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Micronutrients Foliar and Drench Application Mitigate Mango Sudden Decline Disorder and Impact Fruit Yield

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Abstract: Mango sudden death (MSD) or quick decline (QD) is the most destructive disease found in mango orchards of Pakistan and is characterized by collapse of the vascular system by *Ceratocystis fimbriata* and *Lasiodiplodia theobromae*. Cultural practices, chemicals, and biological control are the most valuable tools for the management of MSD, but the role of micronutrient deficiencies has remained an area that is heavily ignored by the farming community. To study the impact of micronutrients, four mango orchards were selected at different locations where different combinations of micronutrients, i.e., Zinc (Zn), Boron (B), and Copper (Cu) in the form of Zinc sulphate (ZnSO₄), Borax/Boric acid (H₃BO₃), and Copper Sulphate (CuSO₄), were applied both foliar and in drench along with the recommended doses of Nitrogen: Phosphorous: Potassium (NPK), and Farmyard manure (FYM), respectively. The quantities of micronutrients were determined from the soil and leaves before and after application of the treatments. The impact of micronutrients was measured in terms of reduction in disease severity and increase in fruit yield. The results revealed that the application of all three micronutrients both in soil drench and in foliar form significantly decreased the disease severity at three locations and increased the yield in all four mango orchards. Application of ZnSO₄ (0.8%), +H₃BO₃ (0.8%), +CuSO₄ (0.5%) and as soil drench ZnSO₄ (400 g) + Borax (200 g) + CuSO₄ 200 g plant^{−1} proved to be the best treatments, with an average of 12.88 and 14.03% reduction in disease severity and with an average yield of 128 and 119 kg, respectively. The application of micronutrients would be a promising solution in an integrated disease management program used to tackle MSD.

Keywords: *Mangifera indica*; *Ceratocystis fimbriata*; *Lasiodiplodia theobromae*; boron (B); zinc (Zn); copper (Cu); farmyard manure (FYM)

1. Introduction

Mango (*Mangifera indica* L.) is the most valuable fruit crop of the family Anacardiaceae and is mostly grown in the tropical and sub-tropical regions of the world. This fruit crop is the second most important tropical crop in terms of production [1]. Mango is the second major fruit crop of Pakistan, and more than 250 varieties of mango are grown in Pakistan.

The overall export of mango has decreased due to low production and poor growth. Mango exports were 103,187 tonnes in 2012–2013 and reduced to 65,311 tonnes in 2014–2015 [2]. Various biotic and abiotic factors are the main cause of the low production of mango in Pakistan. The most important and common diseases present in Pakistan are Powdery mildew (*Oidium mangiferae*), anthracnose (*Colletotrichum gloeosporioides*), fruit rot (*Aspergillus niger*), root rot (*Fusarium* and *Rhizoctinia* species), and tip dieback (*Alternaria alternata*, *Fusarium equiseti*, *Rhizopus nigricans*, and *Aspergillus niger*), stem blight or dieback (*Ceratocystis fimbriata*, *Lasiodiplodia theobromae*), bacterial black spot (*Xanthomonas campestris* pv. *mangiferae*indicae); and mango malformation [3]. Among these, mango sudden death or decline (MSD) is the most destructive disease and is found in almost every mango orchard. The principal pathogen in most countries has been identified to be the fungus called *L. theobromae* [4–7]. There are three other fungal pathogens (*Ceratocystis fimbriata*, *Ceratocystis omanensis*, and *L. theobromae*) that have been linked to the disease [7,8]. *Ceratocystis manginecans* is a causal organism associated with MSD in Oman and Pakistan [9]. *Fusarium* has also been recorded to be associated with the disease [10].

The occurrence of MSD is highly promoted by a beetle (*Xeleborus offinis*) at a relevant humidity above 80% and 25–31 °C temperature [11]. A bark beetle of mango, *Hypocryphalus mangiferae*, is implicated as a vector in the establishment of MSD in Pakistan [7,12,13]. Nutritional deficiency, drought, temperature fluctuations, and mechanical injuries are the principal abiotic factors that speed up MSD [14–16]. Moreover, improper management practices in an orchard, such as poor irrigation, intercropping, damaging of roots caused by intense ploughing, and the existence of tainted plants are the main components of the establishment of the disease [17].

MSD is a complex disease that is believed to cause several yield losses in countries such as Pakistan, Oman, and Brazil. The disease is characterized by the sudden collapse of severely infected mango trees, and disease symptoms initially appear as gum exudation from stem bark and fall off of the branches on trees followed by vascular discoloration beneath the bark. This disease sometimes shows no easily recognizable external symptoms except for stunting, which may be apparent in the field. The disease is recognizable when twigs and branches dry up, combined with complete defoliation, which has the appearance of a tree that has been scorched by fire [18,19]. MSD is caused by *L. theobromae* and when *Ceratocystis fimbriata* attacks the plant, producing a synergistic effect, which leads to the death of the tree within a few days. [13]. Tree plants require an adequate proportion of micronutrients to enhance their growth and yield [20]. Micronutrients play a crucial role and function in plant growth and development by mobilizing different enzymatic pathways to produce various enzymes, which activate the plant's defence mechanism against diseases, whereas micronutrient deficiencies lead to problems that may be incurable. [21].

Micronutrients play a very crucial role in the translocation of macronutrients and functions of many metabolic processes in plant-like respiration, cell wall development, photosynthesis, formation of chlorophyll, hormone synthesis, reduction, fixation of nitrogen, and enzyme activities [22]. A trace amount of micronutrients is required to strengthen the physiological and biochemical exercises in the plants by actuating the enzymes, the osmoregulation of the cells, and changing the permeability of the cell membrane. The general mineral nourishment of a plant may be affected by micronutrients due to catalysing the uptake of macronutrients [23].

For better growth and development of mango trees, nutrients are required in a reasonable amount for plants to cope with the various biotic and abiotic stresses. Therefore, the role of micronutrients in disease control is very important. When micronutrients are applied in a foliar form, they are quickly absorbed by the plant organs and tissues, which improve the quality of fruit. It is becoming common practice to apply micronutrients in the form of a foliar spray to control the deficiency of micronutrients to achieve the best fruit quality. Nutrients are more quickly available to the plants when they are applied in a foliar form compared to soil application [24].

Keeping in view the economic importance of the mango and the devastating effect of MSD, comprehensive research is needed to identify the causes and factors and to develop sustainable disease management practices using balanced nutrition to increase the productivity and income of growers and farmers. Therefore, in this study, the impact of micronutrients (Zn, Cu, and B) was evaluated for the management of MSD.

2. Materials and Methods

2.1. Study Sites

The study was carried out at the orchards of four different farmers, located at Dera Din Panah (30.591° N and 70.943° E), Jalalpur Pirwala (29.622° N and 71.136° E), Tatay Pur (30.212° N and 71.665° E), and Shujaabad (30.266° N and 71.494° E), where normal agro-nomic practices, i.e., irrigation, hoeing, removal of weeds, pruning, etc., were performed. The micronutrients were applied on the leaves and in the soil of the selected tress.

2.2. Micronutrients Application

In each selected orchard, infected mango trees and mango trees vulnerable to disease were selected for the experiment. A trench was made around the trunk under the canopy of each treated mango tree selected for the experiments. Micronutrients were drenched around the trees in January, March, and in the last week of May. Two foliar applications of micronutrients were done before flowering and after fruit setting. All the micronutrients were applied along with a combination of the recommended dose of NPK and FYM. Ten-year old mango trees (cultivar “Summer Bahisht Chaunsa”) were selected from each orchard. All the treatments along with the combination of the recommended doses of NPK and FYM were applied with three replications executed in a randomized complete block design. The details of the experimental treatments are given in Table 1.

Table 1. Details of the experimental treatments for field application of the susceptible mango variety “Summer Bahisht Chaunsa”.

Treatments	NPK	FYM	Nutrition	Application
T1	RD	80 kg plant ⁻¹	-----	Drench
T2	RD	80 kg plant ⁻¹	ZnSO ₄ .H ₂ O (400 g plant ⁻¹)	Drench
T3	RD	80 kg plant ⁻¹	ZnSO ₄ .H ₂ O (0.8%)	Foliar
T4	RD	80 kg plant ⁻¹	Borax (200 g plant ⁻¹)	Drench
T5	RD	80 kg plant ⁻¹	H ₃ BO ₃ (0.8%),	Foliar
T6	RD	80 kg plant ⁻¹	ZnSO ₄ .H ₂ O (400 g plant ⁻¹) + Borax (200 g plant ⁻¹)	Drench
T7	RD	80 kg plant ⁻¹	ZnSO ₄ .H ₂ O (0.8%), + H ₃ BO ₃ (0.8%)	Foliar
T8	RD	80 kg plant ⁻¹	ZnSO ₄ .H ₂ O (400 g plant ⁻¹) + Borax (200 g plant ⁻¹) + CuSO ₄ .5H ₂ O (200 g plant ⁻¹)	Drench
T9	RD	80 kg plant ⁻¹	ZnSO ₄ .H ₂ O (0.8%) + H ₃ BO ₃ (0.8%) + CuSO ₄ .5H ₂ O (0.5%)	Foliar

RD = NPK was applied as per the recommended doses at the rates of 1500, 1000, and 1000 g plant⁻¹, respectively, as NPK 17-17-17, FYM = farmyard manure was applied at a constant dose at the rate of 80 kg plant⁻¹.

2.3. Leaf Analysis for Zn and Cu

Green, healthy, and mature leaves were collected from the trees before application. After 3 to 4 months of micronutrients applications, 4–6 months old middle leaves were collected for the analysis Zn, B, and Cu. The leaves were washed with distilled water, oven dried at 700 °C for 48 h, and ground to powder. One gram of powder from each sample was added to 100 mL beakers and 20 mL of nitric acid and perchloric acid were added at a ratio of 2:1. These beakers were then placed on a hot plate for 2h at 200 °C in an open ventilated place. The temperature was increased gradually until red fumes appeared, followed by white fumes, and finally the volume of each sample was completed to 50 mL by adding distilled water. These processed samples were separated for the detection of different nutrient elements such as Zn and Cu on the atomic absorption spectrophotometer [25].

2.4. Calculation of Boron in the Leaves

Boron was determined by the dry ash method, where 1 g of oven-dried crushed leaves of each sample was taken in a porcelain crucible. In the dry ash method, a muffle type furnace was used to heat the samples. The samples were burnt in the furnace at a temperature of 550 °C for 6 h. A few drops of deionized water (DI) were added to the ash and 10 mL of 0.36 NH₂SO₄ was added to the crucible. The crucible was heated for 20 min in a steam bath, and after 20 min the crucible was removed and cooled at room temperature for 1 h. The solution was then filtered in a volumetric flask of 50 mL, which was made of polypropylene using Whatman filter paper No.1. The readings were measured with the help of an atomic absorption spectrophotometer [26].

2.5. Calculation of Micronutrients in the Soil

Soil samples were collected at three different depths ranges from 0 to 15, 15 to 30, and 30 to 45 cm with the help of auger from four sides of the tree canopies one meter away from the trunk. The soil samples taken from each depth were composited. Micronutrient extraction was done by weighing up to 10 g of air-dried soil into a measuring glass or flask and 20 mL of DTPA-CaCl₂ extraction solution was added to the dried soil. It was then shaken well for 2 h with a mechanical shaker and the atomic absorption spectrophotometer was used to take readings of the samples. A calibration curve was used to measure the concentration of micronutrients, viz., Zn and Cu.

2.6. Determination of Boron in the Soil

The diluted hydrochloric acid (HCl) method was used for the determination of boron from the soil. Ten grams of air-dried soil were added into a polypropylene tube with a volume of 50 mL, then 0.2 g of activated charcoal (B-free) was added to it. Then, 20 mL 0.05 N HCl solution was added and gently shaken for 5 min. The suspension was filtered with the help of filter paper Whatman No. 40. Then, 2 mL of buffer solution was added, followed by 2 mL Azomethine -H, which was then mixed it well. Different standards of the solution were made, and the readings of these solutions were taken with the help of a spectrophotometer at a wavelength of 420 nm to determine the amount of boron [25].

2.7. Disease Measurement

The disease on mango trees was estimated on the basis of symptom severity. Symptoms such as rotting and blackening of the tissues at the collar region of the trunk and crown roots of the mango trees were observed. Other important symptoms were the formation of canker, oozing out of gum from the trunk and branches of the mango trees. Some symptoms were considered as major symptoms, and these include the shedding of leaves, the drying of an entire infected tree in severe cases. In some cases, leaves attachment was also observed after the tree was fully dried. Leaves were observed according to the path indicator from the four sides of a tree, i.e., east, west, north, and south. Mango tree branches showing gummosis were labelled and tagged. A total of 20 branches were selected on a tree in four directions. Data were collected at different dates before and after the application of micronutrients. The formula for the calculation of disease incidence (*D.I*) is given as Equation (1).

$$\text{Disease incidence (D.I)} = \frac{\text{No. of infected plants}}{\text{Total no. of plants assessed}} \times 100. \quad (1)$$

The basic original formula for this calculation was revised by Ref. [27] and reused by Ref. [28], and disease severity percentage was calculated by a specific scale (0–3) used by Ref. [29]. The scale was rated as 0 for no disease, 1 for slightly infected leaves, 2 for dead leaves but still attached to the branch, and 3 as defoliation of leaves and apical necrosis. The disease severity (*D.S*) was calculated on every single tree using Equation (2). The

percentage of the mean severity of each individual tree in terms of yield loss was calculated by Equation (3).

$$\text{Disease Severity (D.S)} = \frac{\text{Sum of numerical ratings on the whole tree}}{\text{Total number of diseased branches on tree}} \times \frac{100}{3}. \quad (2)$$

The percent yield loss was calculated by the following equation:

$$\text{Yield loss (\%)} = \frac{\text{Maximum Yield} - \text{Actual yield}}{\text{Maximum yield}} \times 100. \quad (3)$$

2.8. Statistical Analysis

All the treatment datasets were subjected to analysis of variances (ANOVA). The comparison of disease severity and yield at four different locations were statically analysed, and their means were compared through Duncan's multiple range test at the significance level $p \leq 0.05$, using Origin 2021b software [30].

3. Results

The impact of micronutrients on the MSD-affected trees and on the mango yield was studied through the soil and as foliar applications of micronutrients, as per the recommended doses of NPK and FYM. The results revealed a significant impact of soil and foliar applications of micronutrients at all locations except for Jalalpur Pirwala, where the disease severity was increased after the treatments. At the three other locations, the disease severity was decreased, which resulted in an increased yield of mango fruit as compared to the control (T0), where the disease severity was increased at a tremendous rate because we selected diseased plants for all the treatments.

3.1. Effect of Micronutrients (Zn, Cu, and B) on MSD Disease Severity at Jalalpur Pirwala

At Jalalpur Pirwala, there were significant results of all the treatments in terms of increasing the disease severity. The impact of micronutrients application was not observed to suppress the disease severity, as it increased significantly at this specific location. Although the disease severity increased after the application, the minimum disease severity was observed to be 7.75 and 8.01 on T7 ($\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ (0.8%), + H_3BO_3 (0.8%) and T9 ($\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ (0.8%) + H_3BO_3 (0.8%) + $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.5%), respectively. (Figures 1A and 2A).

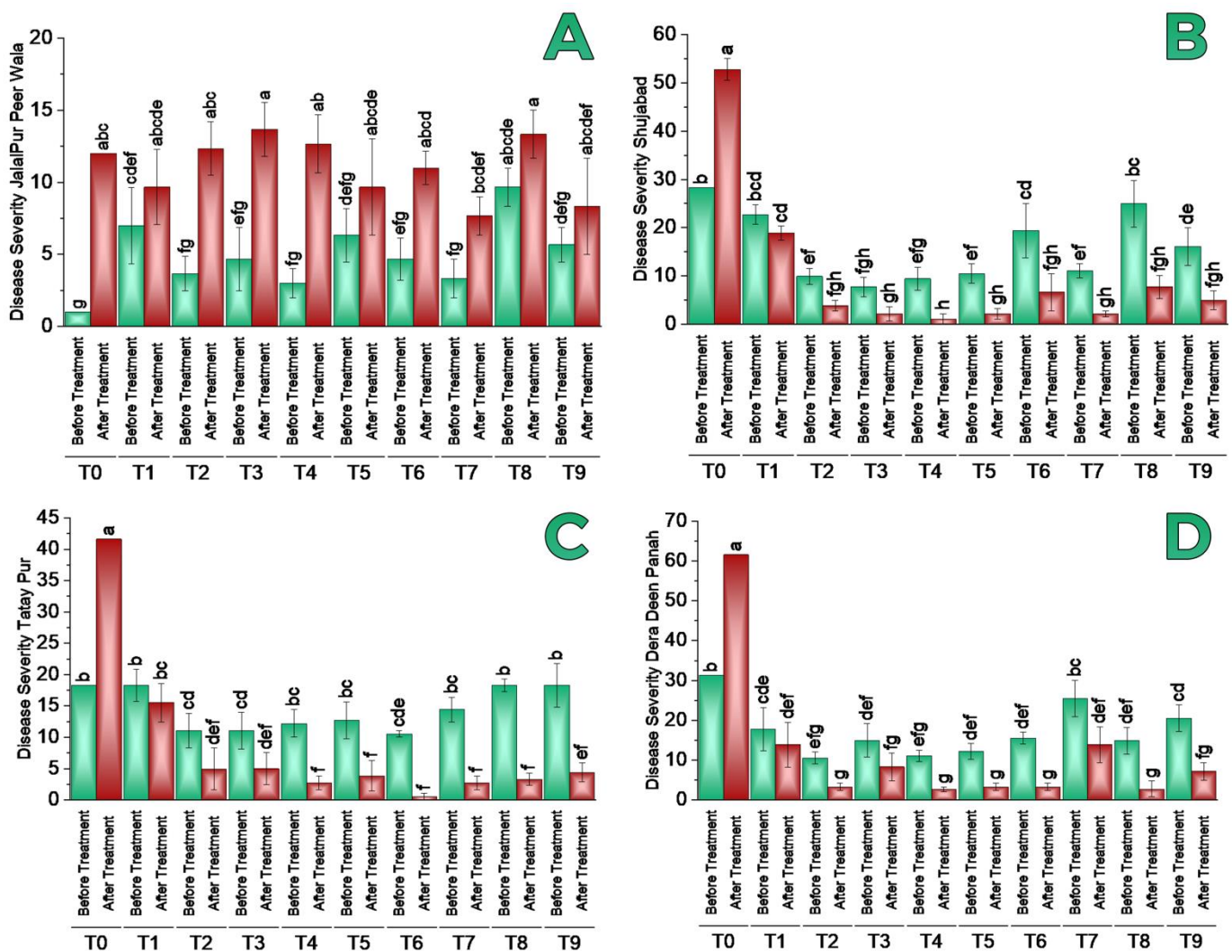


Figure 1. (A–D) Effect of treatments on disease severity before and after the application of nutrients. T0 = control; T1 = recommended dose (RD) of NPK (1500, 1000, 1000 g plant^{−1}) + FYM (80 kg plant^{−1}); T2 = RD + FYM+ ZnSO₄.H₂O (400 g plant^{−1}); T3 = RD + FYM + ZnSO₄.H₂O (0.8% foliar); T4 = RD + FYM + Borax (200 g plant^{−1}); T5 = RD + FYM + H₃BO₃ (0.8% foliar); T6 = RD + FYM + ZnSO₄.H₂O (400 g plant^{−1}) + Borax (200 g plant^{−1}); T7 = RD + FYM + ZnSO₄.H₂O (0.8% foliar) + H₃BO₃ (0.8% foliar); T8 = RD + FYM + ZnSO₄.H₂O (400 g plant^{−1}) + Borax (200 g plant^{−1}) + CuSO₄.5H₂O (200 g plant^{−1}); T9 = RD + FYM + ZnSO₄.H₂O (0.8% foliar) + H₃BO₃ (0.8% foliar) + CuSO₄.5H₂O (0.5% foliar) on location (A–D). Means followed by a similar letter are not statistically different.

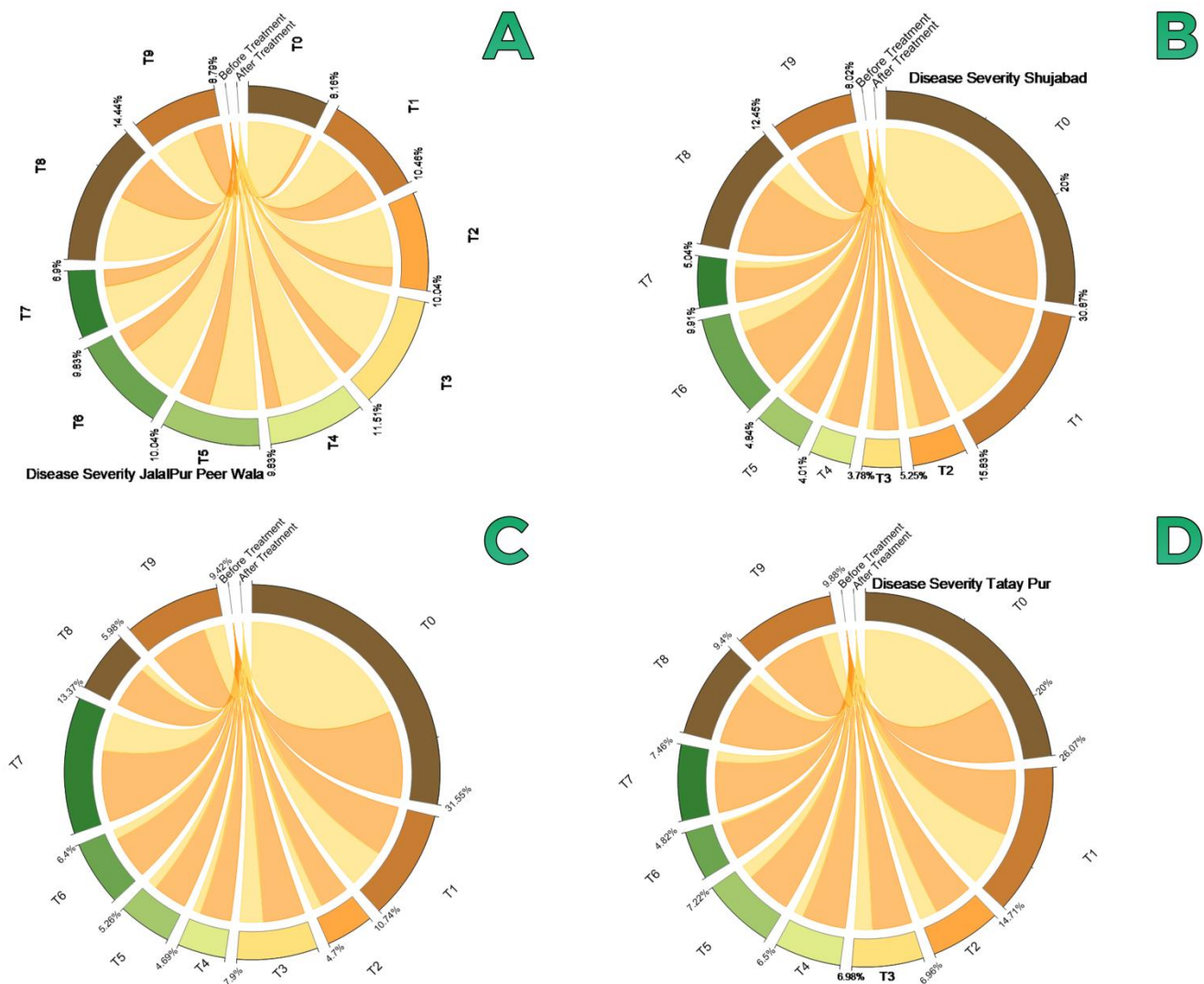


Figure 2. (A–D) Chord analysis: the effect of treatments on disease severity before and after the application of nutrients. T0 = control; T1 = recommended dose (RD) of NPK (1500, 1000, 1000 g plant⁻¹) + FYM (80 kg plant⁻¹); T2 = RD + FYM+ ZnSO₄.H₂O (400 g plant⁻¹); T3 = RD + FYM + ZnSO₄.H₂O (0.8% Foliar); T4 = RD + FYM + Borax (200 g plant⁻¹); T5 = RD + FYM + H₃BO₃ (0.8% foliar); T6 = RD + FYM + ZnSO₄.H₂O (400 g plant⁻¹) + Borax (200 g plant⁻¹); T7= RD + FYM + ZnSO₄.H₂O (0.8% foliar) + H₃BO₃ (0.8% foliar); T8 = RD + FYM + ZnSO₄.H₂O (400 g plant⁻¹) + Borax (200 g plant⁻¹) + CuSO₄.5H₂O (200 g plant⁻¹); T9 = RD + FYM + ZnSO₄.H₂O (0.8% foliar) + H₃BO₃ (0.8% foliar) + CuSO₄.5H₂O (0.5% foliar) on location (A–D). Ratios and proportions among the different treatments show their contribution towards disease suppression.

3.2. Effect of Micronutrients (Zn, Cu, and B) on MSD Disease Severity at Shujaabad

The soil and foliar application of micronutrients as per the recommended doses of NPK and FYM significantly reduced the disease severity of MSD as compared to the control at the experimental site Shujaabad. All the treatments showed a significant impact in mitigating disease severity after the application of micronutrients. A minimum disease severity of 1.1% was observed after the application of treatment T4 (NPK + FYM + Borax) in the soil followed by T7 (NPK + FYM + ZnSO₄.H₂O + H₃BO₃) as foliar with 2.17% disease severity. The disease severity was calculated as 2.20% on T3 (NPK + FYM + ZnSO₄.H₂O) and T5 (NPK + FYM + H₃BO₃) treatments where the disease severity was increased to 52.77% on T1 (NPK and FYM) treatment. The graph shows that T8 appeared to be the best treatment, reducing the disease severity up to 17%, as compared to before and after

the application of micronutrients. Treatment T6 (NPK + FYM + $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ + Borax) in soil and T7 (NPK + FYM + $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ 0.8%) and T9 (NPK + FYM + $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ + Borax) as foliar treatments also showed a drastic reduction in disease severity, up to 12%, 9%, and 11%, respectively, as the result of micronutrient application both in soil and as foliar (Figures 1B and 2B).

3.3. Effect of Micronutrients (Zn, Cu, and B) on (MSD) at TatayPur

There was a significant effect of micronutrients application along with other nutritional requirements for reducing MSD disease severity as compared to the control at Tatay Pur. Disease severity was decreased significantly in the treated plants after the application of micro- and macronutrients. After the application, the minimum disease severity was observed to be 0.53% on T6 (NPK + FYM + $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ 400 g plant⁻¹ + Borax 200 g plant⁻¹). The disease severity was also decreased to 2.73% when the treatments T7 (NPK + FYM + $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ 0.8%) and T4 (NPK + FYM + Borax 200 g plant⁻¹) were applied. The disease severity was increased to 41.60% when the treatment T1 (NPK and FYM) was applied. A maximum reduction of 15% in disease severity was observed in treatment T8 (NPK + FYM + $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ + Borax + $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), which was applied in the soil. The treatment T9 ($\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ + H_3BO_3 + $\text{CuSO}_4 \cdot \text{H}_2\text{O}$), in which micronutrients were applied in foliar form, showed a 13.9% reduction in disease severity, while other treatments, i.e., T2, T3, T4, and T5, also reduced the disease severity to a reasonable extent (Figures 1C and 2D).

3.4. Effect of Micronutrients (Zn, Cu, and B) on MSD at Dera DeenPanah

The soil and foliar applications of micronutrients as per recommended doses of NPK and FYM significantly reduced the disease severity of MSD as compared to the control at the experimental site of Dera Deen Panah. All the treatments showed significant impact on decreasing the disease severity after the application of micronutrients. A minimum disease severity of 2.73% was observed after the application of treatment T4 (NPK + FYM + Borax 200 g plant⁻¹) and T8 (NPK + FYM + $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ + Borax + $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) followed by T6 (NPK + FYM + $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ 400 g plant⁻¹ + Borax 200 g plant⁻¹), T5, and T2 (NPK + FYM + H_3BO_3) with a 3.30% disease severity. A minimum reduction in disease severity of 3.9% was observed on T1 (NPK and Farmyard Manure). The treatment T9 ($\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ + H_3BO_3 + $\text{CuSO}_4 \cdot \text{H}_2\text{O}$) appeared to be the best in reducing the disease severity—up to 13.33%—as compared to before and after the application of micronutrients. Treatments T8 (NPK + FYM + $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ + Borex + $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), and T7 (NPK + FYM + $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ 0.8%) also showed drastic reduction in disease severity—up to 12.23, 12.14, and 11.67%, respectively—as the result of micronutrient application both in soil and as foliar (Figures 1D and 2C).

3.5. Principal Component Analysis

The first principal component (PC1) revealed 55.1% of the total variation. The PC2 explained 25.5% of the total variation in the disease severity at different locations. The loading plots demonstrate that the relationships among disease severity at different locations with a <90° angle of vectors are positively correlated and with a >90° angle of vectors are not correlated. There is a high correlation among the disease severity at three locations, i.e., Dera Deen Panah, Sujhabad, and Tatay Pur, whereas it was negatively correlated with treatment, as disease severity decreased with the application of treatments. The disease severity at the location Jalalpur Pirwala had no correlation with the treatments. The larger the arrows, the greater the relationship (Figure 3).

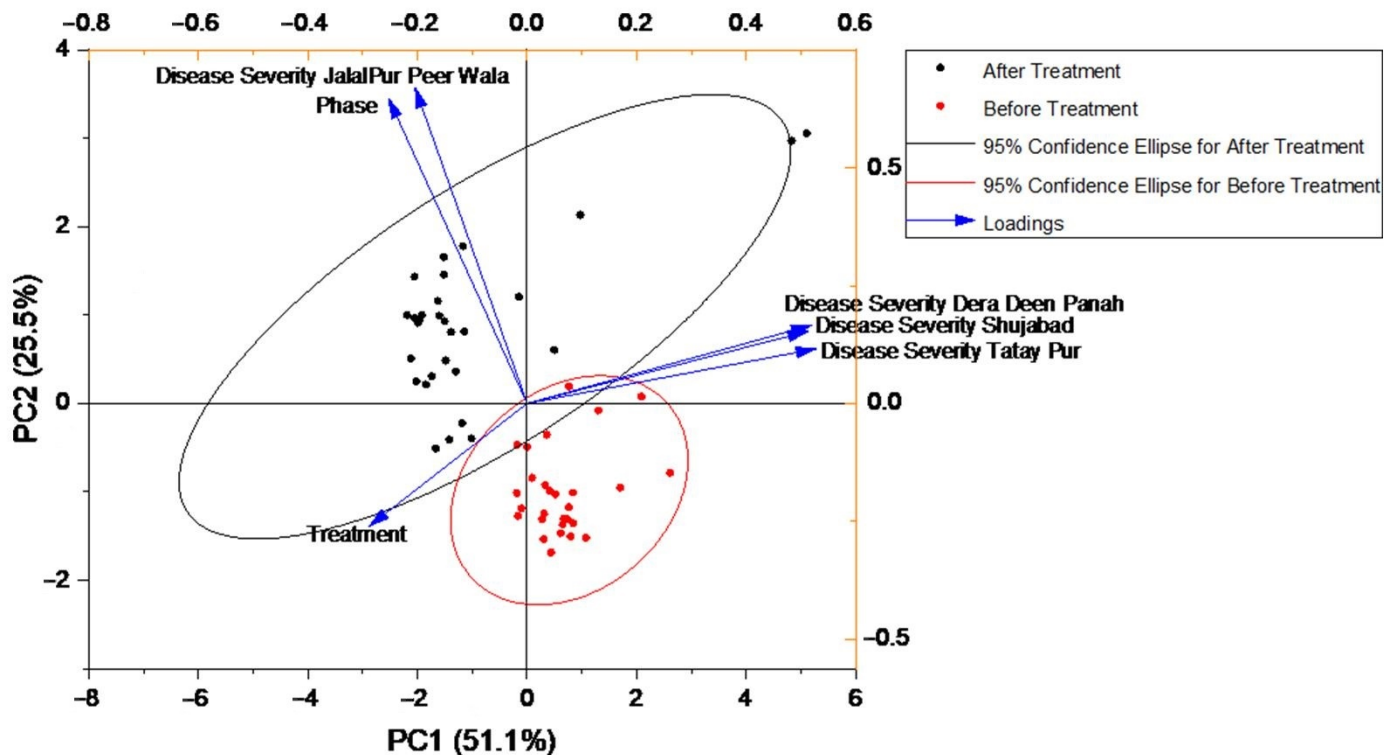


Figure 3. Principle component loading plots and scores of principal component analysis of different treatment applications at four different locations indicate that only one location has non-significant impact of all treatments on disease severity, whereas the three other locations are showing significant observations that all the treatments had a positive impact on the increase of yield and in the reduction of disease severity.

3.6. Effect of Micronutrients (Zn, Cu, and B) on Mango Yield at the Selected Locations

The soil and foliar application of micronutrients along with the recommended doses of NPK and FYM significantly increased the fruit yield as compared to the control at all the experimental sites. Maximum yield obtained was 133 kg at Jalalpur Pirwala and Dera Din Panah and as the result of treatment T9 where micronutrients ($\text{ZnSO}_4 \cdot \text{H}_2\text{O} + \text{H}_3\text{BO}_3 + \text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) were applied in foliar form. The yield recovered from Shujabad and Tatay Pur was recorded at par, i.e., 123 kg in response to the treatment T8 in which micronutrients ($\text{ZnSO}_4 \cdot \text{H}_2\text{O} + \text{Borax} + \text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) were applied in the soil. The minimum yield remained under 13 kg in the control. The yield observed in treatment T1 remained under 95 kg, in which the micronutrients were not given in any form. We assumed that as the result of all the three micronutrient applications in foliar form the average yield per plant increased from 28 kg to 38 kg at all four locations. Similarly, the response of soil drenching of micronutrients also increased the average yield up to 28 kg. Other treatments in which micronutrients were applied alone or in combination either as foliar or as drench also increased the yield, but the combination of three nutrients applied as foliar or drench gave the most promising results (Figure 4A–D).

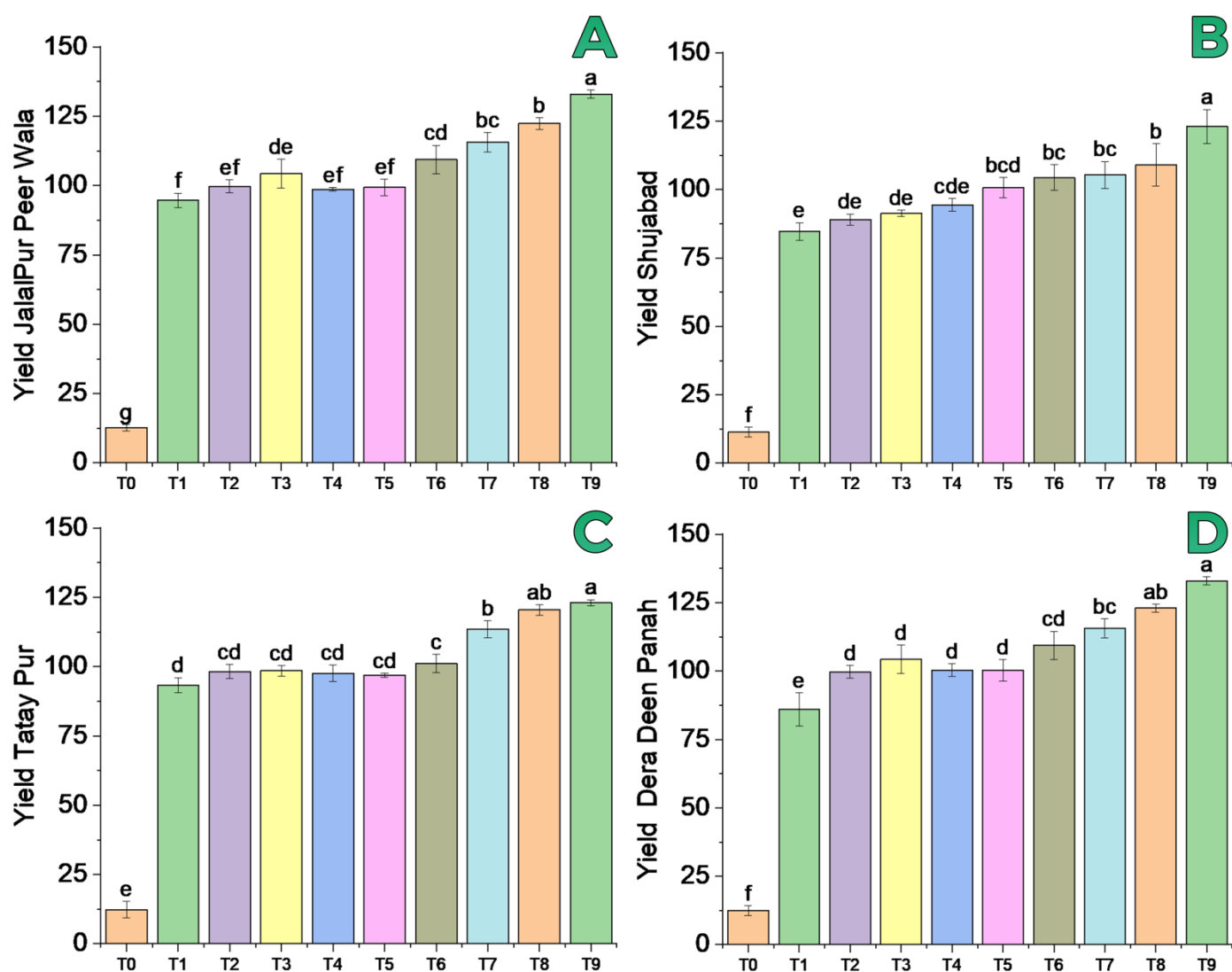


Figure 4. (A–D) Effect of various treatments on fruit yield under T0 = control, T1 = recommended dose (RD) of NPK (1500, 1000, 1000 g plant⁻¹) + FYM (80 kg plant⁻¹); T2 = RD + FYM + ZnSO₄.H₂O (400 g plant⁻¹); T3 = RD + FYM + ZnSO₄.H₂O (0.8% foliar); T4 = RD + FYM + Borax (200 g plant⁻¹); T5 = RD + FYM + H₃B₃ (0.8% foliar); T6 = RD + FYM + ZnSO₄.H₂O (400 g plant⁻¹) + Borax (200 g plant⁻¹); T7 = RD + FYM + ZnSO₄.H₂O (0.8% foliar) + H₃BO₃ (0.8% foliar); T8 = RD + FYM + ZnSO₄.H₂O (400 g plant⁻¹) + Borax (200 g plant⁻¹) + CuSO₄.5H₂O (200 g plant⁻¹); T9 = RD + FYM + ZnSO₄.H₂O (0.8% foliar) + H₃BO₃ (0.8% foliar) + CuSO₄.5H₂O (0.5% foliar) at selected locations. Means followed by a similar letter are not statistically different.

3.7. The Overall Comparison of Treatments on Disease Severity and Yield at All Sites

The comparison of various treatments for the management of MSD in terms of disease severity and yield are shown in Table 2. All the treatments were significantly different as compared to the control at $p \leq 0.05$. The treatments T7 (NPK + FYM + ZnSO₄.H₂O + H₃BO₃ foliar), T8 (NPK + FYM + ZnSO₄.H₂O + Borax + CuSO₄.5H₂O soil), and T9 (ZnSO₄.H₂O + H₃BO₃ + CuSO₄.H₂O foliar) appeared to be the best and statistically at par for the management of MSD. Whereas the disease severity on T2, T3, and T4 statistically remained the same, i.e., these treatments were equally effective for the management of disease as compared to the control. Thus, the application of all three micronutrients both in soil and as foliar significantly reduced the disease compared to other treatments where these nutrients were applied alone or with a combination of the two. The maximum mean yield was calculated from the plants treated with all three micronutrients as foliar application

along with the other recommended doses of NPK and FYM. Following the T9 (ZnSO₄.H₂O + H₃BO₃ + CuSO₄.H₂O foliar) treatment, T8 (NPK + FYM+ ZnSO₄.H₂O+ Borax + CuSO₄.5H₂O) soil and T7 (NPK + FYM+ ZnSO₄.H₂O+ H₃BO₃ foliar) appeared to be the best treatments regarding the yield. The mango yield was statistically the same after the application of treatments T2 (NPK + FYM ZnSO₄.H₂O soil), T3 (NPK + FYM + ZnSO₄.H₂O foliar), T4 (NPK + FYM + Borax2 soil), and T5 (NPK + FYM + H₃BO₃ foliar). The minimum disease was observed as the result of the treatment T9. The fruit yield of the plants was also maximum when treatment T9 (ZnSO₄.H₂O + H₃BO₃ + CuSO₄.H₂O) foliar was applied, which ultimately showed 0% loss of the yield. The yield losses were reduced up to 7% as the result of micronutrients applications, whereas 90% yield losses were calculated from diseased plants without any application (Table 2).

Table 2. Comparison of treatment means for disease severity, yield, and yield loss %.

Treatments	DS (%)	Yield (kg)	Yield Loss (%)
T0 (control)	34.72 a	12.19 g	90.47
T1 (NPK + FYM)	16.55 b	89.64 f	29.96
T2 (NPK + FYM + ZnSO ₄ .H ₂ O) soil	12.72 c	96.64 e	24.5
T3 (NPK + FYM + ZnSO ₄ .H ₂ O) foliar	11.86 c	99.61 e	22.17
T4 (NPK + FYM + Borax) soil	11.47 c	97.72 e	23.65
T5 (NPK + FYM + H ₃ BO ₃) foliar	8.10 de	99.31 e	22.41
T6 (NPK + FYM + ZnSO ₄ .H ₂ O + Borax) soil	9.71 d	106.31 d	16.94
T7 (NPK + FYM + ZnSO ₄ .H ₂ O + H ₃ BO ₃) foliar	7.86 e	112.55 c	12.07
T8 (NPK + FYM + ZnSO ₄ .H ₂ O + Borax + CuSO ₄ . 5H ₂ O) soil	7.77 e	118.70 b	7.26
T9 (NPK + FYM + ZnSO ₄ .H ₂ O + H ₃ BO ₃ + CuSO ₄ . 5H ₂ O) foliar	6.72 e	128.00 a	0.00

Means with the same letter are not significantly different at (LSD test, $p < 0.05$), DS = disease severity.

3.8. Analysis of Zn, B, and Cu in Plant Leaves

The data regarding the analysis of Zn, Cu, and B in the leaves of mango plants from four different orchards where various treatments were applied for the management of MSD revealed that there was an increased level of micronutrients in the leaves from foliar treatments (Table 3), compared to the treatments where micronutrients were applied in the soil (Table 4). This shows that micronutrients are easily available to plant in foliar form as compared to soil application. When compared with disease severity and yield data (Table 2), it shows that when the amount of Zn, B, and Cu was higher, there was minimum disease severity and maximum yield, i.e., treatment T9 (NPK + FYM + ZnSO₄.H₂O + H₃BO₃ + CuSO₄. 5H₂O), while in T0 (control), the micronutrients amount was very low, which resulted in low fruit yield and high disease severity (Table 3).

Table 3. Comparison of the different treatments for Zn, B, and Cu in plant leaves.

List of the Treatments	Zn (mg/kg)	B (mg/kg)	Cu (mg/kg)
T0 (control)	2.16 h	2.16g	2.16 h
T1 (NPK + FYM)	2.53 gh	2.50fg	2.55 gh
T2 (NPK + FYM + ZnSO ₄ .H ₂ O) soil	3.50 f	3.54e	2.83 fgh
T3 (NPK + FYM + ZnSO ₄ .H ₂ O) foliar	18.18 d	2.70 efg	2.17 h
T4 (NPK + FYM + Borax) soil	2.81 fgh	2.87efg	3.50 f
T5 (NPK + FYM + H ₃ BO ₃) foliar	24.39 b	17.21b	2.53 gh
T6 (NPK + FYM + ZnSO ₄ .H ₂ O + Borax) soil	3.91 efg	3.11ef	3.21 fg
T7 (NPK + FYM + ZnSO ₄ .H ₂ O + H ₃ BO ₃) foliar	25.28 a	16.49c	2.98 fgh
T8 (NPK + FYM + ZnSO ₄ .H ₂ O + Borax + CuSO ₄ .5H ₂ O) soil	10.74 e	14.33d	8.12 e
T9 (NPK + FYM + ZnSO ₄ .H ₂ O + Borax + CuSO ₄ .5H ₂ O) foliar	21.40 c	22.51a	14.59 a

Means with the same letter are not significantly different at LSD test, $p < 0.05$, DS = disease severity.

Table 4. Comparison of different treatments for Zn, B, and Cu in the soil.

List of the Treatments	Zn (mg/kg)	B (mg/kg)	Cu (mg/kg)
T0 (control)	0.25 e	0.23 g	0.2 f
T1 (NPK + FYM)	0.27 de	0.26 gf	0.32 de
T2 (NPK + FYM+ ZnSO ₄ .H ₂ O) soil	0.53 b	0.30 de	0.30 ef
T3 (NPK + FYM+ ZnSO ₄ .H ₂ O) foliar	0.28 d	0.29 ef	0.33 d
T4 (NPK + FYM + Borax) soil	0.31 c	0.53 b	0.36 bc
T5 (NPK + FYM + H ₃ BO ₃) foliar	0.33 c	0.34 c	0.36 c
T6 (NPK + FYM+ ZnSO ₄ .H ₂ O + Borax) soil	0.56 a	0.56 a	0.38 b
T7 (NPK + FYM+ ZnSO ₄ .H ₂ O+ H ₃ BO ₃) foliar	0.34 c	0.33 cd	0.36 bc
T8(NPK + FYM + ZnSO ₄ .H ₂ O + Borax+ CuSO ₄ .5H ₂ O) soil	0.57 a	0.58 a	0.48 a
T9 (NPK + FYM+ ZnSO ₄ .H ₂ O+ Borax + CuSO ₄ .5H ₂ O) foliar	0.32 c	0.31 cde	0.38 bc

Means with the same letter are not significantly different at LSD = $p < 0.05$.

3.9. Analysis of Zn, B, and Cu in the Soil

The analysis of Zn, Cu, and B from the soil of four various sites where various treatments were applied showed significant responses (Table 4). If we compare them with the disease severity and yield, it shows that where the amount of Zn, B, and Cu was higher, there was minimum disease severity and maximum yield as mentioned in Table 2 while in T0 (control), the micronutrients amount was a lot less and, as a result, the yield was low and the disease severity was higher. This shows that micronutrients play an important role in MSD. These results also showed that the combined application of Zn, B, and Cu play a role in suppressing the disease and increasing the yield (Table 4).

4. Discussion

Mango is the most important fruit crop of Pakistan and MSD is among one of the main causes behind the low production of mango. The disease is characterized by the sudden collapse of severely affected mango trees. This study observed the impact of micronutrients on this disease. Four experimental sites on the fields of farmers were selected. Symptoms of MSD disease were observed on different parts of the trees, including leaves, the bark of the stem, main trunk, and on the roots, which was found to be common among all the affected trees across four experimental areas. At the early stage of the disease infection, affected leaves on smaller branches became necrotic and gradually progressed to the main branches where gum exudation from bark of main branches was observed [7–12,31].

Subsequently, profuse gum exudation occurs, and this was followed by splitting or cracking of the bark. A related study by Ref. [32] also revealed symptoms of mango tree decline disease to include blight, canker, and gummosis, twig blight, tip die-back and stem bleeding. It was observed during the study that on severely affected trees, gum exudates led to rotten canker and, in the most severe form, the disease caused wilting and defoliation of entire tree leaves. The vascular system was observed to be discoloured upon a longitudinal division of the branches. This observation agrees with the findings of Refs. [32,33], who reported that leaves of trees affected by MSD include defoliation, vascular discoloration, and marginal chlorosis.

The application of nutrition as per micronutrients recommended doses showed a tremendous effect to mitigate MSD. The results revealed that disease severity was minimum on the treatment T9 (NPK + FYM + ZnSO₄.H₂O + Borax) and T8 (NPK + FYM + ZnSO₄.H₂O + Borax), where all these three micronutrients Zn, Cu, and, B were applied as foliar and in the soil. The amount of micronutrients was also found to be higher in the leaves and soil of both treatment types. Furthermore, increased fruit yield was also observed in these two treatments. The role of micronutrients in suppressing diseases is evident from this study. Based on these results, one can conclude that micronutrients are also important for better plant growth, minimizing the infection threat, and for good fruit yield. The foliar application of micronutrients increased the capability of plants to uptake the nutrients. The

nutritional deficiencies of the mango plant decreased and, consequently, the fruit yield and fruit weight improved [34,35].

Zn and B are the most important micronutrients for the best quality of fruit and a good yield. The experimental treatments in which Zn and B were applied in combination in the form of foliar application showed better results as compared to soil application. These findings are supported by the experiment of Ref. [36], who reported that the foliar application of B resulted in good yield and fruit quality. The involvement of micronutrients Zn, Cu, and Mg is evident in various important phenomena such as the setting of fruit, retention, and decrease in fruit drops as well as the growth and development of fruits with improved fruit weight, which surely increased the total yield of the fruit [20,37]. Likewise, foliar sprays of calcium nitrate, boric acid, and zinc sulphate have also improved the fruit yield of guava [38]. Similarly, Ref. [39] found that foliar application of urea, ZnSO_4 , magnesium sulphate, and growth regulators significantly increased the fruit yield of ber.

It was observed that the application of Zn, B, and Cu individually or combined increased the fruit yield compared to the control, in which we applied only the FYM and NPK recommended doses. This was proved by an increase in the yield of apples by foliar sprays of zinc [40] and pistachio [41,42] and a highly positive correlation between the concentration of zinc in leaves and pistachio yield [41]. An almost two-fold increase in maize seed yield per plant was observed [43]. The application of micronutrients individually or combined was involved directly or indirectly with different physiological processes and various enzymatic activities. This may be responsible for better photosynthesis and greater accumulation of starch in the fruits [43].

The significant increase in fruit yield is the result of an increase in the number of fruits due to a reduction of fruit drop, which occurred due to the foliar application of macro- and micronutrients including RDF, which may influence various physiological processes resulting in higher fruit set and in the production of mango. Similar results in mango and kokum were observed by Refs [36,44–47].

The role of micronutrients in mitigating diseases and various physiological disorders is very important, as in the present study it is observed that a balanced and judicious use of micronutrients along macronutrients showed a decrease in disease severity. A 17% decrease was observed in our treatment T9, in which Zn, Cu, and B were applied in combination. Our results are in agreement with the findings of Ref. [16], who showed that the combined application of zinc + copper + humic acid + NP reduced the disease severity up to 30.69% in mango.

The basic and most important aim of the present study was to develop the most effective and least harmful method of plant and environment beneficial disease management through micronutrient application in mango orchards. The result showed that the maximum reduction in disease severity was 17%, which was recorded in treatment T9 ($\text{ZnSO}_4 \cdot \text{H}_2\text{O} + \text{CuSO}_4 \cdot 5\text{H}_2\text{O} + \text{H}_3\text{BO}_3 + \text{NPK}$ and FYM). Zn was used as a defence tool against the management and control of the fungal pathogen in plants. Zn is involved in activating enzymes in various metabolic pathways and enhances the integrity and stability of the cell membrane [48,49].

Zn deficiency leads to increased membrane leakage of low-molecular-weight compounds that provide feeding substrate for the pathogens [48–51]. For example, leakage of sugars due to Zn deficiency on the leaf surface of *Heveabra siliensis* increases the infection of *Oidium* [52]. Similarly, Zn application enhanced the tolerance in wheat to *Fusarium solani* root rot [53]. The phenolic contents of the plant were found to increase with the application of Zn, which reduced the rice sheath blight disease severity [54,55]. Zn also reduced the infection of crown root rot disease in wheat [54–56]. The disease severity of *Macrophomina phaseolina* was reduced by the application of Zn [57].

Ref. [58] found that soil application with Zn at 20 mg kg^{-1} played a pivotal role in the defence mechanism in cluster bean seedlings against the invasion of *Rhizoctonia* root rot by increasing the activity of antioxidative enzymes. Many fungal diseases such as head scab, leaf spot disease, root rot, mildews such as powdery mildew, foot rot, and wilt diseases,

etc., could be managed by the proper application of Zn. It is also noted that in some of the diseases such as leaf rust, mildews, blast, ergot, leaf rust, and wilt diseases, the role of Cu is significant.

B promotes rigidity of the cell wall and thus supports the shape and strength of the plant cell. Furthermore, B is involved in the permeability and integrity of the plasma membrane [48–60]. Infection with powdery mildew of wheat is increased several times more in B deficient than in B-sufficient wheat plants [61,62], which may be due to the increased leakage through the plasma membrane. Further, B significantly affects the number of lesions per leaf during booting and milking stages [51]. In culture medium of *Botrytis cinerea*, B inhibits spore germination, germ tube elongation, and mycelial spread [63]. Application of Cu and B reduces infestation of fungal disease in MR219 rice cultivar and also increases rice yield [64]. Boric acid (1%) can reduce the mycelial growth and infection of *Penicillium digitatum* [65].

Cu is known to have antimicrobial activities long before as it is part of many enzymes (polyphenol oxidase, diamine oxidase, etc.) and is involved in the synthesis of lignin, which gives strength and rigidity to the cell wall [12,60]. Reduced lignification in plants is observed due to low Cu, which is related to increased disease incidence. Cu deficiency modifies the lipid structure, which is essential for the resistance against biotic stress [60]. Diseases such as take-all root rot, stem melanosis, and ergot can occur in Cu-deficient small grains [12]. Cu application in soil depresses leaf infections in wheat by powdery mildew and ergot [66]. Ref. [67] also noted the same results that the combined application of CuSO_4 and ZnSO_4 reduced the risk of plant diseases and showed better results in enhancing the yield of plants.

So, there is a minimum chance of increasing the disease incidence and severity in these cases. Our results resemble the findings of Refs. [51,68–71], who concluded that the combined application of nutrients in a sustainable way has a significant impact against mango diseases. Therefore, for the integrated management of diseases, we cannot ignore the role of micronutrients when only using fungicides for the control of diseases.

5. Conclusions

MSD is characterized by the collapse of the vascular system due to *Ceratocystis fimbriata* and *Lasioidiplodia theobromae*. The role of micronutrient deficiencies is an area that is heavily ignored area by farmers; hence, different combinations of micronutrients, i.e., Zn, B, and Cu in the form of ZnSO_4 , H_3BO_3 , and CuSO_4 applied as foliar and as in drench as per the recommended doses of NPK and FYM gave an extraordinary result. The application of 0₃ micronutrients either in soil drench or in foliar form significantly decreased the disease severity and increased the yield. The application of ZnSO_4 (0.8%) + H_3BO_3 (0.8%) + CuSO_4 (0.5%) and as soil drench of ZnSO_4 (400 g) + Borax (200 g) + CuSO_4 200 g plant^{−1} proved to be best treatments, with an average of 12.88 and 14.03% reduction in disease severity.

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