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# Influence of foliar applications of chelator and micronutrients on antioxidants in green chilli

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**The aim of the present work was to study the influence of foliar applications of chelator [Humic acid: $HA_1$(0%) and $HA_2$(0.05%)] and micronutrients [Zinc: $Zn_1$(0%); $Zn_2$(0.05%) and Boron: $B_1$(0%); $B_2$(0.02%)] on the antioxidant compounds in green mature chilli fruits cv. Bullet (*Capsicum annum* L.), a pot experiment in factorial randomized block design with three replications was conducted in the net house of Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal and India. In this experiment, some non enzymatic antioxidants, enzymatic antioxidants and total antioxidant activity were analyzed. It was also suggested that the ascorbic acid content significantly positively influenced the application of $Zn_2$, $HA_2Zn_2$ and $HA_2B_2$. The free phenol content exerted significantly positive induction by application of $B_2$. The total phenol content significantly increased the application of $HA_2Zn_2$ and $HA_2B_2$. The applications of $HA_2$, $Zn_2$ and $HA_2B_2$ could produce significantly increasing effects in concentration of carotene content. While, the highest values of capsaicin content was obtained by application of $HA_1B_2$. The highest activity of superoxide dismutase, peroxidase and catalase were exhibited by the application of $HA_2B_2$, $HA_1Zn_2$ and $HA_1B_2$, respectively. The highest total antioxidant activity was observed by application of $HA_2B_2$. The carotene and peroxidase activity had a significant positive association with ascorbic acid, total phenol and carotene had a significant positive correlation with superoxide dismutase, and free phenol and capsaicin had a significant positive association with total antioxidant activity. Based on principal component analysis and average values, foliar application of $HA_2B_2$ followed by $HA_2$ and $B_2$ had good performers with respect to all variables, which may bring about the proper value addition in green chilli by enhancing the antioxidant constituents and antioxidant activities.**

**Key words:** *Capsicum annum* L., chelator, micronutrients, antioxidants, enzymes, molybdate reducing antioxidant potential.

## INTRODUCTION

From time immemorial, chilli (*Capsicum annum* L.) has been used as a common vegetable cum spice because of its colour, test, pungency, flavor and aroma. Apart from providing basic nutrition, chilli is well known for its health benefits; antioxidative potential powers in terms of antioxidant constituents and antioxidant activities have

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been reported in green chilli (Bhattacharya et al., 2010; Biswas et al., 2011). Food rich in antioxidants play a role in prevention of cardiovascular diseases and cancers (Garber et al., 2002) and neurodegenerative diseases (Di-Matteo and Esposito, 2003), as well as prevention of inflammation and problems causes by cell and cutaneous aging (Ames et al., 1993).

Humic acids (HAs) are multi-substituted poly-aromatic heterocyclic macromolecules that incorporate proto-catechuic acid, vanillic acid, vanillin, resorcinol, ferulic acid, benzoic acid, and other cyclic polyphenols resulting from the degradation of the lignin in plant cell walls. Humic acid are rich in carboxyl, hydroxyl, and carbonyl groups as well as in phenols, quinones and semi-quinones (Bruneton, 1995; Bravo, 1998; Yoshino, 1998). The stimulatory effects of humic substances have been directly correlated with the enhanced uptake of micronutrients such as Fe, Zn, Cu, and Mn (Chen et al., 1999). The main enzymes involved in the antioxidative defense, such as super oxide dismutase (SOD), catalase, ascorbate peroxidase and peroxidase were also monitored by humic acid (Kesba and El-Beltagi, 2012).

Zinc (Zn) is one of the essential micronutrients playing a significant role in many vital metabolic processes (Rout and Das, 2003; Aravind and Prasad, 2005a, b). An increase in the level of reactive $O_2$ species (ROS) may appear in Zn-deficient plants. Zn deficiency enhances $O_2^{\bullet-}$ generation by enhancing NADPH-dependent oxidase activity (Cakmak, 2000). Moreover, as it is an integral constituent of Cu/Zn super oxide dismutase (Cu/Zn SOD), Zn plays an important role in the detoxification of the $O_2^{\bullet-}$ (Apel and Hirt, 2004). Besides Zn deficiency, there was an increase in the activity of POD as reported by Kosesakal and Unal (2009), while Hajiboland and Amirazad (2010) also reported that under Zn deficiency, there was decrease in the activity of POD and CAT. Excess Zn can also affect the uptake of other nutrient elements. Thus, deficiency of the other elements may cause oxidative stress (del-Rio et al., 1991; Bonnet et al., 2000). These oxygen species are highly reactive and cause the death of plants by damaging membrane lipids, proteins, pigments and nucleic acids (Chaoui et al., 1997; Weckx and Clijsters, 1997; Bonnet et al., 2000; Cuypers et al., 2002).

There is evidence that B is one of the micronutrient responsible for the changes in concentration and a number of metabolic pathways such as carbohydrate metabolism, nitrogen metabolism, phenol metabolism and ascorbate metabolism in plants (Marschner, 1995; Dordas and Brown, 2005; Luaszewski and Blevins, 1996). In fact, it is well known that B deficiency causes an accumulation of phenolics through the stimulation of the enzyme phenylalanine-ammonium lyase (PAL) (Cakmak et al., 1995; Ruiz et al., 1998b; Camacho-Cristobal et al., 2002). Other reports have shown that B deficiency does not only induced quantitative changes but also qualitative changes in the phenolic pool of plants (Camacho-Cristobal et al., 2002; Camacho-Cristobal et al., 2004; Karioti et al., 2006). However, B deprivation also increased the activity of polyphenol oxidase (PPO) (Pfeffer et al., 1998; Camacho-Cristobal et al., 2002) enzyme that catalyses the oxidation of phenolic com-pounds into quinones. Besides yield increase, a relation-ship also exists between vitamin C content and boron treatment in different vegetables like summer squash, beet, tomato and potato (Luaszewski and Blevins, 1996; Govindan, 1950; Mondy and Munshi, 1993).

The present study was to investigate the effects of foliar applications of chelator (humic acid) and micro-nutrients (Zn and B) with respect to level of antioxidant constituents and antioxidant activities of green chilli fruits in order to identify the best treatment that could be used to develop improved quality.

## MATERIALS AND METHODS

### Field experiment

The seedlings that were grown in nursery beds were prepared in a sandy loam soil and were 12 cm tall and 1.0 m wide. Weathered cow dung manure, 4 kg/m$^2$, was mixed into the beds. Beds were drenched with formaldehyde (4%) and covered with polythene sheets for one week to avoid damping off disease. Seedling was treated with Dithane M-45 (2.5 g/kg of seed) (Hindustan Pulverizing Mills Industrial Growth Centre, Sumba, Jammu, India) prior to sowing. Fresh seeds of chilli cv. bullet (*Capsicum annuum* L.) collected from AICRP on Vegetable Crops were sown at 10 mm and 5 cm apart. After sowing, beds were covered with straw until seed germination and hand water regularly. Seedlings were hardened by withholding water four days before transplanting.

### Pot experiment

A pot experiment was conducted in the net house of Bidhan Chandra Krishi Viswavidyalaya (BCKV), Mohanpur, Nadia, West Bengal and India. The 40 days old seedlings were transplanted in earthen pot of 15 cm size (one plant/pot) having a central drainage hole. Soil was prepared by mixing appropriate amount of well rotted cow dung and manures (Soil 700 g/pot, Cow manure 100 g/pot, Urea 5 g/pot, Single super phosphate 20 g/pot, and Muriate of potash 6 g/pot) (Department of Spices and Plantation Crops, BCKV, Mohanpur, Nadia, West Bengal and India). Additional fertilizer 5 g/pot was applied 21 days after transplanting. The experiment consisting of six treatments including control (only tap water sprayed) were arranged in a factorial randomized block design with three replications. The detail treatments are summarized as shown in Table 1.

To prevent blossom and fruit drop, supplementary irrigation was required (hand water was applied at an interval of 1 day with the first being immediately after transplanting). There was no weeding required. The insecticide Rogor at 2.5 ml/L (Rallis India Ltd., Mumbai, Maharashtra, India) was applied three times beginning just from flowering stage and at 15 days intervals to control aphids.

About 400 g edible green mature chilli fruits from each replication were collected. Fresh materials were chopped with a sharp knife into small pieces before analysis of non enzymatic antioxidants (ascorbic acid, phenols, and carotene), enzymatic antioxidants (SOD, POD and CAT) and total antioxidant activity (as MRAP). Samples were shredded and dried at 40°C for 96 h. This material was prepared for capsaicin analyses by grinding to a fine powder using an electric grinder. A subsample of the dried shredded

**Table 1.** Detail doses of treatments which used as foliar spray for green chilli.

| Treatment | | Humic acid (HA) (%) | Zinc (Zn) (%) | Boron (B) (%) |
|---|---|---|---|---|
| Control | $HA_1Zn_1B_1$ | 0.0 | 0.0 | 0.0 |
| HA | $HA_2Zn_1B_1$ | 0.05 | 0.0 | 0.0 |
| Zn | $HA_1Zn_2B_1$ | 0.0 | 0.05 | 0.0 |
| B | $HA_1Zn_1B_2$ | 0.0 | 0.0 | 0.02 |
| HA×Zn | $HA_2Zn_2B_1$ | 0.05 | 0.05 | 0.0 |
| HA×B | $HA_2Zn_1B_2$ | 0.05 | 0.0 | 0.02 |

Source of chelator was humic acid (granular form), that of Zn was $ZnSO_4 \cdot 7H_2O$ and B from $Na_2B_4O_7 \cdot 10H_2O$. Each spraying was done four times with sticker starting from 25 days after transplanting and subsequent ones at an interval of 10 days during vegetative stage.

material was further dried at 100°C to constant dry weight to determine percent moisture.

## Chemical analysis

### *Analysis of ascorbic acid*

One gram of finely chopped green chilli was extracted with 20 ml of 4% oxalic acid following maceration in a pestle and mortar and the material centrifuged for 30 min at 10,000 *g*. Ascorbic acid content was determined using the dichlorophenol indophenol titration procedure (Casanas et al., 2002).

### *Analysis of total phenol and free phenol*

Total phenol was extracted in 50% methanolic 1.2 N HCl and free phenol was extracted in 50% aqueous methanol by boiling 1 g of finely chopped tissue for 1.5 h at 80 to 90°C following the method of Vinson et al. (1995) and subsequent analysis was with the Folin-Ciocalteau reagent using gallic acid as standard.

### *Analysis of carotene*

Carotene was extracted in 50% alcoholic and 10% KOH by boiling 2 g of finely chopped green chilli tissue for 1.5 h at 80 to 90°C followed by centrifugation at 10,000 *g* for 30 min and subsequent analysis was monitored according to the method of Singh and Bradbury (1988). Total carotene was analyzed spectrophotometrically using β-carotene as the standard.

### *Analysis of capsaicin*

Pungency of chilli was determined as capsaicin (phenolic compound) was extracted in 10 ml aqueous ethanol by shaking 0.5 g powdered chilli sample for 1 h at mechanical shaker and centrifuged for 10 min at 10,000 *g*. Tubes containing 1 ml extract, 5 ml of 0.4% NaOH and 3 ml of 3% phosphomolybdic acid were incubated at room temperature for 60 min. Absorbance at 650 nm was recorded according to Sadasivam and Manickam (1992). The values were converted from dry to fresh weight using the moisture content of green chilli fruits.

### *Analysis superoxide dismutase (SOD) activity*

One gram finely chopped bulb tissue extracts was used to determine SOD activity by inhibiting photochemical reduction of nitroblue tetrazolium (NBT) in riboflavin light NBT system (Beauchamp and Fridovich, 1971). Absorbance at 560 nm was recorded and percentage inhibitory activity was calculated as $[(A_0 - Ae)/A_0] \times 100$, where $A_0$ = absorbance without extract and Ae = absorbance with extract.

### *Analysis of peroxidase (POD) activity*

One gram of freshly harvested green chilli pepper tissue from each treatment was macerated in a pestle and mortar, and extracted with 10 ml tris-HCL buffer (pH 7.6) to determine POD activity. Triturated samples were centrifuged at 10,000 *g* for 30 min at 4°C and supernatants were assessed for enzyme activity. The POD was estimated by mixing 0.1 ml chilled enzyme extract with 2.8 ml reaction mixture (0.5% *o*-dianisidine dissolved in methanol, 0.28 ml Na-acetate buffer, 2.4 ml water). The reaction was initiated by adding 0.1 ml $H_2O_2$ (30%). Changes in absorbance at 430 nm were monitored at 1 min intervals for up to 3 min. POD activity was measured using *o*-dianisidine as the substrate (Bhattacharya et al., 2010) and expressed as mM *o*-dianisidine oxidized/min/g of fresh tissue using the extinction coefficient $1.13 \times 10^4$ M/cm.

### *Analysis of catalase (CAT) activity*

An assay mixture of 3 ml of phosphate buffer, 2 ml of $H_2O_2$, and 1 ml of enzyme source were pipette into a porcelain crucible and incubated for 1 min at 20°C to estimate CAT activity. The reaction was stopped with addition of 10 ml of 0.7 N $H_2SO_4$ and the reaction mixture titrated against 0.01 N $KMnO_4$ to determine residual $H_2O_2$ until a faint pink color that persisted for at least 15 s. One unit of CAT activity was defined as the amount of enzyme that destroyed 1 µmol of $H_2O_2$ min/g of fresh tissue. Changes in activity were measured using the method of Kar and Mishra (1976).

### *Analysis of total antioxidant activity*

A 0.5 g freshly chopped green chilli pepper sample was extracted by macerating with 10 ml distilled water for estimation of total antioxidant activity. Tubes containing extract and reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) were incubated at 95°C for 90 min. After incubation, the mixture was cooled to room temperature; absorbance of each solution was measured at 695 nm against a blank (Prieto et al., 1999). Antioxidant capacity was expressed as ascorbic acid equivalent and gallic acid equivalent.

## Statistical analyses

Data were subjected to analysis of variance (ANOVA) of a factorial randomized block design, simple correlation were calculated and tested for significance and means were separated by Duncan's multiple range test. Principal component analysis (PCA), as the method of identifying the factor dimension of the data, was used to summarize the treatment producing in a reduced number of factors for selection of the best performing treatment. Statistical analyses were done using SPSS Professional Statistics ver. 7.5 (SPSS Inc., Irvine, California).

## RESULTS AND DISCUSSION

### Non enzymatic antioxidants

Ascorbic acid is one of the most important hydrophilic antioxidants that scavenge harmful free radicals and other ROS and also regenerate other antioxidants like tocopherol to its functional state. In the present experiment (Table 2), ascorbic acid content is known to show remarkable variation among the treatments. The applications of Zn ($Zn_2$), HA×Zn ($HA_2Zn_2$) and HA×B ($HA_2B_2$) did to show significantly positive influences with ~1.16, ~1.20 and ~1.04 fold, respectively as compared to that of their corresponding values of control ($Zn_1$, $HA_1Zn_1$ and $HA_1B_1$) treatments. The highest value was obtained from application of $HA_1Zn_2$ followed by $Zn_2$, $HA_2Zn_2$, $HA_1B_2$ and $HA_2B_1$, respectively. Results were found to be in agreement with that of Tariq et al. (2007), who reported that foliar application of Zn significantly increased and B application increased but not significantly in the concentration of ascorbic acid in juice of sweet orange as compared to control treatment. Rehman (1992) and Khan et al. (2012) also reported that increase in concentration of ascorbic acid content in citrus fruit due to foliar application of Zn. Regarding, the beneficial effect of Zn (Alloway, 2008) and HA (Muscolo et al., 1999) on production of auxin in plant species, as the production of auxin increases ascorbic acid content in Kinnow mandarin as reported by Nawaz et al. (2008). The higher ascorbic acid content was observed in treatment of $HA_1Zn_2$.

Phenols are ubiquitous photochemical that contribute largely to antioxidant potential of any plant. In the present experiment (Table 2), free (FPC) and total (TPC) phenols were estimated to assess the contents of unconjugated (free) and conjugated phenols in the cv. Bullet of green mature chilli fruits. The free phenol content did not show significantly remarkable effects among the Zn and B applications only, in which it significantly decreased ~1.08 fold in Zn and significantly increased ~1.11 fold in B applications as compared to that of their corresponding control treatments. On the other hand with respect to total phenol content, the just opposite trend did not show results of free phenol content, in which, significant effects showed the application of HA, HA×Zn and HA×B, while the HA application did not show significant negative influence (~1.11 fold) as compared to that of the control treatment. The maximum values of free and total phenol content were obtained by applications of $HA_1B_2$ (~ 1.15 fold) and $HA_2B_2$ (~1.13 fold), respectively.

Concerning the foliar application of Zn is known to elevate the carotene concentration in Irish plant as compared to the control treatment (Khalifa et al., 2011). However, increased carotene concentration by foliar application of Zn was reported by Abd El-Baky et al. (2010) in sweet potato. In the present experiment (Table 2), results showed that, without these particular treatments, that is, B and HA×Zn, all others treatments showed significantly positive influences among the concentration of carotene content, in which HA ~1.33, Zn ~1.22 and HA×B ~1.34 fold better influence than that of their corresponding control treatments. The highest value was observed by application of $HA_2Zn_2$ followed by $HA_2B_1$.

Capsaicin, the phenolic pungent principle of pepper competes with phenol synthesis (Sukrasno and Yeoman, 1993). In the present experiment (Table 2), it was observed that the capsaicin content is known to show remarkable variation among the treatments. Herewith, only B and HA×B applications produced significantly positive influences among the concentration of capsaicin content as compared to that of their corresponding control treatments. The highest value was obtained from application of $HA_1B_2$ (~1.77 fold) followed by $B_2$ (~1.41 fold).

### Enzymatic antioxidants

The level of oxygen free radicals in cells can also be enhanced by a decrease of the enzymatic antioxidants involved in their detoxification such as SOD, POD and CAT. Superoxide dismutase is a key enzyme in protecting cells against oxidative stress. This enzyme catalyses the dismutation of $O_2^{\bullet-}$ to $H_2O_2$ and $O_2$, which is ultimately neutralized to $H_2O$, a safe product by CAT. There are three SODs in plants according to co factors: Mn-SOD, Fe-SOD and Cu/Zn SOD. In the present experiment (Table 2), all the treatments except B produced significantly remarkable effects on the activity of SOD, whereas decreasing $IC_{50}$ value are indicative of increasing SOD activity. The highest activity was observed from application of $HA_2B_2$ (~2.95 fold) followed by $HA_2Zn_2$ (~2.51 fold). However, the stimulatory effects of humic substances have been directly correlated with the enhanced uptake of micronutrients such as Fe, Zn, Cu, and Mn (Chen et al., 1999) which are cofactor of SOD. The highest activity of SOD was obtained by application of $HA_2B_2$.

Regarding POD activity (Table 2), is known to show remarkable variation among the treatments. The applications of Zn ($Zn_2$), HA×Zn ($HA_2Zn_2$) and HA×B ($HA_2B_2$) showed the significantly positive influences with ~1.64, ~2.01 and ~1.10 fold, respectively as compared to that of that of their corresponding values of control ($Zn_1$, $HA_1Zn_1$ and $HA_1B_1$) treatments. The highest activity was obtained from application of $HA_1Zn_2$ followed by $Zn_2$. Moreover, Bybordi and Memadov (2010) reported that Zn foliar application causes increase in POD activity.

Zinc foliar application decreased CAT activity in canola as compared to the control (Bybordi and Mamedov, 2010) treatment. Decreased CAT activity by application of Zn was reported by Ozdener and Aydin (2010) in rocket leaves. In the present study (Table 2), the CAT activity showed significantly remarkable effects among the Zn and B applications only, which significantly decreased ~1.20 fold in Zn and significantly increased

**Table 2.** Influence of foliar application of chelator and micronutrients on antioxidant compounds in fresh green chilli fruits.

| Main effect | AAC (mg/g) | FPC (mg/g) | TPC (mg/g) | Carotene (mg/g) | Capsaicin (mg/g) | SOD ($IC_{50}$: mg/ml) | POD (mM *o*-dianisidine oxidized/min/g) | CAT (units) | MRAP (mg/g AAE) | MRAP (mg/g GAE) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Chelator** | | | | | | | | | | |
| $HA_1$ | 1.268 | 1.137 | 2.283[az] | 0.500[b] | 0.830 | 5.890[a] | 1.192 | 153.940 | 3.462[b] | 7.363[b] |
| $HA_2$ | 1.297 | 1.133 | 2.058[b] | 0.665[a] | 0.743 | 2.442[b] | 1.263 | 146.790 | 3.761[a] | 8.135[a] |
| **Micronutrients** | | | | | | | | | | |
| $Zn_1$ | 1.186[b] | 1.183[a] | 2.173 | 0.524[b] | 0.838 | 3.853[b] | 0.928[b] | 163.804[a] | 3.690 | 7.957 |
| $Zn_2$ | 1.380[a] | 1.087[b] | 2.168 | 0.641[a] | 0.735 | 4.478[a] | 1.527[a] | 136.926[b] | 3.532 | 7.542 |
| $B_1$ | 1.271 | 1.075[b] | 2.191 | 0.585 | 0.652[b] | 4.156 | 1.208 | 138.184[b] | 3.284[b] | 6.911[b] |
| $B_2$ | 1.294 | 1.195[a] | 2.151 | 0.580 | 0.922[a] | 4.176 | 1.247 | 162.546[a] | 3.938[a] | 8.588[a] |
| **$HA_1$** | | | | | | | | | | |
| $Zn_1$ | 1.124 | 1.190 | 2.122 | 0.427 | 0.880 | 4.687 | 0.664 | 168.818 | 3.548 | 7.592 |
| $Zn_2$ | 1.412 | 1.077 | 1.995 | 0.573 | 0.780 | 7.093 | 1.720 | 139.062 | 3.375 | 7.135 |
| **$HA_2$** | | | | | | | | | | |
| $Zn_1$ | 1.247 | 1.177 | 2.225 | 0.622 | 0.797 | 3.020 | 1.192 | 158.790 | 3.832 | 8.322 |
| $Zn_2$ | 1.347 | 1.097 | 2.342 | 0.708 | 0.690 | 1.863 | 1.335 | 134.790 | 3.689 | 7.948 |
| **$HA_1$** | | | | | | | | | | |
| $B_1$ | 1.186 | 1.053 | 2.083 | 0.467 | 0.600 | 5.187 | 1.079 | 134.838 | 3.220 | 6.745 |
| $B_2$ | 1.351 | 1.213 | 2.033 | 0.533 | 1.060 | 6.593 | 1.305 | 173.042 | 3.703 | 7.982 |
| **$HA_2$** | | | | | | | | | | |
| $B_1$ | 1.357 | 1.097 | 2.218 | 0.703 | 0.703 | 3.125 | 1.338 | 141.530 | 3.348 | 7.077 |
| $B_2$ | 1.237 | 1.177 | 2.348 | 0.627 | 0.783 | 1.758 | 1.188 | 152.050 | 4.173 | 9.193 |
| **Analysis of variance (F values)** | | | | | | | | | | |
| HA | NS | NS | 92.985** | 75.392** | NS | 1160.517** | NS | NS | 13.443** | 13.967** |
| Zn | 33.796** | 12.072** | NS | 37.692** | NS | 38.124** | 158.372** | 10.719** | NS | NS |
| B | NS | 18.603** | NS | NS | 24.451** | NS | NS | 8.806* | 64.352** | 65.939** |
| HA × Zn | 7.934* | NS | 27.189** | NS | NS | 309.803** | 91.727** | NS | NS | NS |
| HA × B | 18.192** | NS | 14.878** | 14.223** | 12.108** | 187.662** | 15.585** | NS | NS | NS |

*Significant at 5%, **Significant at 1%, NS= Non significant. [z]Values followed by the same letter in a column are not significant at P < 0.05, Duncan's Multiple Range Test (DMRT). $HA_1$=Control of humic acid (0.0%), $HA_2$=dose of humic acid (0.05%), $Zn_1$= control of zinc (0.0%), $Zn_2$= dose of zinc (0.05%), $B_1$= control of boron (0.0%), $B_2$= dose of boron (0.02%).

**Table 3.** Pearson's correlation matrix of all variables.

| Variable | AAC[a] | FPC[a] | TPC[a] | Carotene | Capsaicin | SOD[a] | POD[a] | CAT[a] | MRAP (AAE)[a] |
|---|---|---|---|---|---|---|---|---|---|
| AAC[a] | | | | | | | | | |
| FPC[a] | -0.267 | | | | | | | | |
| TPC[a] | 0.004 | 0.109 | | | | | | | |
| Carotene | 0.726** | -0.274 | 0.495* | | | | | | |
| Capsaicin | -0.147 | 0.891** | -0.003 | -0.232 | | | | | |
| SOD[a] | -0.002 | -0.185 | -0.929** | -0.493* | 0.013 | | | | |
| POD[a] | 0.818** | -0.568* | -0.004 | 0.691** | -0.463 | 0.070 | | | |
| CAT[a] | -0.409 | 0.870** | 0.006 | -0.331 | 0.863** | -0.072 | -0.654** | | |
| MRAP (AAE)[a] | -0.319 | 0.738** | 0.410 | -0.034 | 0.613** | -0.392 | -0.293 | 0.624** | |
| MRAP (GAE)[a] | -0.333 | 0.728** | 0.418 | -0.034 | 0.606** | -0.393 | -0.298 | 0.629** | 0.998** |

**Significant at 1%, *Significant at 5%, Student's t-test. [a]AAC: Ascorbic acid content; FPC: Free phenol content; TPC: Total phenol content; SOD: Super oxide dismutase, POD: Peroxidase; CAT: Catalase; MRAP: Molybdate reducing antioxidant potential; AAE: Ascorbic acid equivalent; GAE: Gallic acid equivalent.

~1.18 fold in B applications as compared to that of their corresponding control treatments. The same trend has been seen in concentration of ascorbic acid content. The highest activity was observed from application of $HA_1B_2$ followed by $B_2$. Moreover, increase in the CAT activity in response to B application was reported by Ardic et al. (2009) in chickpea cv. Gokce and Cervilla et al. (2007) in tomato cv. Kosaco.

## Total antioxidant activity

The antioxidant activity of fruits and vegetables is important for assessing their nutritional value (Rice-Evans et al., 1996) and its measurement allows the evaluation of this nutritional variable without analysis of each antioxidant compound (Pellegrini et al., 1999; Scalfi et al., 2000). Antioxidant activity was analyzed under different systems of assay. In the present experiment (Table 2), antioxidant activity was expressed as Molybdate reducing antioxidant potential (MRAP). In green chilli, the MRAP values exerted significantly positive influence by application of HA ($HA_2$) (~1.09 fold) and B ($B_2$) (~2.61 fold) alone as compared with that of their corresponding control ($HA_1$ and $B_1$) treatments. The highest activity was observed from application of $HA_2B_2$ followed by $B_2$ and $HA_2Zn_1$, respectively.

## Correlation among variables

There are correlations between pairs of variables (Table 3). Most hydrophilic antioxidant ascorbic acid had significantly (at 1% level) positive correlations with carotene and POD. β-carotene and ascorbic acid were also significantly positive correlated in tomato fruits reported by Hanson et al. (2004). There were also significant (at 1% level) positive correlations of FPC with capsaicin, FPC with CAT and FPC with MRAP (AAE and GAE). Moreover, significant positive correlations between total phenolics with anti radical power (Hanson et al., 2004) and total phenolic content with antioxidant activity (Connor et al., 2002; Prior et al., 1998). In this study, there is also significant positive correlation between TPC and carotene (at 5% level). These relationships indicate that improving phenolics concentration could accompany improvement of fruit quality as well as improvement in antioxidant activity. Moreover, there was a significant negative correlation between TPC with SOD, POD with CAT at 1% level and carotene with SOD, FPC with POD at 5% level. While Marschner (1995) reported that phenol is a substrate of peroxidase (POD).

## Principal component analysis (PCA)

In this experiment, PCA was used to summarize the treatment information in a reduced number of components, components, where a total of three components were chosen (PC1, PC2 and PC3) due to their Eigen value being greater than 1.0 and they together explained 94.477% of total variance (Table 4).

The first component (PC1) explained 52.480% of total variance, in which an increase in free phenol (GAE), total phenol (GAE), capsaicin, CAT, MRAP (AAE) and MRAP (GAE) was associated with a decrease in ascorbic acid, carotene, SOD and POD (Table 4). Therefore, on the basis of the first component, the treatments $HA_2B_2$ and $B_2$ can be selected as having all desirable traits.

The second component (PC2) explained an additional 29.010% of total variance, in which ascorbic acid, total phenol (GAE), free phenol (GAE), carotene, POD and MRAP (AAE) (GAE) were positively loaded in contrast to capsaicin, SOD and CAT, which were negatively loaded

**Table 4.** Results of principal component analysis (PCA) for influence of foliar applications of chelator and micronutrients on antioxidant constituents and antioxidant activity in green chilli.

| Principal component | Eigen value | Variance (%) | Cumulative variance (%) |
|---|---|---|---|
| **Eigen values and variance accounted for percent by PCA based on correlation matrix** | | | |
| 1 | 5.248 | 52.480 | 52.480 |
| 2 | 2.901 | 29.010 | 81.490 |
| 3 | 1.299 | 12.987 | 94.477 |
| | | | |
| **Variable** | **PC1** | **PC2** | **PC3** |
| **Factor loadings due to PCs with Eigen values greater than 1** | | | |
| Ascorbic acid | -0.625 | 0.514 | 0.563 |
| Free phenol(GAE) | 0.969 | 0.051 | 0.208 |
| Total phenol (GAE) | 0.285 | 0.866 | -0.390 |
| Carotene | -0.422 | 0.854 | 0.227 |
| Capsaicin | 0.848 | -0.035 | 0.464 |
| SOD | -0.255 | -0.823 | 0.489 |
| POD | -0.720 | 0.441 | 0.451 |
| CAT | 0.946 | -0.110 | 0.220 |
| MRAP (AAE) | 0.854 | 0.367 | 0.121 |
| MRAP (GAE) | 0.855 | 0.367 | 0.119 |

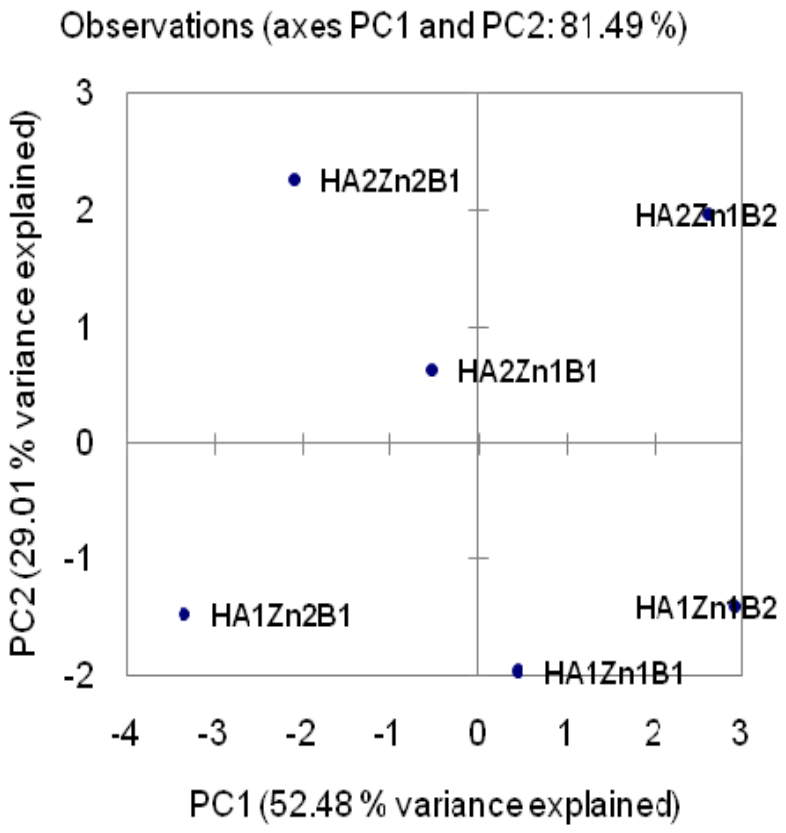


**Figure 1.** Biplot of the regression factor scores for the first and second components produced by PCA. Treatments: $HA_1Zn_1B_1$: Control, $HA_2Zn_1B_1$: Humic acid (0.05%), $HA_1Zn_2B_1$: Zinc (0.05%), $HA_1Zn_1B_2$: Boron (0.02%), $HA_2Zn_2B_1$: Humic acid (0.05%) × Zinc (0.05%), $HA_2Zn_1B_2$: Humic acid (0.05%) × Boron (0.02%).

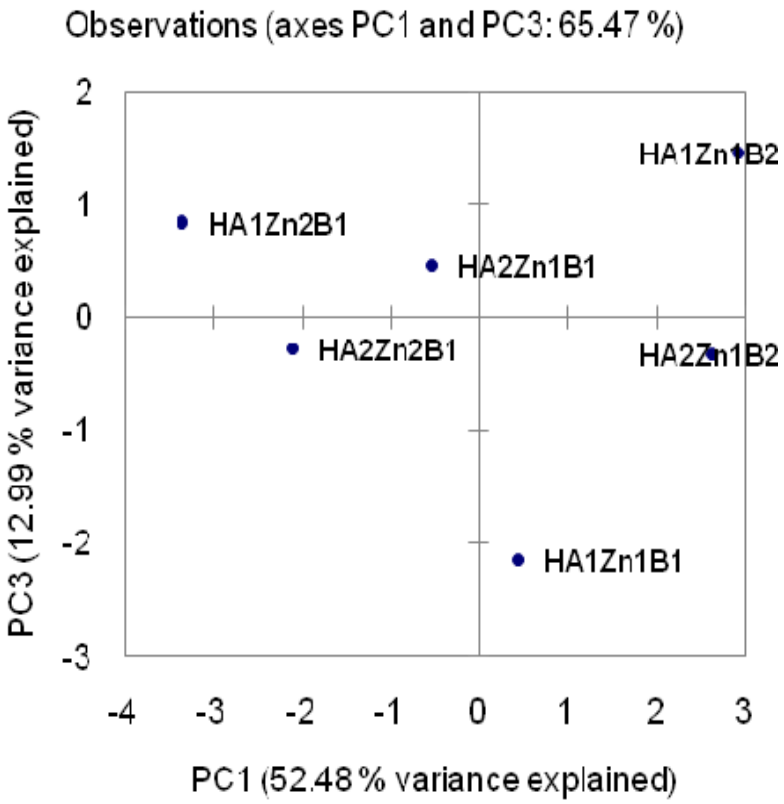


**Figure 2.** Biplot of the regression factor scores for the first and third components produced by PCA. Treatments: $HA_1Zn_1B_1$: Control, $HA_2Zn_1B_1$: Humic acid (0.05%), $HA_1Zn_2B_1$: Zinc (0.05%), $HA_1Zn_1B_2$: Boron (0.02%), $HA_2Zn_2B_1$: Humic acid (0.05%) × Zinc (0.05%), $HA_2Zn_1B_2$: Humic acid (0.05%) × Boron (0.02%).

(Table 4). Based on PC1 vs. PC2, the performing treatments would be $HA_2$, $HA_2Zn_2$ and $HA_2B_2$ (Figure 1).

The third component (PC3) explained another 12.987% of total variance, in which all the variables other than TPC are positively loaded meaning that all other variables restrict TPC, in which the former is not desirable. According to the plot of regression factor scores due to PC1 vs. PC3 (Figure 2), the treatment $HA_2$, $Zn_1$ and $B_2$ can be selected as performers.

## Conclusion

Based on principal component analysis and average values, foliar application of $HA_2B_2$ followed by $HA_2$ and $B_2$ had good performers with respect to all variables, which may bring about the proper value addition in green chilli fruit by enhancing the antioxidant constituents and antioxidant activities.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

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