An increase in the yield of crops has also been observed in combined application of iron and zinc than their individual application (Chavan and Banerjee, 1980). Moreover, iron can effectively adjust manganese deficiency in soils containing appreciable quantities of higher oxides of manganese.

Therefore, nutrient's application is highly encouraged for improving tree vigor against the management of sudden death of mango. The results of leaf analysis, soil analysis and disease severity made clear that that balanced nutrition plays a significant role in disease management.

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