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Effect of foliar application of humic acid, zinc and boron on biochemical changes related to productivity of pungent pepper (*Capsicum annuum* L.)

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To study the effect of foliar application of humic acid at 0.05%, zinc at 0.05% and boron at 0.02% on biochemical changes related to productivity of pungent pepper cv. Bullet (*Capsicum annuum* L.), a pot experiment in randomized block design with three replications was conducted in the net house of Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia-741252, West Bengal, India. In This experiment, we observed some physiological and biochemical characteristics of pungent pepper. Obtained results revealed that the highest value of height of plant, numbers of leaf plant^{-1}, leaf area, numbers of branch plant^{-1}, numbers of fruit plant^{-1} and fruit length were exhibited by foliar application of HA + Zn + B. The highest value of fruit diameter, weight of 20 fresh fruits, numbers of seed fruit^{-1} and weight of 100 seeds were exhibited by foliar application of Zn + B and HA + Zn + B, respectively. The total chlorophyll content significantly increased in HA + Zn + B followed by HA + B and Zn + B application. The highest value of reducing sugar, total sugar and starch content were obtained by application of HA + Zn + B. The highest content of protein, fat, energy and total phenol were exhibited by foliar application of Zn + B, Zn, HA + Zn + B and HA + Zn + B, respectively. The activity of polyphenol oxidase was significantly 'good performer' in HA + Zn + B followed by HA and HA + B application. There are significant and positive correlation of starch with some physiological characteristics such as leaf area, number of fruits plant^{-1}, fruit diameter and total chlorophyll. The correlation of energy value with carbohydrates, fat, protein, total chlorophyll and leaf area were positive and significant. Based on principle component analysis and considering average values of all variables, our results suggested that the good promising treatment in HA + Zn followed by Zn + B, HA + Zn + B and Zn alone application may bring about the proper value addition in quality as well as productivity of crop by enhancing the some physiological and biochemical characteristics in pungent pepper.

Key words: *Capsicum annuum* L., humic acid, zinc, boron, quality, productivity.

INTRODUCTION

Pungent pepper (*Capsicum annuum* L.) is one of the most important and popular crop grown in India and many countries all over the world. From time immemorial, it has been used as a common vegetable cum spice because of its color, test, pungency, flavor and aroma. Apart from basic quality, productivity of crop depends on their physical and biochemical properties. Concentrations of these properties in plant may be modified by various factors including foliar nutrition with organic component. Some studies estimates indicate that a large number of diverse materials can serve as sources of plant nutrients. The majority of nutrient input to agriculture comes from commercial mineral fertilizers. Organic manures are considered to play a significant but lesser role in nutrient contribution, leaving aside their beneficial effects on soil physicochemical and biological properties. Foliar feeding is a relatively new and controversial technique of feeding plants by applying liquid fertilizer directly to their leaves.

Most of the used organic-mineral fertilizers are humic substances, since, humic acid is one of the major components of humic substances. Nowadays, the use of humic acid has increased with increase in the agricultural production and the most economical humic acid almost applied directly to the soil and/or as a foliar application to the plants. The mode of action of humic acid on plant growth can be divided into direct and indirect effects as it affects the membranes resulting in improved transport of nutritional elements, enhanced protein synthesis, enhanced photosynthesis, solublization of micronutrients, reduction of active levels of toxic elements, enhancement of microbial population, enhanced soil structure improvement and increased both cation exchange capacity and water retention. MacCarthy et al. (1990). Singaroval et al. (1993) claimed that the increase in dry matter production with humic acid might be due to its direct action on plant growth auxin activity, contributing to increase in the dry matter.

Moreover, application of humic acid also increased the seed weight due to better mobilization of nutrients to seeds. Nardi et al. (1999) found that the biological activity of the humic acid was attributed to their chemical structure and their functional groups, which could interact with harmonic-binding proteins in the membrane system, evoking a hormone-like response.

Micronutrients have received a great deal of importance in crop production during recent years because of the widespread occurrences of their deficiencies from different parts of the country. Researchers from almost all the states in the country have also reported significant responses of many crops to micronutrient fertilization. Zinc (Zn) and boron (B) is an essential trace element for plants, being involved in many enzymatic reactions and is necessary for their good growth and development. The foliar application of micronutrients (Zn and B), increased total sugars (TS), reducing sugar (RS), non-reducing sugar (NRS), ascorbic acid (AA) and TSS/acid ratio of papaya fruit (Sing et al., 2002). The foliar application of micronutrients (Zn and B) also significantly enhanced fruit juice content, TSS, AA and NRS of sweet orange fruits.

Zn is also involved in regulating the protein and carbohydrate metabolism (Swietlik, 1999). Under Zn deficiency, the leaves shoot tips do not elongate fully, resulting in compressed internodes length and a tuft or rosette of leaves at the terminal (Tariq et al., 2007). However, in the light of better fruit quality development, Zn holds more significance besides imparting sustainability in production/productivity by reducing the fruit drop (Malik et al., 1999) and granulation (Kaur et al., 1990). External symptoms of Zn-deficiency in citrus as described by Wutscher (1979) are reduction in leaf size somewhat in proportion to the Zn-concentration in leaf. Zinc rates from 4 to 12 kg ha^{-1} had beneficial effect on physical and chemical characters of fruits, but best results with regard to flavor and contents of juice, vitamin-C, and total sugars were obtained from the lowest Zn-rate (Mdwaradze, 1981). Application of Zn at 0.6% plus 2,4-D at 20 ppm as foliar spray gave the best results with regard to fruit weight, diameter, juice percentage, TSS and ascorbic acid content in Kagzi lime (Batru et al., 1984). Bahadur et al. (1998) claimed that the Zn uptake rate was faster in mango trees when zinc sulfate was foliar applied as compared with its soil application. While, the boron requirement is much higher for reproductive growth than for vegetative growth and increases flower production and retention, pollen tube elongation and germination, and seed and fruit development (Peres and Reyes, 1983). The application of boron as foliar spray also enhanced the fruit set in papaya (Jeyakumar et al., 2001). Furthermore, the supply of boron needed for reproductive growth in many crops is more needed than that needed for vegetative growth (Mengel and Kirkby, 1982; Marschner, 1986; Hanson, 1991) and same may be true in citrus. In citrus B deficiency leads to low sugar content, granulation and excessive fruit abortion (Reuther et al., 1968) as well as rind thickening. There is also concern 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; Lukaszewski and Blevins, 1996).

Therefore, in the present work, we have studied this response in pungent pepper grown at different levels of foliar application of HA and micronutrients (Zn and B), as well as the physiological and biochemical changes that take place during vegetative growth and fruit formation, in order to improve the quality, as well as productivity of crop.

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Table 1. Detail doses of treatments which used as foliar spray for pungent pepper.

Treatment		Humic acid (HA) (%)	Zinc (Zn) (%)	Boron (B) (%)
Control :	$HA_0Zn_0B_0$	0.0	0.0	0.0
HA:	$HA_1Zn_0B_0$	0.05	0.0	0.0
Zn:	$HA_0Zn_1B_0$	0.0	0.05	0.0
B:	$HA_0Zn_0B_1$	0.0	0.0	0.02
HA+Zn:	$HA_1Zn_1B_0$	0.05	0.05	0.0
HA+B:	$HA_1Zn_0B_1$	0.05	0.0	0.02
Zn+B:	$HA_0Zn_1B_1$	0.0	0.05	0.02
HA+ Zn+B:	$HA_1Zn_1B_1$	0.05	0.05	0.02

The source of humic acid was granular form, that of zinc (Zn) was $ZnSO_4.7H_2O$ and boron (B) from $Na_2B_4O_7.10H_2O$. 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.

MATERIALS AND METHODS

Field experiment

The seedling was grown in nursery beds prepared with a sandy loam soil and were 12 cm tall and 1.0 m wide. Weathered cow dung manure 4 kg/m, was mixed into the beds. Beds were drenched with formaldehyde (4.0%) and covered with polythene sheets for one week to avoid damping off disease. Seedling was treated with Dithane M-45 (2.5 g.kg^{-1} of seed) (Hindustan Pulverizing Mills Industrial Growth Centre, Sumba, Jammu, India) prior to sowing. Fresh seeds of pungent pepper cv. bullet (*C. 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 watering was done 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, Mohanpur, Nadia, West Bengal and India. The 40 days old seedlings were transplanted in earthen pot of 15 cm size (one plant.pot^{-1}) having a central drainage hole. Soil was prepared by mixing appropriate amount of well rotted cow dung and manures (soil 700 g.pot^{-1}; cow manure 100 g.pot^{-1}; urea 5 g.pot^{-1}; single superphosphate 20 g.pot^{-1} and muriate of potash 6 g.pot^{-1}) (according to guidance of Department of Spices and Plantation Crops, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India). Additional fertilizer 5 g.pot^{-1} was applied 21days after transplanting. The experiment consisting of eight treatments including control (only tap water sprayed) were arranged in a randomized block design with three replications. The detail treatments are summarized as 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). No weeding was required. The insecticide Rogor at 2.5 ml.L^{-1} (Rallis India Ltd., Mumbai, Maharashtra, India) was applied three times beginning just from flowering stage at 15 days intervals to control aphids. The following physiological and biochemical qualities were determined in the present experiment.

Physiological qualities

All the recommended cultural practices for pungent pepper production were followed according to advice of Horticulture Faculty, Department of Spices and Plantation Crops, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia- 741252, West Bengal, India. Data were recorded as follows:

Plant growth characteristics

At the end of the growing season, (~90 days after transplanting) samples of ten selected plants were taken at random from each replication to determine the following characteristics:

1. Average height of plant (cm)
2. Average number of leaves plant^{-1}
3. Average leaf area (sq.cm) [Leaf area was determined followed the procedure of McKee (1964)]
4. Average number of branches.plant^{-1}

Fresh fruit yield and its components

Fresh fruit yield was started andharvested at 25 days after initiation of flowering. Samples of ten selected plants of each replication were counted until final harvesting to determine the following characteristics:

1. Average number of fresh fruits plant^{-1}
2. Average length of fresh fruit (cm)
3. Average diameter of fresh fruit (cm)
4. Weight of 20 fresh fruits (g)

Seed yield and its compositions

Twenty pods were taken at random from each replication to determine the following characteristics:

1. Average number of fresh seeds fruit^{-1}
2. Weight of 100 fresh seeds (g)

Biochemical qualities

About 300 g weighed edible pungent pepper from each replication was collected. Fresh materials were chopped with a sharp knife into small pieces before analysis of total phenol and one oxidative enzyme [polyphenol oxidase (PPO)]. Samples were shredded and dried at 40°C for 96 h. This material was prepared for reducing sugar, total sugar, starch, protein and fat analyses by grinding to a fine powder using an electric grinder. A subsample of the dried shredded material was further dried at 100°C to constant dry weight to determine percent moisture.

Analysis of chlorophyll a, chlorophyll b and total chlorophyll

One gram fresh leaf mass of pungent pepper (*C. annuum* L.) was extracted by macerating with 10 ml of 80% solution of acetone. The procedure was repeated until the leaf mass was completely decolorized. The resulting extracts are kept in the dark in order to prevent the destruction of the chlorophyll molecules. The extracts are spectrophotometrically measured at the wave length of 663 and 645 nm (Arnon, 1956). The concentration (content) of chlorophylls in the solution was calculated with the following formulas:

Chlorophyll a (mg.g^{-1}) = [12.7(A_{663}) - 2.69(A_{645})] × V/1000×W

Chlorophyll b (mg.g^{-1}) = [22.9(A_{645}) - 4.68(A_{663})] × V/1000×W

Total chlorophyll (mg.g^{-1}) = [20.2(A_{645}) - 8.02(A_{663})] × V/1000×W

Where, A = Absorbance of specific wavelength, V = final volume of chlorophyll extract in 80% acetone, W = weight of sample.

Analysis of reducing sugar

Reducing sugar were extracted in 10 ml of 80% alcohol by boiling 0.1 g dry powdered sample for 30 min at 80-90°C followed by centrifugation at 5,000 *g* for 10 min and subsequent analysis was followed using the Nelson-Somogyi's method (Sadasivam and Manickam, 1992). The amount of reducing sugar was determined against a glucose solution (0-500 µg) as standard curve.

Analysis of total sugar and starch

Sugar and starch were extracted in 10 ml of 80% anhydrous alcohol by boiling 0.1 g dry powdered sample for 30 min at 80°C followed by centrifugation at 5,000 *g* for 10 min and subsequent procedures was followed using the Anthrone reagent method (Sen et al., 2005). The amount of concentration was determined against a glucose solution (0-500 µg) as standard curve.

Analysis of total protein

Total nitrogen (N) was analyzed by the Kjeldahl method (Casanas et al., 2002) and used for the calculation of the protein concentration by multiplying with a conversion factor of 6.25.

Analysis of fat

Fat content of dry fruit of pungent pepper was analyzed following extraction with petrol ether in a Soxhlet apparatus and subsequent proceeding was followed by method of AOAC (1984).

Analysis of energy

The energy value was calculated using Atwater factors of 17 kJ/g of protein, 38 kJ/g of fat, 17 kJ/g of starch and 16 kJ/g of sugar (FAO/WHO/UNU, 1985).

Analysis of total phenol

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

Analysis PPO Activity

One gram of fresh tissue from each treatment was macerated in a pre-chilled pestle and mortar and extracted with 10 ml phosphate buffer (pH 6.0) to determine PPO activity. Triturated samples were centrifuged at 10,000 *g* for 30 min at 0°C and supernatants were assessed for enzyme activity. Estimation of polyphenol oxidase was made by adding 2 ml phosphate buffer and 0.5 ml (0.01 M) catechol solution with 0.5 ml extract. Changes in activities were measured following the procedure of Matto and Diamond (1963).

Statistical analysis

Data were subjected to ANOVA of a randomized block design, simple correlation were calculated and tested for significance and means. Principal component analysis (PCA), as the method of identifying the effect dimension of the data, was used to summarize the treatment information in a reduced number of effects 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

Physiological qualities

The changes in physiological qualities in response to foliar applications of HA, Zn and B in plants and fresh fruits of pungent pepper (*C. annum* L.), are shown in Tables 2, 3 and 4.

Plant growth qualities

The plant growth quality aspects were determined with regard to average height of plant, average number of leaves plant^{-1}, average leaf area and average number of branches plant^{-1} (Table 2). In our present experiment, results revealed that with respect to plant height, there was significantly positive influence in all treatments except Zn and B individual application as compared to that of their corresponding control (only tap water spray) treatment, in which (Zn and B) treatments also positive influenced the height of plant, apparently, though not significantly. The higher performer was observed, when plants are treated with HA + Zn + B followed by HA + Zn and Zn + B.

On the other hand, in respect to number of leaves plant^{-1}, there was significantly positive effect in all treatments except B application alone over the control treatment, in which B treatment also positively enhanced, apparently, but not significantly. The maximum value of number of leaves was observed in HA + Zn + B treatment.

Regarding leaf area, significantly positive influence was

Table 2. Effect of foliar application of HA, Zn and B on plant growth quality in pungent pepper.

Treatment	Average height of plant (cm)	Average number of leaves plant^{-1}	Average leaf area (sq.cm)	Average number of branches plant^{-1}
Control	45.60	30.46	1.19	27.31
HA	53.02*	40.85*	1.40^{NS}	45.87*
Zn	51.09^{NS}	46.34**	1.28^{NS}	49.02*
B	48.27^{NS}	39.39^{NS}	1.33^{NS}	35.95^{NS}
HA+Zn	58.00**	56.34**	1.46*	63.04**
HA+B	51.62*	42.63*	1.69**	56.26**
Zn+B	57.00**	53.91**	1.71**	71.17**
HA+Zn+B	64.03**	58.57**	1.80**	75.11**
SE(m)±	1.96	3.21	0.07	5.56
LSD (0.05)	5.89	9.64	0.22	16.68
LSD (0.01)	8.47	13.87	0.30	24.02

*Significant at 5%, **Significant at 1% and ^{NS}Non significant

seen in all combination treatments of HA, Zn and B (HA + Zn, HA + B, Zn + B and HA + Zn + B) as compared to that of their corresponding control treatment. While, individual treatments of HA, Zn and B also exerted positive influence, through not significantly. The best performer was observed, when plants are treated with HA + Zn + B followed by Zn + B and HA + Zn application.

In the case of number of branches plant^{-1}, it was significantly induced in all treatments except B application alone than the control treatment. While, B application alone also induced the number of branches but not significantly. The highest number was recorded from HA + Zn + B followed by Zn + B treatment.

Our results also indicated that, in respect to growth quality, Zn application gave the better flourishing than the HA and B application over the control treatment. Moreover, under HA treatment, the plant height and number of branches plant^{-1} were increased significantly in pea plant, that reported by El-Hak et al. (2012). Azarpour et al. (2011) also reported that the foliar application of HA gave the best and highest plant height values of cowpea. Kaya et al. (2005) reported that, HA and Zn both alone and/or combination treatments significantly enhanced plant height as compared to control treatment. While, Hatwar et al. (2003) reported that, Zn (0.1%) and B (0.1%) both alone and/or combination treatments exerted significantly positive influence on the plant height and number of branches plant^{-1} as compared to the control. Improvement of plant growth might be due to enhancement in photosynthetic and other metabolic activities which led to an increase in various plant metabolites responsible for cell division and cell elongation. Enhanced photosynthetic reactions in the presence of Zn and B might have increased the plant growth (Rawat and Mathpal, 1984). The further height of plant due to application of Zn explained by Mallick and Muthukrishnan (1979) was due to an active synthesis of tryptophan, an amino acid in the presence of Zn and it is the precursor of IAA which stimulates the growth of plant tissues. Besides, the Zn and B also plays an essential role in the development and growth of new cells in the plat. Plant requires B for synthesis of amino acids and proteins and regulation of carbohydrates metabolism (Dyar and Webb, 1961).

Fresh fruit yield and it's components

The fruit yield aspects were determined with regard to average number of fresh fruits plant^{-1}, average length of fresh fruit, average diameter of fresh fruit and average weight of 20 fresh fruits (Table 3).

Our present experiment indicated that, with respect to number of fresh fruits plant^{-1}, all the treatments except HA application alone exerted significantly positive influence as compared to control. The HA application also exerted positive influence, through not significantly. The maximum number of fruit plant^{-1} was observed by application of HA + Zn + B.

In the case of length of fruit, Zn, B, HA + B, Zn + B and HA + Zn + B application exerted significantly positive influence over the control. The remaining treatments, HA and HA + Zn application also influenced, apparently, though not significantly. The highest values of length of fruit were exhibited by HA + Zn + B followed by Zn + B and HA + B application.

All the treatments except HA alone exerted significantly positive influence on the diameter of fresh fruit as compared to control treatment, in which HA had positive influence but not significantly. The maximum value was recorded by Zn + B application.

Regarding, weight of 20 fresh fruits, HA + Zn + B, Zn + B and HA + Zn application exerted significantly positive effect as compared to the control. The remaining treatments also had positive effect, though not significantly.

The best performer was observed by HA + Zn + B

Table 3. Effect of foliar application of HA, Zn and B on fresh fruit yield and its components in pungent pepper.

Treatment	Average number of fresh fruits plant^{-1}	Average length of fresh fruit (cm)	Average diameter of fresh fruit (cm)	Average weight of 20 fresh fruits (g)
Control	3.37	4.47	3.00	45.67
HA	5.50^{NS}	5.27^{NS}	3.50^{NS}	48.33^{NS}
Zn	6.58*	5.90**	4.10**	52.73^{NS}
B	5.78*	5.57*	4.00**	49.60^{NS}
HA+Zn	8.66**	5.06^{NS}	4.70**	60.33*
HA+B	8.33**	6.20**	4.60**	57.60NS
Zn+B	9.53**	6.90**	4.80**	69.33**
HA+Zn+B	9.80**	7.30**	4.50**	81.60**
SE(m)±	0.75	0.31	0.21	4.05
LSD (0.05)	2.25	0.95	0.63	12.16
LSD (0.01)	3.24	1.34	0.91	17.50

*Significant at 5%, **Significant at 1% and ^{NS}Non significant.

followed by Zn + B application.

Whatsoever, Halwar et al. (2003) reported that, Zn and B both alone and/or combination treatments exerted significantly positive influence on the number of fruit plant^{-1} as compared to control. While Dongre et al. (2000) reported that the application of $ZnSO_4$ (0.10, 0.25 and 0.50%) exerted significantly positive influence on the average length of fruit and average diameter of fruit as compared to control. Concerning the effect of foliar application with HA, number of fruits plant^{-1}, fruit length, fruit diameter and 20 fruits weight were significantly increased during first season, 2009/2010 in pea (El-Hak et al., 2012). Azarpour et al. (2011) reported that HA foliar application gave significant increasing results on number of pods plant^{-1} and pod length of cowpea. Kaya et al. (2005) reported that HA + Zn treatments significantly enhanced number of pods plant^{-1} over the control treatment. Many researchers e.g., Zaky et al. (2006) on beans, Neri et al. (2002) on strawberry and Forgac and Czimbalmos (2011) on pea plants used the HA foliar application in green house and/or open field cultivations and found that number of pods plant^{-1}, total yield plant^{-1} and average pod fresh weight were markedly increased by the treatment at the rate of 1 g.L^{-1} combined with irrigation water.

Fresh seed yield and its compositions

Data regarding number of fresh seeds fruit^{-1} and weight of 100 fresh seeds are presented in Table 4. Analysis of variance revealed that the number of seeds fruit^{-1}, was significantly positive as observed by foliar application of Zn + B, HA + Zn + B, HA + B, HA + Zn and B as compared to control. HA and Zn application also occurred the positive effect, though not significantly. The good promising result was recorded by Zn + B followed by HA + Zn + B, HA + B and HA + Zn application.

On the other hand, in respect to average weight of 100 fresh seeds, all the combination treatments (HA + Zn, HA + B, Zn + B and HA + Zn + B) exerted significantly positive influence as compared to control treatment. While, individual application of HA, Zn and B also had positive influence, though not significantly. The maximum weight of 100 fresh seeds were recorded by application of HA + Zn + B. Moreover, Dongre et al. (2000) reported that the application of $ZnSO_4$ (0.10, 0.25 and 0.50%) exerted significantly positive influence on the average number of seeds fruit^{-1} and weight of 500 seeds as compared to the control. While, number of fresh seeds pod^{-1}, seeds weight and green pods yield significantly increased in pea (El-Hak et al., 2012). Similar results were obtained by Malik and Azam (1986) on wheat; Putintsev and Platonova (1991) on pea; Salib (2002) on peanut; Habashy et al. (2005) on peanut and faba bean and Azarpour et al. (2011) on cowpea plants as they reported that foliar spray with humic acid increased the dry seed yield and its parameters.

Biochemical qualities

Green pigments content

Beneficial effect of Zn on photosynthetic pigments may be due to its role in increasing the rates of photochemical reduction (Kumar et al., 1988), chloroplast structure, photosynthetic electron transfer as well as photosynthesis (Romheld and Marschner, 1991). In our present experiment, results revealed that, all the treatments exerted significantly positive influence on content of chlorophyll a, chlorophyll b and total chlorophyll as compared to control treatment (Table 5). The maximum chlorophylls (chl. a, chl. b and total chl.) content were recorded from HA + Zn + B application. Increased chlorophyll concentration by foliar application of B and Zn were reported in Irish plant over the control treatment (Khalifa et al., 2011). However

Table 4. Effect of foliar application of HA, Zn and B on fresh seed yield and its compositions in pungent pepper.

Treatment	Average number of fresh seeds.fruit^{-1}	Average weight of 100 fresh seeds (g)
Control	56.06	0.51
HA	61.46^{NS}	0.54^{NS}
Zn	59.26^{NS}	0.54^{NS}
B	66.37*	0.53NS
HA+Zn	71.52**	0.57*
HA+B	72.41**	0.58*
Zn+B	78.43**	0.62**
HA+Zn+B	76.54**	0.65**
SE(m)±	2.75	0.02
LSD (0.05)	8.26	0.05
LSD (0.01)	11.88	0.08

*Significant at 5%, **Significant at 1% and ^{NS}Non significant.

Table 5. Effect of foliar application of HA, Zn and B on green pigments content in pungent pepper.

Treatment	Chlorophyll a [mg.g^{-1} (FW)]	Chlorophyll b [mg.g^{-1} (FW)]	Ratio of chlorophyll a:b	Total Chlorophyll [mg.g^{-1} (FW)]
Control	0.91	0.44	2.07	1.32
HA	1.26*	0.71*	1.78*	1.97*
Zn	1.23*	0.70*	1.75**	1.93*
B	1.38**	0.89**	1.56**	2.27**
HA+Zn	1.45**	1.05**	1.46**	2.25**
HA+B	1.56**	1.05**	1.51**	2.72**
Zn+B	1.55**	1.04**	1.49**	2.60**
HA+Zn+B	1.72**	1.15**	1.50**	2.83**
SE(m)±	0.08	0.08	0.07	0.17
LSD (0.05)	0.25	0.24	0.21	0.50
LSD (0.01)	0.36	0.34	0.30	0.73

*Significant at 5%, **Significant at 1% and ^{NS}Non significant.

Farouk et al. (2011) reported that the application of HA also enhanced the chlorophyll concentration in radish plant. Whereas, in the case of values of chlorophyll a:b ratio opposite trend result of total chlorophyll were given, which exerted significantly negative influence in all treatments as compared to that of control (only water sprayed) treatment.

Moisture content

There are differences in moisture content and nutrient contents among the several treatments are presented in Table 6. In our present experiment, foliar applications of HA, Zn and B through different treatments could produce negative significant differences in the content of moisture. Therefore, no positive significant effects could be observed by applications of HA, Zn and B on the content of moisture.

Carbohydrates constituents

The beneficial effect of B may be due to its role in facilitating transport of carbohydrates, that is, starch and sugar (Donald et al., 1998). The obtained results are in conformity with those of Farahat et al. (2007) on *Cupressus sempervirens* and Nahed and Laila (2007) on *Salvia farinacea*. In our present study, results showed that in respect to reducing sugar, significantly enhanced effects were seen in all treatments except HA alone as compared to the control, in which (HA) also positive enhanced, apparently, though not significantly (Table 6). The maximum content of reducing sugar was recorded by HA + Zn + B and HA + B application.

Regarding, total sugar content (Table 6), exerted significantly positive influenced results were observed in HA + Zn + B, Zn + B, HA + B and B applications as compared to control. HA, Zn and HA + Zn also had positive effect but not significantly. The highest concentration of total

Table 6. Effects of HA, Zn and B on biochemical qualities in pungent pepper cv. Bullet (*C. annuum* L.)

Treatment	Moisture content (%)	Reducing sugar [mg.g^{-1} (DW)]	Total sugar [mg.g^{-1} (DW)]	Starch [mg.g^{-1} (DW)]	Protein [mg.g^{-1} (DW)]	Fat [mg.g^{-1} (DW)]	Energy [kJ.g^{-1} (DW)]	Total phenol [mg.g^{-1} (QE) (FW)]	Polyphenol oxidase (ΔA/min/g)
Control	86.02	7.49	26.65	213.80	111.30	71.00	8.65	0.97	0.56
HA	82.30*	8.90^{NS}	33.40^{NS}	270.60**	177.67**	93.00^{NS}	11.69**	1.14^{NS}	1.7**
Zn	83.22*	9.99*	29.21^{NS}	251.60*	200.67**	122.00**	12.79**	1.15^{NS}	0.67^{NS}
B	82.37*	11.61**	39.27*	301.40**	153.67*	60.00^{NS}	10.65*	1.58*	0.66^{NS}
HA+Zn	79.66**	12.21**	35.67^{NS}	291.70**	211.00**	111.00**	13.33**	1.40^{NS}	0.95^{NS}
HA+B	81.01**	13.05**	44.80**	308.23**	203.74**	91.00^{NS}	12.88**	1.86**	1.61**
Zn+B	80.59**	12.21**	47.85**	314.76**	221.11**	115.83**	14.28**	1.99**	1.12*
HA+Zn+B	79.03**	13.05**	52.00**	322.67**	215.32**	121.20**	14.58**	2.25**	1.72**
SE(m)±	0.76	0.68	3.01	12.33	12.56	7.82	0.66	0.15	0.16
LSD (0.05)	2.23	2.05	9.03	36.93	37.68	23.45	1.97	0.46	0.49
LSD (0.01)	3.28	2.94	13.00	53.26	54.26	33.78	2.85	0.65	0.69

*Significant at 5%, **Significant at 1% and ^{NS}Non significant.

sugar was observed from application of HA + Zn + B followed by Zn + B and HA + B.

On the other hand, the starch contents (Table 6), exerted significant positive influence in application of HA, Zn and B alone and/or combination treatments. The highest concentration of starch was observed from HA + Zn + B followed by Zn + B and HA + B application. However Khan et al. (2012) observed a decreased trend in reducing sugar concentration in relation to foliar application of Zn and B in citrus fruit. While, Kalifa et al. (2011) also reported that application of Zn and B increased the carbohydrates percentage in Irish plant. A similar trend of results was found by El-Khayat (1999), Gomaa (2001) and Samia and Mahmoud (2009) showing that Zn increased total carbohydrate in *Antholyza aethiopica* and *Tritonia crocata* plants, respectively. However, under Zn treatment, the values of total and non-reducing sugar increase were observed in citrus fruit (Khan et al., 2012). Increased total sugar concentration by application of HA was reported in radish plant (Farouk et al., 2011).

Protein content

Protein content of groundnut pod was significantly influenced by B application as reported by many workers (Bhuiyan et al., 1997; Murthy, 2006). Application of B stimulated nitrogen content of potato tubers and might have increased protein synthesis and subsequent storage of protein as suggested by Yadav and Manchanda (1979). In our present study, results observed that with respect to protein content, significant increasing trend were observed in all treatments over the control treatment (Table 6). The highest concentration of protein was recorded from Zn + B followed by HA + Zn + B and HA + Zn application. Moreover, increasing the concentration of protein by application of HA was reported in radish plant (Farouk et al., 2011) and in common vetch (*Vicia sativa* L.) (Saruham et al., 2011).

Lipid content

In the case of fat content (Table 6), HA, B and HA+B foliar spray failed to produce any significant effect over the control. The highest value of fat was exhibited by Zn followed by HA + Zn + B and Zn + B application. However, foliar application of Zn and B significantly increased the oil content in flowers of Irish plant over the control as observed by Khalifa et al. (2011).

Energy content

Regarding energy values in pungent pepper fruits (Table 6), all the treatments produced significantly increasing effects over the control treatment. Whereas, plants treated with foliar application of combination treatments gave best results than individual treatments. The highest value of energy was observed from application of HA + Zn + B followed by Zn + B and HA + Zn.

Total phenol

Phenols are ubiquitous phytochemicals that contributed largely to antioxidant potential of any plant. The B is presumably responsible for the metabolism changes and cell damages in boron deficient tissue (Marschner, 1986) and it is thought that B complexes the phenolic compounds in plant cells, reducing their potential toxicity (Lee and Arnoff, 1967). However, the increase in total phenol effectuated by Zn application in wheat leaves was reported by Vinod et al. (2012). Hamid et al. (2010) found that phenolic content of plant were increasing with increasing level of heavy metal. In our present experiment, results showed that the total phenol content significantly increased in all treatments exception HA, Zn and HA + Zn application as compared to the control, which also followed an increasing trend over the control, apparently, though not significantly (Table 6). The highest concentration of total phenol was observed from HA + Zn + B followed by Zn + B and HA + B application.

Polyphenol oxidase

Polyphenol oxidases (PPO) proteins containing copper catalyze oxidation of hydroxyphenols to their quinine derivatives, which then spontaneously polymerize. Enzymatic browning in fruits and vegetables are due to presence of PPO and is often responsible for unpleasant sensory qualities and losses in nutrient quality (Sanchez-Amat and Solano, 1997). Cakmak et al. (1995) reported that under B-deficiency conditions, sunflower leaves are characterized by a rapid loss of K^+ and a strong discoloration (browning), as occurs with high PPO activity. Our present study showed that the polyphenol oxidase (PPO) activity was significantly increased in all treatments except Zn, B and HA + Zn application as compared to control, where Zn, B and HA+Zn also increased the activity of PPO but not significantly (Table 6). The highest activity of PPO was observed in foliar spray with HA+Zn+B followed by HA and HA + B. While, the PPO activity declined significantly in plant with B deficiency as reported by Cara et al. (2002). However, recently Pfeffer et al. (1998), working with B-deficient sunflower leaves, observed that B does not maintain plasma-membrane integrity by complexing phenols and inhibiting PPO activity to prevent damage by oxygen-free radicals or by regulating ascorbate metabolism. The authors concluded that the resupply of B to deficient leaves might be the result of the inactivation (detoxification) of phenolics by the formation of stable phenol-borate complexes and, thus, repression of phenol oxidation.

Correlation among variables

Correlation coefficients (Table 7) showed that the number of branches plant^{-1} had positive and significant correlation with length of plant (r^2=0.932), number of leaves plant^{-1} (r^2=0.943) and leaf area (r^2=0.882). While number of fruits plant^{-1} had positive and significant correlation with length of fruit, number of leaves plant^{-1}, leaf area and number of branches plant^{-1}. The correlation of number of seeds fruit^{-1} with fruit length (r^2=0.774) and fruit diameter (r^2=0.882) were positive and significant. There are significant and positive correlation between fruit length and weight of 100 seeds (r^2=0.890), plant height and weight of 100 seeds, total chlorophyll and leaf area (r^2=0.921). The correlation of energy with carbohydrates, fat, protein, total chlorophyll and leaf area were positive and significant. Another, significant and positive correlation was between polyphenol oxidase and leaf area (r^2=0.742). Moreover, there was significant and positive correlation of starch with some physiological characteristics such as leaf area, number of fruits.plant^{-1}, fruit diameter and total chlorophyll. The correlation of total phenol with carbohydrates and chlorophyll were positive and significant. Our results revealed that the positive and significant correlation between number of fruits plant^{-1} and 100 seeds weight (r^2=0.919), agree with that of result by Kaya et al. (2005), they observed positive and non significant correlation. The significant inverse correlation between moisture and starch (r^2=0.901) obtained supports results of Casanas et al. (2002) and Guchhait et al. (2008). Above relationships indicate that improving number of leaves and leaf area as well as increasing number of fruit and chlorophyll contents could accompany improvement in energy value as well as quality of fruits.

Principal component analysis (PCA)

In our present experiment, PCA was used to summarize the treatment information in a reduced number of 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 93.07% of total variance (Table 8).

The PC1 explained 80.99% of total variance where all the variables other than ratio of chlorophyll a:b and moisture content are positively loaded meaning that all other variables limit ratio of chlorophyll a:b and moisture content, in which the former is not desirable (Table 8). Therefore, on the basis of the first component, the treatments such as HA + Zn + B, Zn + B, HA + Zn and HA + B can be selected as having all desirable traits.

PC2, on the other hand, explained another 7.53% of total variance in which an increase in length of plant, number

Table 7. Pearson's correlation matrix of all variables.

Variable	Plant height	Number of leaves	Leaf area	Number of branches	Number of fruits	Fruit length	fruit diameter	Weight of 20 fruits	Number of seeds
Plant height									
Number of leaves	0.936**								
Leaf area	0.787*	0.711*							
Number of branches	0.932**	0.943**	0.882**						
Number of fruits	0.861**	0.919**	0.892**	0.970**					
Fruit length	0.699	0.681	0.856**	0.801*	0.810*				
Fruit diameter	0.684	0.838**	0.765**	0.856**	0.948**	0.694			
Weight of 20 fruits	0.914**	0.861**	0.874**	0.916**	0.884**	0.855**	0.717*		
Number of seeds	0.767*	0.787*	0.922**	0.875**	0.930**	0.774*	0.882**	0.849**	
Weight of100 seeds	0.900**	0.841**	0.947**	0.945**	0.919**	0.890**	0.759*	0.979**	0.900**
Chlorophyll a	0.803*	0.804*	0.915**	0.869**	0.931**	0.837**	0.874**	0.834*	0.927**
Chlorophyll b	0.780*	0.821*	0.870**	0.855**	0.938**	0.734*	0.922**	0.797*	0.945**
Ratio of chlorophyll a:b	-0.673	-0.784*	-0.747*	-0.775**	-0.886**	-0.648	-0.938**	-0.662	-0.885**
Total chlorophyll	0.714*	0.721*	0.921**	0.820*	0.902**	0.850**	0.865**	0.785*	0.918**
moisture	-0.888**	-0.900**	-0.834**	-0.902**	-0.932**	-0.683	-0.871**	-0.806*	-0.888**
Reducing sugar	0.655	0.736*	0.818*	0.772*	0.897**	0.725*	0.928**	0.732*	0.903**
Total sugar	0.711*	0.649	0.947**	0.783*	0.833*	0.877**	0.732*	0.850**	0.930**
Starch	0.691	0.707	0.856**	0.766*	0.855**	0.772*	0.836**	0.728*	0.921**
Protein	0.797*	0.892**	0.759*	0.924**	0.928**	0.736*	0.898**	0.728*	0.760*
Fat	0.760*	0.819*	0.514	0.813*	0.714*	0.590	0.600	0.680	0.447
Energy	0.875**	0.932**	0.821*	0.969**	0.958**	0.817*	0.883**	0.838**	0.812*
Total phenol	0.702	0.663	0.931**	0.783*	0.845**	0.894**	0.756*	0.873**	0.924**
Polyphenol oxidase	0.605	0.384	0.742*	0.575	0.510	0.528	0.318	0.504	0.502

*Significant at 5%, **Significant at 1%, Student's t-test.

Table 7. Contd.

Variable	Weight of 100 seeds	Chlorophyll a	Chlorophyll b	Ratio of chlorophyll a:b	Total Chlorophyll	Moisture	Reducing sugar	Total sugar	Starch	Protein	Fat	Energy	Total phenol
Plant height													
Number of leaves													
Leaf area													
Number of branches													
Number of fruits													
Fruit length													
Fruit diameter													
Weight of 20 fruits													

Table 7. Contd.

Variable	Weight of 100 seeds	Chlorophyll a	Chlorophyll b	Ratio of chlorophyll a:b	Total chlorophyll	Moisture	Reducing sugar	Total sugar	Starch	Protein	Fat	Energy	Total phenol
Number of seeds													
Weight of 100 seeds													
Chlorophyll a	0.883**												
Chlorophyll b	0.837**	0.977**											
Ratio of chlorophyll a:b	-0.707*	-0.926**	-0.968**										
Total chlorophyll	0.852**	0.988**	0.958**	-0.911**									
moisture	-0.838**	-0.946**	-0.961**	0.924**	-0.893**								
Reducing sugar	0.767*	0.947**	0.975**	-0.959**	0.951**	-0.885**							
Total sugar	0.903**	0.928**	0.881**	-0.784*	0.941**	-0.802*	0.853**						
Starch	0.792*	0.968**	0.957**	-0.941**	0.969**	-0.901**	0.931**	0.923**					
Protein	0.785*	0.828*	0.815*	-0.810*	0.796*	-0.865**	0.766*	0.643	0.734*				
Fat	0.670	0.492	0.457	-0.402	0.423	-0.580	0.381	0.332	0.313	0.832*			
Energy	0.877**	0.869**	0.841**	-0.803*	0.829*	-0.890**	0.781*	0.727*	0.767*	0.983**	0.846**		
Total phenol	0.908**	0.922**	0.880**	-0.781*	0.935**	-0.784*	0.874**	0.990**	0.901**	0.643	0.359	0.732*	
Polyphenol oxidase	0.615	0.630	0.528	-0.397	0.627	-0.608	0.418	0.617	0.565	0.519	0.337	0.546	0.540

*Significant at 5%, **Significant at 1%, Student's t-test.

Table 8. Results of PCA for effect of foliar application of HA, Zn and B on biochemical changes related to productivity of pungent pepper (*C. annuum* L.).

Principle component	Eigen value	Variance	Cumulative variance (%)
Eigen value and variance accounted for (%) by PCA based on correlation matrix			
1	18.63	80.99	80.99
2	1.73	7.53	88.53
3	1.04	4.53	93.07
Variables	**PC1**	**PC2**	**PC3**
Factor loadings due to PCs with Eigen values greater than 1			
Length of plant	0.872	0.324	-0.143
Number of leaves	0.889	0.369	0.139
Leaf area	0.933	-0.118	-0.261
Number of branches	0.953	0.281	-0.049
Number of fruits	0.984	0.111	0.092
Length of fruit	0.857	-0.019	-0.309
Diameter of fruit	0.909	0.012	0.361
Weight of 20 fruits	0.904	0.147	-0.304
Number of seeds	0.950	-0.185	-0.012
Weight of 100 seeds	0.944	0.092	-0.301

Table 8. Contd.

Principle component	Eigen value	Variance	Cumulative variance (%)
Chlorophyll a	0.975	-0.177	0.045
Chlorophyll b	0.960	-0.188	0.186
Ratio of Chl. a/Chl. b	-0.896	0.209	-0.389
Total chlorophyll	0.950	-0.267	0.031
Moisture	-0.948	-0.010	-0.177
Reducing sugar	0.913	-0.279	0.251
Total sugar	0.906	-0.333	-0.252
Starch	0.915	-0.350	0.101
Protein	0.889	0.322	0.225
Fat	0.638	0.754	-0.011
Energy	0.936	0.309	0.082
Total phenol	0.907	-0.311	-0.233
Polyphenol oxidase	0.590	-0.049	-0.358

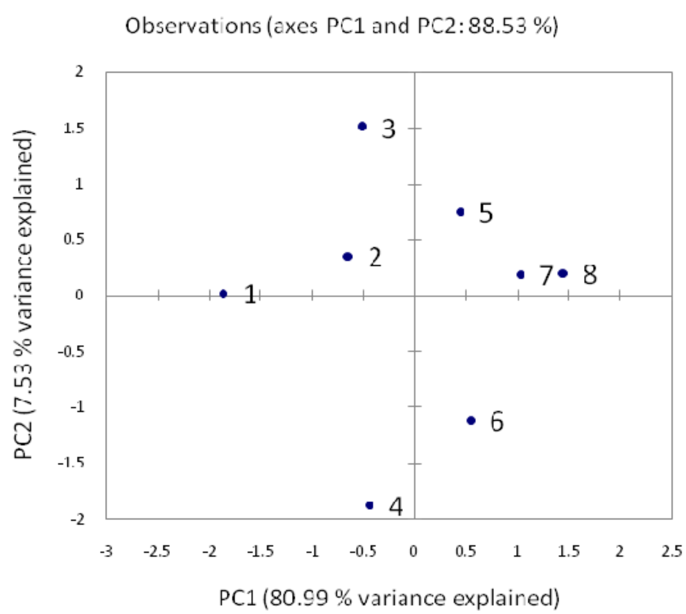

Figure 1. Biplot of regression factor scores for the first and second components produced by PCA: Legend: 1 (Control); 2 (HA); 3 (Zn); 4 (B); 5 (HA+Zn); 6 (HA+B); 7 (Zn+B); 8 (HA+Zn+B).

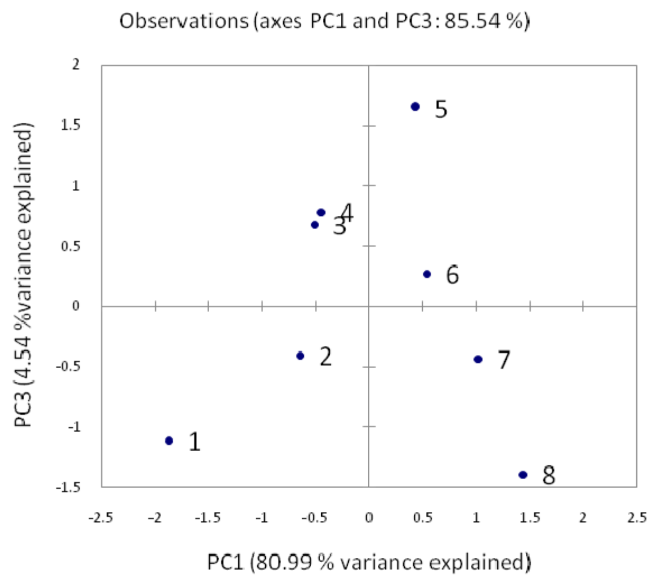

Figure 2. Biplot of regression factor scores for the first and third components produced by PCA: Legend: 1 (Control); 2 (HA); 3 (Zn); 4 (B); 5 (HA+Zn); 6 (HA+B); 7 (Zn+B); 8 (HA+Zn+B)

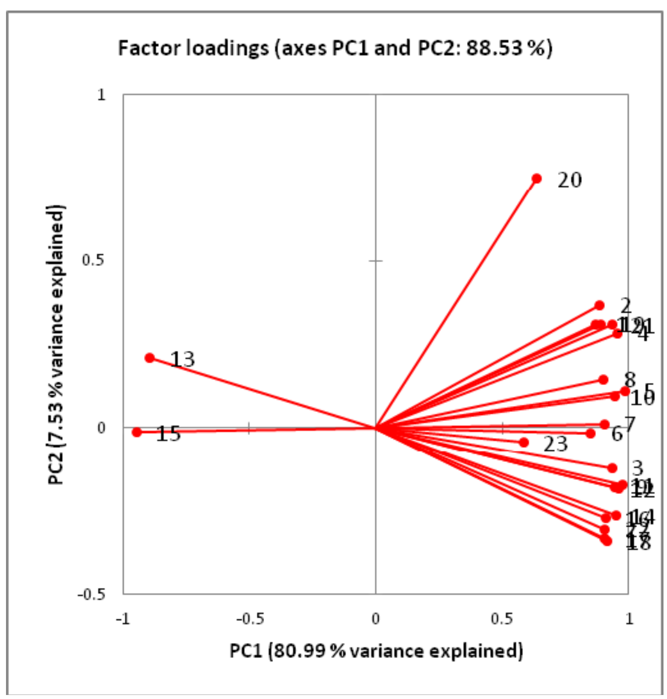

Figure 3. Scatter diagram of correlations between variables and factors loading for the first and second components produced by PCA: Legend: 1 (length of plant); 2 (number of leaves); 3 (Leaf area); 4 (number of branches); 5 (number of fruits); 6 (Length of fruit); 7 (diameter of fruit); 8 (weight of 20 fruits); 9 (Number of seeds); 10 (weight of 100 seeds); 11 (chlorophyll a); 12 (Chlorophyll b); 13 (ratio of Chl. a/Chl. b); 14 (total chlorophyll); 15 (moisture); 16 (reducing sugar); 17 (total sugar); 18 (starch); 19 (protein); 20 (fat); 21 (energy); 22 (total phenol); 23 (polyphenol oxidase).

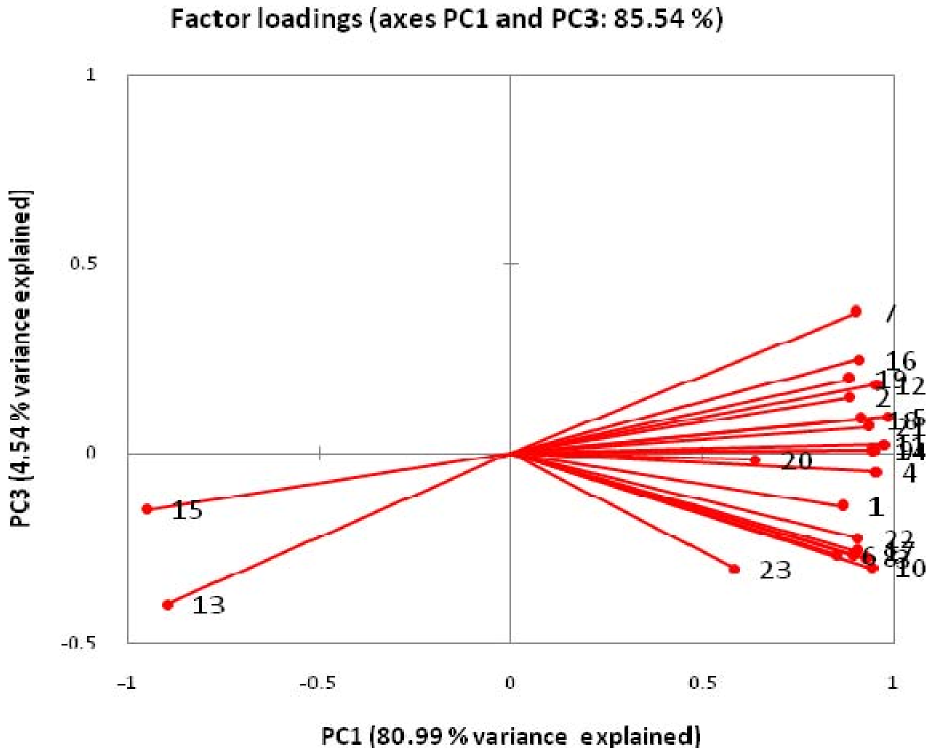

Figure 4. Scatter diagram of correlations between variables and factors loading for the first and third components produced by PCA: Legend: 1 (Length of plant); 2 (Number of leaves); 3 (leaf area); 4 (number of branches); 5 (number of fruits); 6 (Length of fruit); 7 (Diameter of fruit); 8 (weight of 20 fruits); 9 (number of seeds); 10 (weight of 100 seeds); 11 (chlorophyll a); 12 (chlorophyll b); 13 (ratio of Chl. a/Chl. b); 14 (total chlorophyll); 15 (moisture); 16 (reducing sugar); 17 (total sugar); 18 (starch); 19 (protein); 20 (fat); 21 (energy); 22 (total phenol); 23 (polyphenol oxidase).

of leaves, number of branches, number of fruits, diameter of fruit, weight of 20 fruits, weight of 100 seeds, ratio of chlorophyll a:b, protein, fat and energy were associated with a decrease in leaf area, length of fruit, number of seeds, chlorophyll a, chlorophyll b, total chlorophyll, moisture, reducing sugar, total sugar, starch, total phenol and polyphenol oxidase (Table 8 and Figure 3). Based on PC1 and PC2, the treatments such as HA + Zn + B followed by Zn + B and HA + Zn can be selected as performers (Figure 1).

The PC3 explained an additional 4.53% of total variance, in which number of leaves, number of fruits, diameter of fruit, chlorophyll a, chlorophyll b, total chlorophyll, reducing sugar, starch, protein, and energy were positively loaded in contrast to length of plant, leaf area, number of branches, length of fruit, weight of 20 fruits, number of seeds, weight of 100 seeds, ratio of chlorophyll a:b, moisture, total sugar, fat, total phenol and polyphenol oxidase, which were negatively loaded (Table 8 and Figure 4). According to the plot of regression factor scores due to PC1 and PC3, the performing treatment such as HA + Zn followed by Zn can be selected as having all desirable trails (Figure 2).

Conclusions

Based on principle component analysis and considering average values of all variables, our results suggested that the good promising treatment in HA + Zn followed by Zn + B, HA + Zn + B and Zn alone application may bring about the proper value addition in quality as well as productivity of crop by enhancing some physiological and biochemical characteristics in pungent pepper.

Conflict of Interests

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

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