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Zinc (Zn) and Iron (Fe) Fertilization for Improving the Antioxidant Enzyme Activity and Biochemical Constituents in Capsicum Hybrids

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Micronutrients, particularly Iron (Fe) and Zinc (Zn), play a vital role in the growth and development of plants due to their catalytic effect on many metabolic processes. However, the biochemical responses to the applied micronutrients vary with cultivars and their species. A screening experiment was conducted during 2020 to know the antioxidants enzyme activities and biochemical constituents in response to iron and zinc fertilization by six capsicum hybrids grown in grow bags under shade net conditions. The experiment consists of three treatments *viz.,* Control (No Fe & Zn), 50 kg $FeSO_4$ and 37.5 kg $ZnSO_4$ ha^{-1} as a basal soil application with six capsicum hybrids *viz.,* Indra, Priyanka, Inspiration, Massilia, Bachata, and Local green. Leaf samples of the capsicum hybrids were collected at Fruiting stage and analysed for antioxidant enzyme activities. The fruit samples were used for quantifying the biochemical constituents. The results revealed that, application of ferrous sulphate ($FeSO_4$) and zinc sulphate ($ZnSO_4$) to capsicum hybrids increased

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the biochemical constituents in fruits and the antioxidant enzyme activities in leaves. Out of the six hybrids tested, Indra possessed higher ascorbic acid content (9.20 mg 100 g^{-1} fresh weight), acidity (6.0), and total soluble solids (6.1^0 Brix) in the fruits, which was followed by Inspiration and Bachata. The superoxide dismutase (6.70 unit's mg^{-1} protein) and peroxidase (6.90 unit's g^{-1} fresh weight) activities were also higher with the same genotypes. The biochemical constituents and antioxidant enzyme response to Zn addition was better than Fe. There was 13.2, 10.9 and 9.5 per cent increase in titratable acidity, total soluble solids, ascorbic acid content in the fruits of Indra due to $ZnSO_4$ application.The Principal Component Analysis (PCA) and hierarchical clustering revealed that Indra is highly responsive to Zn and Fe fertilization, while the local green showed very less response. The rest of the genotypes such as Inspiration, Bachata, Massilia, and Priyanka, are medium responsive for Zn and Fe fertilization.

Keywords: Biochemical constituents; antioxidant enzyme activities; capsicum hybrids; Fe/Zn fertilization; Principal Component Analysis (PCA).

1. INTRODUCTION

Capsicum is one of the most popular vegetables commercially grown under protected cultivation because of its adaptability in different protected structures. Due to its qualitative and quantitative advantages, a hike in demand increased the farmer's interest in cultivating this crop under a protected structure [1]. It is grown on a large scale as an off-season vegetable crop. Protected cultivation of Capsicum for fresh vegetable marketing and processing has recently gained economic importance [2]. Capsicum hybrids are a rich source of vitamin C (ascorbic acid); they also contain antioxidants with important health-related implications [3]. The levels of these compounds are modified by growing conditions and nutrient application and depend on the genotype and fruit maturity [2]. Fertilizers and irrigation are the basic requirements for increasing the fruit yield of capsicum. Capsicum responds well to fertilizer applications and is reported to have a high demand for major nutrients (NPK). Micronutrients are usually required in minute quantities but essential for various activities; particularly iron (Fe) and Zinc (Zn) play vital roles in the growth and development of plants due to their catalytic effect on many metabolic processes. Improvement in growth characters due to the application of micronutrients such as zinc and iron may be due to enhanced photosynthetic and other metabolic activity, leading to an increase in various plant metabolites responsible for cell division and elongation [4]. Iron is an essential element for almost all living organisms as it participates in oxygen transport, DNA synthesis, and electron transport. Many enzymes require iron as a co-factor for their function, which is involved in oxidative phosphorylation, a metabolic pathway that converts nutrients into energy [5]. In plants, iron is involved in the synthesis of chlorophyll and is essential for maintaining chloroplast structure and function [6]. Iron (Fe) also affects fruit quality parameters such as colour, firmness, acidity and affects fruit production, dropping the number of fruits per plants, fruit size, and yield [7]. Likewise, Zn activates the electrophile and nucleophiles as a component of plant carbonic anhydrase and many other photosynthetic enzymes, which influences the photosynthetic efficiency, chlorophyll structure and content. It is also involved in sucrose and starch formation, protein metabolism, membrane integrity, auxin metabolism, defense mechanism, flowering, and seed production of crop plants [8]. The current study showed that, soils with deficient Zn and Fe could increase the yield by the addition of Zn and Fe fertilizers [9]. It also increases the plant height, number of side branches, and leaf area [10]. Singh et al., [11] found that zinc application at 0.6% was most effective in increasing the total soluble solids and ascorbic acid content. Meena et al., [12] observed that, application of ferrous sulphate and borax at (0.6%) improved the fruit setting time, increased the total soluble solids, total sugar and decreased the acidity in fruits. Hasani, et al., [13] examined the response of capsicum for the foliar spraying of micronutrients on fruit yield and quality and reported that, foliar sprays had positive and significant effects on the fruit yield, total soluble solids, weight of fruit, juice content, anthocyanin index, fruit diameter, and leaf area. Increased fruit yield and quality as well as Nitrogen (N), Phosphorus (P), Potassium (K) intake [14], and water use efficiency [15] were also reported with the foliar spraying of Zn and salicylic acid. The role of these nutrients in various plant metabolic processes was studied by several authors such as Tamilselviet al., [16]; Hatwaret al., [17] and Shaheenet al., [18] and the results revealed that application of micronutrients as foliar spray caused an improvement in plant growth, fruit yield and its physical and chemical properties of fruits. In the same way, Bhatt et al. [19] studied the effect of foliar application of micronutrients and reported significant improvement in yield, which might be attributed to the increased photosynthetic activity and production and accumulation of carbohydrates. Also, Malawadiet al. [20] studied the effect of soil application of micronutrients on the yield and quality of capsicum and noticed higher fruit weight, yield, and maximum ascorbic acid content. Likewise, Batraet al. [21] and Savitha [22] reported that, foliar application of iron after transplanting resulted in a significant improvement in the ascorbic acid content in fruits due to increased activity of ascorbic acid oxidizing enzyme. Regarding the major quality parameters, Tamilselvi et al., [13] observed maximum total soluble solids (TSS), acidity, and ascorbic acid contents in the fruits with micronutrients application. From the literature, it was perused that, different forms and levels of Zn and Fe give differential responses to plants' physiology [23], particularly the biochemical responses which vary with cultivars. Hence the present investigation was carried out, to understand the differential biochemical responses of capsicum hybrids in improving fruit biochemical constituents and leaf antioxidant enzyme activities due to zinc and iron fertilization.

2. MATERIALS AND METHODS

2.1 Experimental Details

A screening experiment was conducted to understand the antioxidant enzyme activity and biochemical responses of six capsicum hybrids to iron and zinc fertilization grown in grow bags under shade net condition in the farmer's field (110 48' 15.8" N 770 59' 25.3" E) at Thalavadi, Erode district. The experiment consists of three treatments *viz.,* control (No Zn &Fe), 50 kg $FeSO_4$ and 37.5 kg $ZnSO_4$ application ha^{-1} and six capsicum hybrids *viz.,* Indra, Priyanka, Inspiration, Massilia, Bachata, and local green. The experiment was laid out in a Randomised Block Design with three replications. Recommended fertilizer nutrients such as Nitrogen, Phosphorus, and Potassium were applied as per soil test recommendation. About 45 days old seedlings of all the capsicum hybrids were transplanted in each grow bag, and the treatments were imposed. Necessary plant protection measures were carried out as and when needed. The antioxidant enzyme activity in plants and biochemical changes in the fruits of capsicum hybrids for Zn and Fe fertilization was determined and reported.

2.2 Physico-chemical Properties of the Experimental Soil

The experimental soil was sandy loam in texture with neutral pH (7.42) and lesser electrical conductivity (0.33 dS m^{-1}). The organic carbon content of the soil was low (0.40%) and non-calcareous in nature (2.50%). The soil had low available nitrogen (157 kg ha^{-1}), low available phosphorus (12.0 kg ha^{-1}), and medium available potassium (280 kg ha^{-1}) status. Regarding the available micronutrient status, zinc was deficient (0.68 mg g^{-1}), and other micronutrients were sufficient (6.50, 3.48 1.00, and 0.52 mg kg^{-1} for iron, manganese, copper, and boron, respectively) in the soils.

2.3 Estimation of Biochemical Constituents and Antioxidant Enzyme Activities

The fruit's biochemical constituents, such as acidity, total soluble solids (TSS), and ascorbic acid content were determined after the harvest. The acidity in the fruit juice was estimated by titration with a standard alkali using phenolphthalein as an indicator. From the homogenized fruit juice, 25 ml was taken and titrated against 0.1 N potassium hydroxide in the presence of phenolphthalein indicator till a permanent pink color is obtained. The result was expressed in citric acid per 100 g of fruit sample [24]. Total soluble solids (TSS) content was determined by means of hand held Refractometer, which was calibrated at 20^0C and a few drops of fruit juice sample was placed in between the prisms and read at the demarcation line and expressed as Brix [24]. Ascorbic acid content was estimated by titration with an oxidizing agent viz., indophenol dye, and expressed in mg per 100 gram of fresh weight of fruit sample [25].

The Zn and Fe requiring enzymes in the leave samples at fruiting stage were determined to understand the role of applied Zn and Fe in plant nutrition. Peroxidase activity was estimated using leaf samples homogenized in phosphate buffer. Then, one mL of supernatant was taken and to this 3 mL of 0.05 M pyrogallol and 0.5 mL of 30% H_2O_2 were added. The change in absorbance was measured at 430 nm for every 30 seconds up to 180 seconds. The enzyme activity was calculated and expressed as Units min^{-1} mg^{-1} fresh weight of leaf sample [25]. The superoxide dismutase activity was measured using the Nitro blue tetrazolium (NBT) method [26]. Five hundred milligram of leaf sample was macerated using 10 ml HEPES-KOH buffer containing 0.1mM EDTA and centrifuged at 15000 rpm for 15 min. The supernatant was collected and made up to 50 ml volume. One ml of the enzyme extract was mixed with 3 ml of the reaction mixture, and the absorbance was recorded at 560 nm. One unit of SOD activity was defined as the amount of enzyme required for 50% inhibition of NBT activity at 560 nm. The result was expressed in units per gram of fresh leaf weight.

2.4 Statistical Analysis

The data obtained from the study were subjected to the analysis of variance to find out the significance as suggested by Panse and Sukhatme [27] using SPSS software. Principal component analysis and hierarchical clustering were plotted using R studio software [28].

3. RESULTS AND DISCUSSION

3.1 Titrable acidity

The characteristic flavour of each horticultural product must not only be associated with the presence of sugars such as sucrose, glucose, galactose, and fructose [29] but also due to the accumulation of some organic acids (citrate and malate) [30]. The acidity data obtained for the fruits expressed as the prevailing organic acid function are shown in Fig. 1. The titrable acidity of the capsicum hybrids increased with zinc (Zn) and iron (Fe) fertilization regardless of capsicum hybrids. The hybrid Indra exhibited higher fruit acidity (6.00, 5.80% citric acid) followed by Inspiration (5.80, 5.60% citric acid) and Bachata (5.70, 5.50% citric acid), respectively, with $ZnSO_4$ and $FeSO_4$ application. The increased acidity in the fruit might be due to the metabolic transformation of sugar into organic acids by the addition of Zn and Fe [31].

Lesser sugar conversion into organic acid was observed with the local green (5.0 % citric acid) at no Zn and Fe application. It was reported that, application of non-chelated forms of micronutrients (Fe + Zn) produced higher acidic fruits compared to chelated forms [32]. However, the results of the present study are similar to the findings of Dhotra *et al*., [33], who reported that plants sprayed with $ZnSO_4$ and $FeSO_4$ produced more acidic fruits than control.

3.2 Total Soluble Solid (TSS)

The accumulation of solutes during the maturation process is one of the parameters with greater precision and reliability when used as a harvest index for fruits and vegetables [34]. Its rapid determination, relatively low cost, correlates directly with flavour [27]. The capsicum hybrid Indra had higher TSS (6.10, 5.80 ^{0} Brix) followed by Inspiration (5.90, 5.75 ^{0} Brix) and Bachata (5.80, 5.70 ^{0} Brix) in response to $ZnSO_4$ and $FeSO_4$ application (Fig. 2). Total soluble solids were found to be higher with the $ZnSO_4$ application than $FeSO_4$ with a mean of 5.77 and 5.90 ^{0} Brix, respectively. Lesser TSS content in the fruit was noticed with local green (5.10 ^{0} Brix) in no Zn and Fe applied control. It was reported that TSS of the fruit is an important quality parameter [35], which could be increased with Zn and Fe application [36]. This results in increasing photosynthesis activity, translocation of sugars from source to sink and conversion of sugars from the complex form (polysaccharides) to simple form (glucose and fructose) in fruits [37].

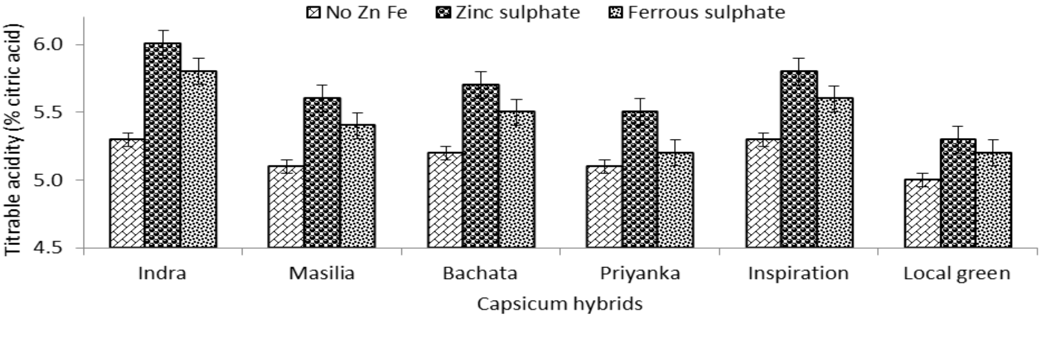

Fig.1. Changes in titrable acidity in fruits of different capsicum hybrids due to Zn and Fe fertilization (Error bars represents standard error n=3)

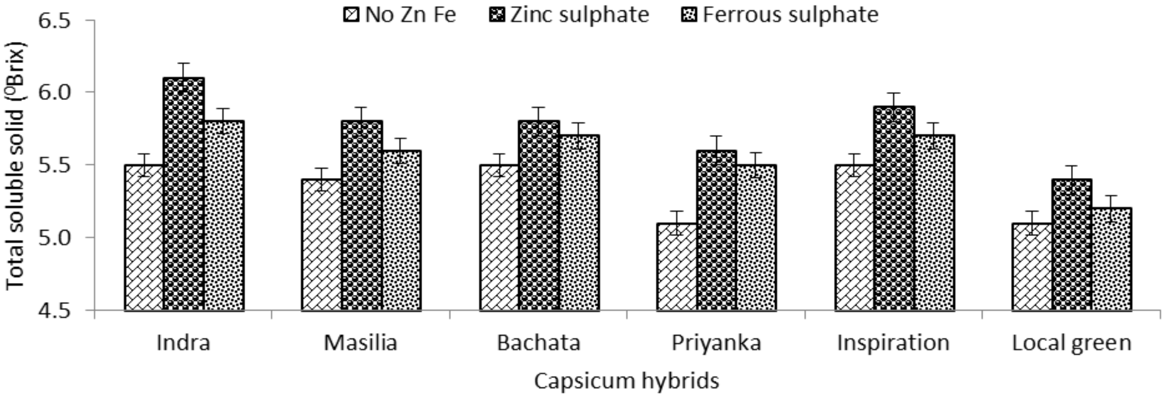

Fig. 2. Changes in total soluble solid content in fruits of different capsicum hybrids due to Zn and Fe fertilization (Error bars represents standard error n=3)

3.3 Ascorbic Acid Content

The quality of capsicum fruit is defined mainly by the concentration of vitamin C, acidity, soluble solids, and carotenoids. The former being valuable, particularly in the human body, makes it essential to evaluate fruit quality assessment [38]. Ascorbic acid content is highly correlated with antioxidant activity and application of micronutrients also significantly affects its availability in fruits [39]. Among the capsicum hybrids, the fruits of Indra contains higher ascorbic acid (9.20, 8.60 mg 100 g^{-1} fresh weight) followed by Inspiration (8.70, 8.60 mg 100 g^{-1} fresh weight) and Bachata (8.70, 8.50 mg 100 g^{-1} fresh weight) respectively with $ZnSO_4$ and $FeSO_4$ application (Fig. 3). The improvement in ascorbic acid content with Zn application was greater than Fe with a mean of 8.70 and 8.47 mg 100 g^{-1} fresh weight, respectively. Lesser ascorbic acid content was noticed with local green (8.20 mg 100 g^{-1} fresh weight) at no Zn and Fe applied control. Higher sugar levels with micronutrients might be the possible cause for increased ascorbic acid content, which is synthesized from sugar [40]. Zinc plays an active role in the synthesis of Auxins, and increased synthesis of Auxins has been reported to enhance the accumulation of ascorbic acid content. Hence, the increased ascorbic acid content with the application of zinc might be due to the higher synthesis of auxin [41]. Batra *et al.* [21] reported that foliar application of iron at 40, 50, and 60 days after transplantation significantly improved the ascorbic acid content of tomato fruits. The most probable increase in ascorbic acid content might be due to the increased activity of ascorbic acid oxidase enzyme [22].

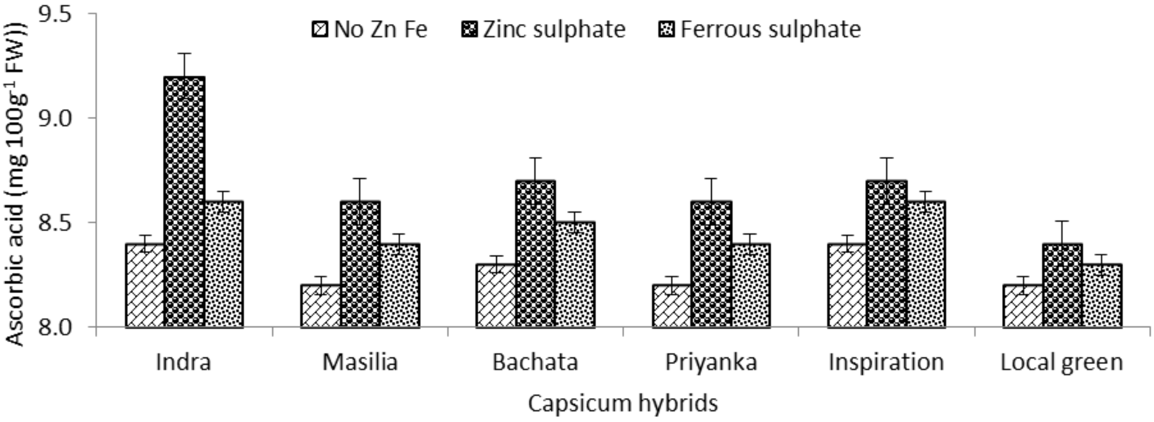

Fig. 3. Changes in ascorbic acid content in the fruits of different capsicum hybrids due to Zn and Fe fertilization (Error bars represents standard error n=3)

3.4 Superoxide Dismutase Activity (SOD)

The generation of reactive oxygen species such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide causes oxidative damage to plants. Plants with high levels of antioxidants, either constitutive or induced, have been reported to have greater resistance to oxidative damage. Plants have evolved specific protective mechanisms involving antioxidant molecules and enzymes to defend against oxidants [42]. The application of iron and zinc increased the superoxide dismutase activity in the leaves of all capsicum hybrids (Fig. 4). All the genotypes exhibited higher superoxide dismutase activity with Zn application than Fe (6.70 to 6.30 Units g^{-1}) and the highest superoxide dismutase activity was exhibited by Indra (6.90, 6.70 Units g^{-1}), followed by Inspiration and Bachata. Lesser SOD activity was measured with Local green hybrid at no Zn, and Fe applied control (5.10 Units g^{-1}).Zinc has an essential role on enzymes including Cu/Zn-SOD [43], and the enhanced enzyme activity in the present study is due to involvement of Zn as a co-factor for the functioning of SOD.

Moreover, activities of SOD depend on the availability of Zn from soil which differs among the genotypes [44]. Several studies reported an 89% correlation with the increased SOD activity in various plant species due to Zn fertilization [45]. Kaya *et al.* [46] said that pepper plants (*Capsicum annum* L.) treated with high Zn concentration decreased H_2O_2 concentrations and MDA and stimulated the SOD activity. The lesser activity of SOD was observed in untreated plants [47]. Wu et al., [48] and Sida-Arreola et al., [49] also reported an increase in SOD activity with Zn fertilizer application.

Similarly 80% of the studies shows increased SOD activity[45] by evaluating the physiological effect of Fe in plants.Rout et al. [50]reported that applying Fe at different concentrations (0, 25, 50, 100, and 200 µM of $FeSO_4$) increased the SOD activities to mitigate the oxidative damage caused by ROS in plants.

3.5 Peroxidase Activity (POX)

Peroxidase (POX) enzyme has a protective effect and is involved in chlorophyll degradation. ROS generation and membrane lipid peroxidation which are responsive to injury and a product of senescence [51]. With the application of iron and zinc, peroxidase activity in leaves of capsicum hybrids increased substantially irrespective of genotypes (Fig. 5). The observation also depicted that iron application had a higher impact on peroxidase activity than zinc application with a mean of 6.72 and 6.63 Units min^{-1} g^{-1} Fresh weight, respectively. The higher peroxidase activity was observed with Indra (7.00, 6.90 Units min^{-1} g^{-1} Fresh weight) followed by Inspiration and Bachata. Lesser POX activity was observed with Local green hybrid at no Zn and Fe applied control (6.30 Units min^{-1} g^{-1} Fresh weight). This might be due to the de novo biosynthesis of enzyme by Fe^{+2} activities in response to the presence or absence of Fe in leaves [52,53]. Michael et al. [54] also observed increased POX activity in the plants treated with 50 mg kg^{-1} of Zn. Kaya et al.[46] reported decreased H_2O_2 concentrations and stimulation of enzymatic antioxidants enzyme POD in response to Zn application in pepper plants (*Capsicum annum* L.). Iron (Fe) is a constituent of enzymes associated with the cellular antioxidant system, such as Fe-SOD; hence, plants exposed to Fe application show variation in POD activities [55]. Tewari et al. [56] and Jucoski et al., [57] also reported similar results with theapplication of Fe fertilizers.

3.6 Principal Component Analysis and Hierarchical Clustering

The principal component scatter plots for individuals' capsicum hybrids were studied, and found that the individuals lying closer to each other seemed to be similar based on the variables studied. Indra and Inspiration were most distant from the origin in the direction of variable vectors, making it the most responsive hybrids to Zn and Fe fertilization. Individuals falling far from the origin and opposite the variable vectors were less responsive. Further cluster analysis (Fig. 6) was studied for grouping the capsicum hybrids based on their behaviour. The results revealed that the genotype Indra was a highly responsive hybrid, whereas local green was less responsive to Zn and Fe fertilization. The rest of the genotypes, viz., Inspiration, Bachata, Massilia, and Priyanka were grouped as medium responsive to antioxidant enzyme activity and biochemical changes due to zinc and iron fertilization.

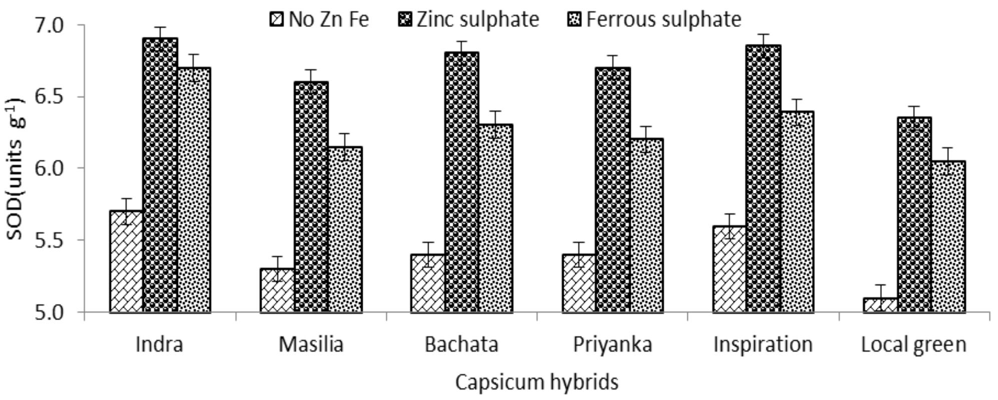

Fig. 4. Changes in leaf superoxide dismutase activity in capsicum hybrids due to Zn and Fe fertilization (Error bars represents standard error n=3)

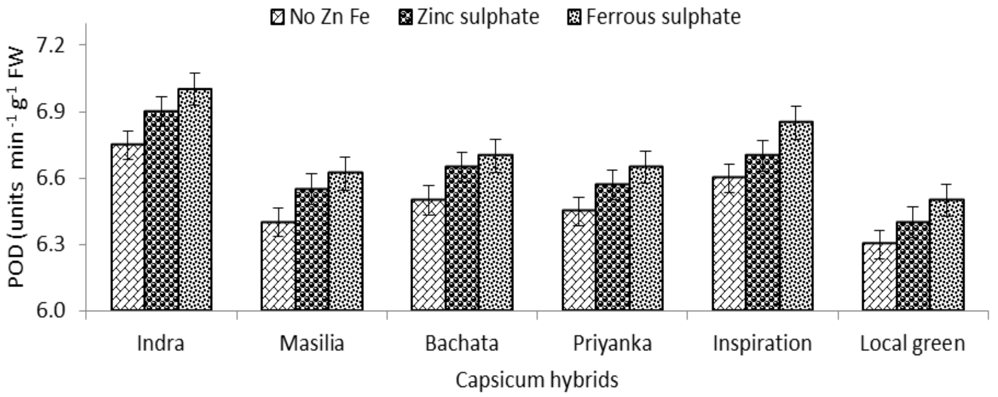

Fig.5. Changes in the leaf peroxidase activity in different capsicum hybrids due to Zn and Fe fertilization (Error bars represents standard error n=3)

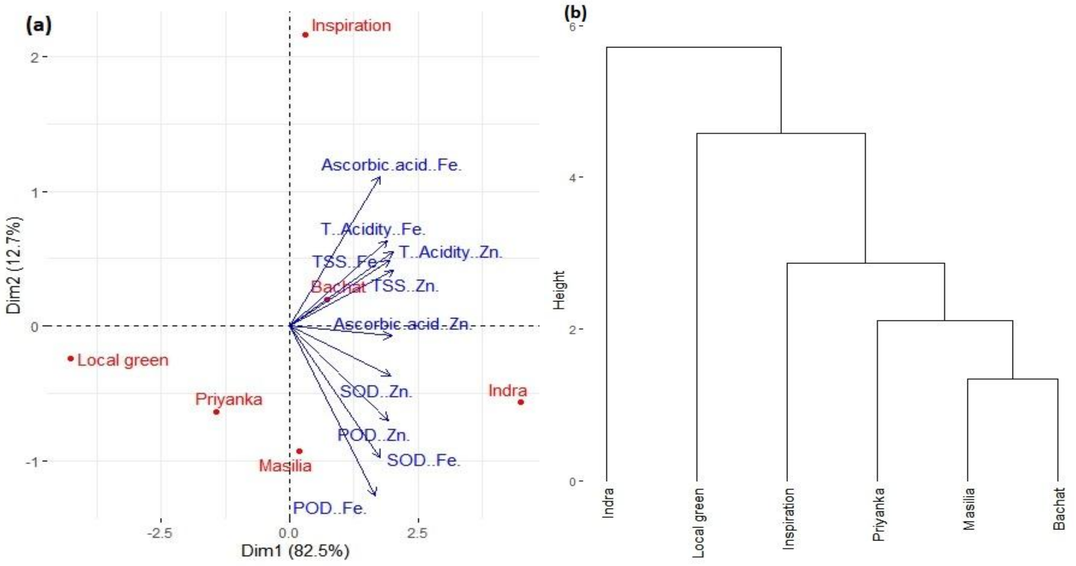

Fig.6. (a) Principal Component Analysis and (b) Hierarchical clustering for grouping the capsicum hybrids based on their antioxidant enzymes and biochemical responses to Zn and Fe fertilization

4. CONCLUSION

It can be concluded from the study that, application of ferrous sulphate and zinc sulphate to capsicum hybrids increased the biochemical constituents in fruits and antioxidant enzyme activities in the leaves. Out of the six capsicum hybrids, higher ascorbic acid content (9.20 mg 100 g^{-1} fresh weight), acidity (6.0% citric acid), and total soluble solids (6.1 ^{0}Brix) in the fruits were observed with Indra followed by Inspiration and Bachata. Lesser biochemical and antioxidant enzyme activity for the Zn and Fe application was registered with local green. The capsicum hybrid Indra was highly responsive based on the principal component analysis and hierarchical clustering. At the same time, Inspiration, Bachata, Massilia, and Priyanka were responsive to the antioxidant enzyme and biochemical changes upon zinc and iron fertilization. However, the local green genotype was lesser responsive to Zn & Fe fertilization, which was evident from the lesser antioxidant enzyme activity in plants and biochemical constituents in fruits.

DISCLAIMER

The products used for this research are commonly and predominantly used in our area of study and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for litigation but knowledge advancement. Also, the research was not funded by the producing company rather, it was financed by the personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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