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POTENTIAL OF FOLIAR APPLICATION OF MICRONUTRIENTS ON GROWTH, YIELD, QUALITY AND NUTRACEUTICAL PROPERTIES OF PEA (*PISUM SATIVUM* L.)

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Abstract

Nutrients management has prime importance in agricultural crops for imperative yield. Micronutrients, though needed in low amounts, are essential for different physiological processes, thus improve, growth, yield and nutritive value of food crops. Hence, current study aimed to evaluate the effect of foliar application of different micronutrients on two pea cultivars. Three micronutrients; zinc sulphate ($ZnSO_4$), copper sulphate (Cu_2SO_4) and boric acid (H_3BO_3) were sprayed alone as well as in combinations, each at the level of 3 mg L^{-1}. Meteor cultivar plants had taller plants, and had maximum number of leaves, while those of Pencil produced greater fresh and dry biomass, more number of branches, number of pods and number of seeds per pod, and higher pod fresh and dry weights and leaf chlorophyll content. However, quality traits did not differ between the cultivars. Plant height, fresh as well as dry plant biomass, number of branches as well as number of leaves/ plant were increased through foliar sprays of $ZnSO_4$ + Cu_2SO_4 and $ZnSO_4$ + H_3BO_3. Foliar sprays of $ZnSO_4$ alone greatly increased the length pods as well as 100 seed weight. Plant height revealed significant association with all the studied growth as well as yield traits. Moisture content of seeds had significant relationship with all studied quality traits. Biplot analysis, a multivariate approach, confirmed the mean performance of genotypes as in mean tables and traits relationship as in trait association matrix. Conclusively, current study encourages the use of foliar spray of different micronutrients as mixture for attaining higher pea yields.

Key words: Biplot analysis, Foliar spray, Mixture of micronutrients, Trait association.

Introduction

Green pea (*Pisum sativum* L.), a leguminous vegetable crop, belongs to the family *Fabaceae*. It has good association with nitrogen fixing bacteria, which is an important component for low-input cropping systems worldwide. It is a highly self-pollinated crop (Amjad *et al*., 2004). Green pea is frequently used as human diet because of higher contents of protein, carbohydrates, sugars, vitamin A and C, phosphorus, calcium and amino acids (Bhat *et al*., 2013). Sandy to clayey soils is favorable for successful pea production. Shallow root system increases its water use efficiency. Hence, it is cultivated as a good rotational crop in those areas where water conservation is challenging (Nielsen, 2001). Pea production depends on growing region, growing season, rainfall, climate, soil health, nutritional balance, genetic make-up of genotypes and disease outbreak (Bhat *et al*., 2013). Hence, good management practices can increase the pea production (Bhat *et al*., 2013). Among those, nutrients management has prime importance for higher pea production (Fageria, 2012).

Foliar application of nutrients is one of the imperative methods for sustainable as well as crops production management (Noack *et al*., 2010). Leaching and soil blockages are major constrains that limit the accessibility of soil nutrients to plants. Nutritional problems of crops can be recovered through foliar application (Fernández and Brown, 2013). Hence, foliar application has potential to provide optimum nutrients to plants through leaf to fulfill their nutrient requirements. Plants roots are not active during cold periods, therefore, soil nutrients are useless and not up-taken through roots. However, leaf application is best solution to apply nutrients to the plants for better growth as well as development (Ali *et al*., 2015). The performance of foliar application of nutrients relies on physico-chemical properties of the formulation and absorbance behavior of plants to which nutrients are sprayed. These are factors which influence the efficacy of foliar fertilization (Fernández and Brown, 2013). Foliar fertilization has very high commercial importance all over the world. The use of micronutrients through foliar application is going to increase with passage of time because plant leaves had excellent potential for utilization of nutrients resulting in higher crop growth and production (Hasani *et al*., 2012; Ali *et al*., 2015).

Different micronutrients i.e. zinc (Zn), copper (Cu) and boron (B) have significance importance in higher pea production. Zn plays an imperative role in metabolism regulation, photosynthesis maintenance and regulation of carbohydrate synthesis (Ali *et al*., 2009). The use of zinc sulphate ($ZnSO_4$) as a source of Zn in field crops is very common because of low market prices and easy availability. Its foliar application is very helpful for leaf greening of many crops. Cu is applied to regulate the photosynthesis mechanism, oxidation process, enzyme activity and mitochondrial inhalation in young plants (Quartacci *et al*., 2001). Deficiency or toxicity of Cu limits the protein movement or enzymes action, defective cell movement and imbalance oxidation mechanism. It is usually applied in the form of copper sulphate (Cu_2SO_4). B is involved in root growth, cell wall development and healthy fruits. Boric acid (H_3BO_3) is used as a source of B to increase the production of pea (Fageria *et al*., 2007). Thus, micronutrients especially Zn, Cu and B can be applied to improve growth and increase pea production worldwide.

Huge research work has been conducted on production technology of pea crop, while optimum use of micronutrients through foliar application is still limited. Foliar application of various nutrients is very common method of fertilization in developed countries and $ZnSO_4$, Cu_2SO_4 and H_3BO_3 are regularly applied to some crops. However, application of micronutrients i.e. $ZnSO_4$, Cu_2SO_4 and H_3BO_3 through foliar method is very negligible in pea crop. Soil compaction and high pH are major causes of unavailability of micro-nutrients to plants resulting in poor crop production. Therefore, current work successfully explored the performance of two pea cultivars and three different micronutrients i.e. $ZnSO_4$, Cu_2SO_4 and H_3BO_3.

Materials and Methods

Plant materials: Monthly metrological data was collected from sowing to harvesting of the crop during the both years (2017-18 and 2018-19) and presented in Fig. 1. Seeds of two pea cultivars i.e. Meteor and Pencil were purchased from vegetable seed market, Multan. The seeds were sown on 3rd November 2017 for first year crop and on 5th November 2018 for second year crop. Fruit were picked three times and the last picking was performed on 19th March 2018 and 23rd March 2019, respectively for first and second year crops.

Seeds sowing: Clay pots (45 × 30 cm) were filled with 8 kg loamy soil. Seeds were sown at depth of 2 - 3 cm and three seeds were sown in each pot.

Crop maintenance: Pots were irrigated regularly after every 2 to 3 days without any application of fertilizer. Hoeing and weeding practices were done regularly, when required. After three weeks of germination, thinning was practiced to maintain one uniform plant size in each pot. Confidor 200 SC insecticide was applied to control sucking pest attack on the plants, when needed.

Treatments

T_0 = Control
T_1 = Zinc sulphate ($ZnSO_4$) 3 mg L^{-1}
T_2 = Copper sulphate (Cu_2SO_4) 3 mg L^{-1}
T_3 = Boric acid (H_3BO_3) 3 mg L^{-1}
T_4 = $ZnSO_4$ 3 mg L^{-1} + Cu_2SO_4 3 mg L^{-1}
T_5 = $ZnSO_4$ 3 mg L^{-1} + H_3BO_3 3 mg L^{-1}
T_6 = Cu_2SO_4 3 mg L^{-1} + H_3BO_3 3 mg L^{-1}
T_7 = $ZnSO_4$ 3 mg L^{-1} + Cu_2SO_4 3 mg L^{-1} + H_3BO_3 3 mg L^{-1}

Application of treatments: 1st application of micronutrients was made after four weeks of seed germination, 2nd application at flowering stage and 3rd application was made during pod formation. The experiment was designed on the basis of completely randomized design (CRD) with 3 repeats. Randomly twelve plants were selected and tagged in each treatment for estimation of growth, yield, quality and nutraceutical traits.

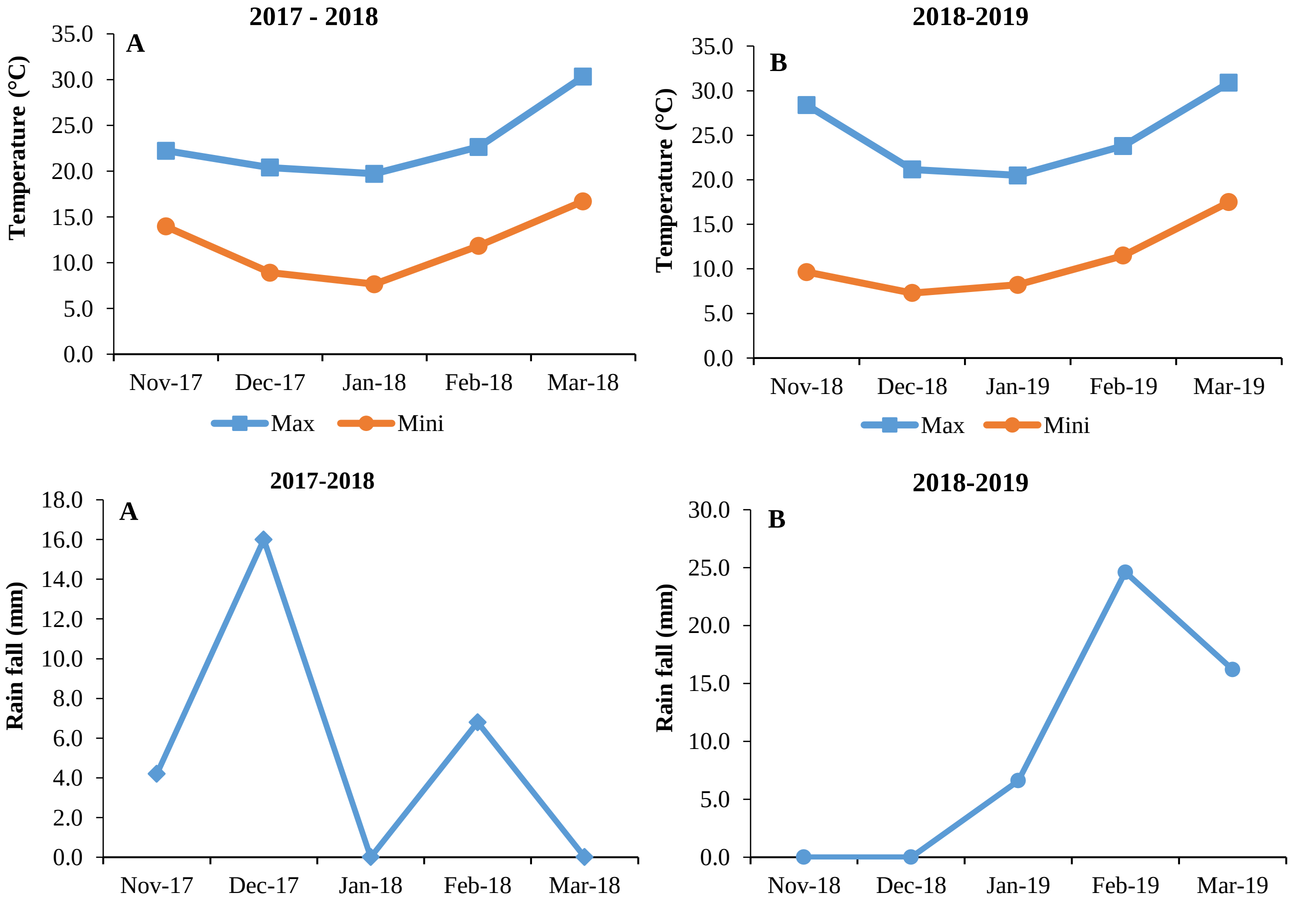

Fig. 1. Monthly maximum and minimum temperatures (A & B) and rainfall data (C & D) collected from sowing to harvesting during 2017-2018 and 2018-2019.

Data recorded

Growth and yield related traits: Plant height was measured through measuring scale. Fresh as well as dry plant biomass, and pods weights were recorded through weighing balance (WT6002-D). Relative leaf chlorophyll content was estimated through chlorophyll meter (SPAD-502 MINOLTA, Japan). Number of branches and leaves per plant, and days to first pod formation were counted. Pod length was measured through Vernier caliper (IKKEGOL).

Quality and nutraceutical related traits: Seed moisture and ash contents were determined by using the following formulas:

$$\text{Seed moisture content (\%)} = \frac{Seed\ weight\ before\ drying - Seed\ weight\ after\ drying}{Seed\ weight\ before\ drying} \times 100$$

$$\text{Ash content of seeds (\%)} = \frac{Weight\ of\ ash}{Weight\ of\ leaf\ sampe} \times 100$$

Table 1. Statistical analyses of different growth, yield and quality traits of pea cultivars.

Trait	F value with significance level			Coefficient of variance
	Cultivars	Treatments	Cultivars × Treatments	
Plant height (cm)	5.77*	39.84**	6.54**	4.21
Fresh plant biomass (g)	25.52**	56.62**	12.08**	5.80
Dry plant biomass (g)	12.19**	40.78**	5.27**	10.88
Number of branches/ plant	9.09**	5.59**	4.73**	10.86
Number of leaves/ plant	18.15**	12.79**	3.11*	6.73
Leaf chlorophyll content (SPAD)	113.68**	36.06**	21.51**	4.31
Days to first pod formation	0.81ns	13.81**	8.65**	1.77
Number of pods/ plant	40.61**	22.75**	2.98*	10.95
Fresh weight of pods/ plant (g)	4.58*	30.06**	4.71**	11.21
Dry weight of pods/ plant (g)	11.35**	40.87**	4.67**	11.55
Pod length (cm)	0.15ns	55.08**	26.65**	3.93
Number of seeds/ pod	17.26**	58.86**	6.44**	7.08
1000 seeds weight (g)	2.07ns	65.28**	9.31**	2.15
Seeds moisture content (%)	2.55ns	151.63**	17.73**	1.42
Ash content of seeds (%)	1.67ns	11.35**	3.10*	7.85
Vitamin C (mg 100 mL^{-1})	2.70ns	39.28**	5.97**	2.84
Phenolics (µg GE mL^{-1})	3.77ns	6.84**	4.67**	7.55
Carotenoids (µg g^{-1})	3.98ns	8.32**	5.48**	10.55

ns = Non-significant, * = Significant at $p = 0.05$, and ** = Significant at $p = 0.01$

100 seed weight was measured using weighing balance (WT6002-D). Vitamin C content in pea seeds was determined by titrating the known quantity of juice against 2, 6-dichlorophenol indophenol (Anjum *et al*., 2020). Phenolics of seeds were recorded through Folin-Ciocalteu reagent method and absorbance reading was noted at 700 nm through spectrophotometer (Anjum *et al*., 2020). Carotenoids were determined by using acetone as well as separated with hexane (Ndawula *et al*., 2004).

Data analysis

The collected data of pea crop were analyzed using a computer software Statistix 8.1 (Tallahassee Florida, USA) applying two way analysis of variance to evaluate the performance of two cultivars under foliar application of different micronutrients. Two years data were pooled and then analyzed because year effect was non-significant. LSD test was applied to separate means at 5% level of probability. Trait association analyses were carried out through R software. Biplot analyses were constructed through XLSTAT, 2019.

Results

Crop growth and some of the yield and quality traits were significantly affected by the cultivars, micronutrients as well as their interaction (Table 1).

Growth traits: Between the cultivars, Meteor had larger plant height than Pencil cultivar. Greater plant height was recorded from application of $ZnSO_4$ + H_3BO_3 and $ZnSO_4$ + Cu_2SO_4, while shorter plant height was measured in control plants (Table 2). The largest plant height was recorded from combined effect of Pencil × $ZnSO_4$ + Cu_2SO_4 and Meteor × $ZnSO_4$ + H_3BO_3, whereas the shortest plant height was measured in Meteor and Pencil cultivars' untreated control plants (Fig. 2A). Pencil had greater fresh as well as dry plant biomass than Meteor. The greater fresh plant biomass was recorded from application of $ZnSO_4$ and $ZnSO_4$ + Cu_2SO_4, followed by $ZnSO_4$ + H_3BO_3, while significantly lower fresh plant biomass was estimated in control (Table 2). The maximum dry plant biomass was observed from application of $ZnSO_4$ + Cu_2SO_4, followed by $ZnSO_4$, while the minimum dry plant biomass was achieved by control plants and H_3BO_3 (Table 2). The highest fresh as well as dry plant biomass were recorded in cultivar Pencil × $ZnSO_4$, followed by Meteor × $ZnSO_4$ + H_3BO_3 application, whereas the lowest fresh as well as dry plant biomass was recorded in cultivar Meteor under control and Meteor × H_3BO_3 application (Fig. 2B & C). Pencil had greater number of branches per plant than Meteor. The higher number of branches per plant was found in $ZnSO_4$, Cu_2SO_4 + H_3BO_3, $ZnSO_4$ + H_3BO_3, followed by Cu_2SO_4, $ZnSO_4$ + Cu_2SO_4 and $ZnSO_4$ + Cu_2SO_4 + H_3BO_3, while lower number of branches per plant was observed in controlled plants, followed by spray of H_3BO_3 treated plants (Table 2). The higher number of branches per plant was counted in Pencil × Cu_2SO_4, whereas lower number of branches per plant was counted in Meteor × control and Meteor × H_3BO_3 (Fig. 2D). Cultivar Meteor had more number of leaves than Pencil. The maximum number of leaves were found in Cu_2SO_4 + H_3BO_3, followed by $ZnSO_4$ and Cu_2SO_4 and $ZnSO_4$ + H_3BO_3, while the minimum was present in control plants (Table 2). Higher number of leaves/ plant was counted in Pencil × Cu_2SO_4 + H_3BO_3 as well as Pencil × $ZnSO_4$ + H_3BO_3, followed by Pencil × $ZnSO_4$ + Cu_2SO_4. However, meager number of leaves/ plant was recorded in Meteor × control and Pencil × control (Fig. 2E). Leaf chlorophyll content was higher in Pencil as compared to the cultivar Meteor. The higher chlorophyll content of leaves was recorded in $ZnSO_4$ + H_3BO_3, while lower chlorophyll content of leaves was measured in control plants (Table 2). Leaf chlorophyll content were more in combined effect of Pencil × $ZnSO_4$ + H_3BO_3 and Pencil × Cu_2SO_4 + H_3BO_3, whereas less leaf chlorophyll content was found in Meteor × control (Fig. 2F).

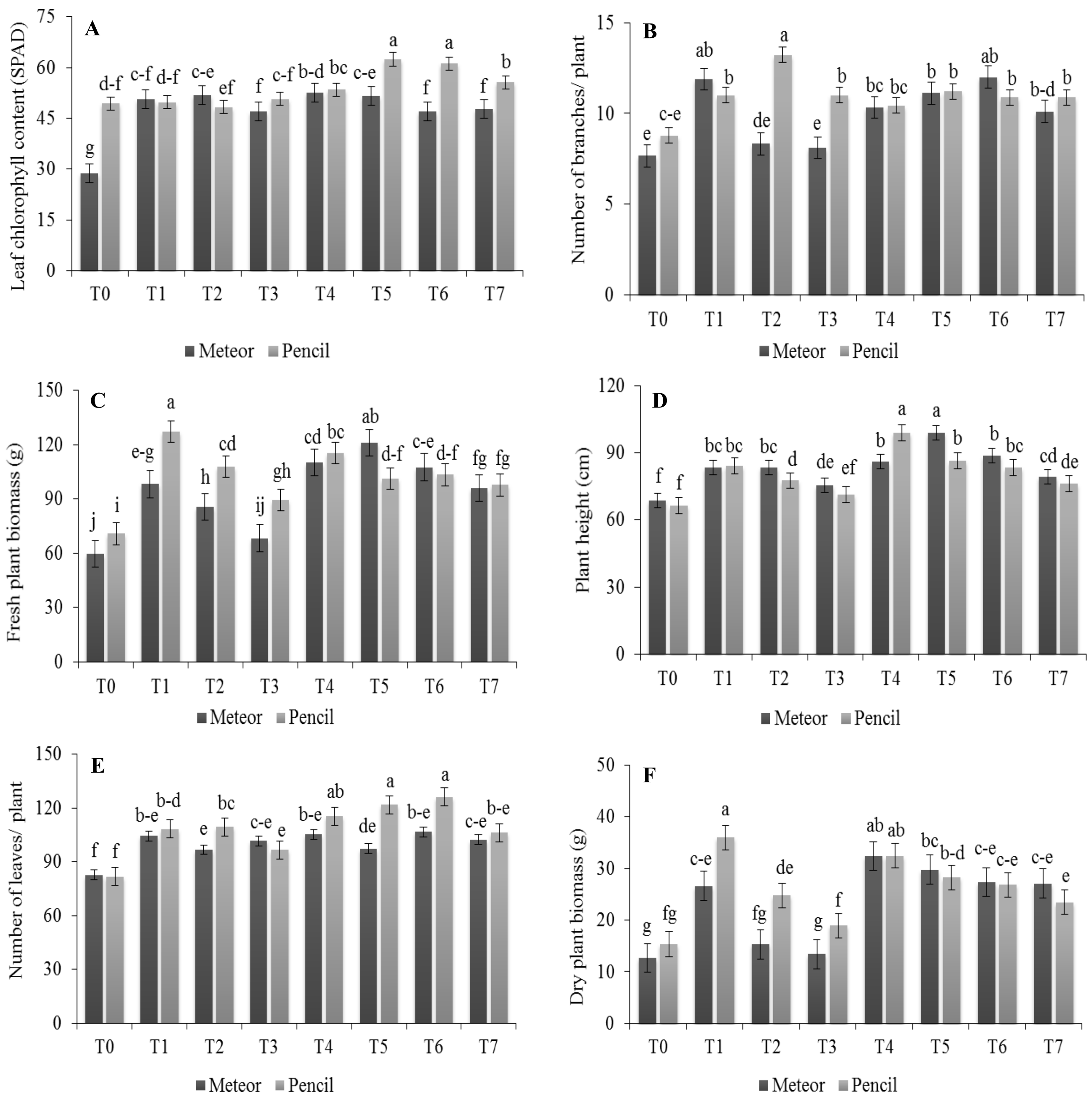

Fig. 2. Growth traits of pea as affected by cultivars and micronutrients. Vertical bars indicate standard errors of the means and data are mean of three biological replicates. Different letters indicate significant differences among the means according to LSD test $p \leq 0.05$. T_0 = control, T_1 = $ZnSO_4$, T_2 = Cu_2SO_4, T_3 = H_3BO_3, T_4 = $ZnSO_4$ + Cu_2SO_4, T_5 = $ZnSO_4$ + H_3BO_3, T_6 = Cu_2SO_4 + H_3BO_3 and T_7 = $ZnSO_4$ + Cu_2SO_4 + H_3BO_3.

Table 2. Growth related traits of pea as affected by cultivars and micronutrients.

Cultivar/ Treatment	Plant height (cm)	Fresh plant biomass (g)	Dry plant biomass (g)	Number of branches/ plant	Number of leaves/ plant	Leaf chlorophyll content (SPAD)
Meteor	82.96 a	93.38 b	23.06 b	9.94 b	108.15 a	47.17 b
Pencil	80.57 b	101.63 a	25.74 a	10.93 a	99.56 b	53.86 a
LSD value	*2.03*	*3.34*	*1.56*	*0.67*	*4.12*	*1.28*
Control	67.50 f	65.16 e	14.00 f	8.22 c	82.17 d	39.00 e
$ZnSO_4$	83.78 bc	112.80 a	31.27 ab	11.45 a	106.33 bc	50.22 cd
Cu_2SO_4	80.44 cd	96.82 c	20.03 e	10.78 ab	103.00 bc	50.11 cd
H_3BO_3	73.33 e	78.85 d	16.18 f	9.55 bc	99.00 c	48.89 d
$ZnSO_4 + Cu_2SO_4$	92.61 a	112.78 a	32.42 a	10.39 ab	110.28 ab	53.00 b
$ZnSO_4 + H_3BO_3$	92.72 a	111.23 ab	28.98 bc	11.17 a	109.44 ab	57.06 a
$Cu_2SO_4 + H_3BO_3$	85.94 b	105.50 b	27.07 cd	11.44 a	116.33 a	54.17 b
$ZnSO_4 + Cu_2SO_4 + H_3BO_3$	77.78 d	96.91 c	25.26 d	10.50 ab	104.28 bc	51.667 bc
LSD value	*4.06*	*6.67*	*3.13*	*1.34*	*8.24*	*2.56*

*Mean values sharing similar letter(s) in a column separately for cultivars and micronutrient treatments are statistically non-significant at $p = 0.05$ (LSD test)

Table 3. Yield related traits of pea as affected by cultivars and micronutrients.

Cultivar/ Treatment	Days to first pod formation	Number of pods/ plant	Fresh weight of pods/ plant (g)	Dry weight of pods/ plant (g)	Pod length (mm)	Number of seeds/ pod
Meteor	69.68 a	8.68 b	25.76 b	10.62 b	83.71 a	6.25 b
Pencil	69.36 a	10.62 a	27.61 a	11.89 a	83.35 a	6.80 a
LSD value	*0.73*	*0.62*	*1.76*	*0.37*	*1.94*	*0.22*
Control	66.44 d	5.55 e	13.00 d	6.27 f	63.17 g	4.17 e
$ZnSO_4$	68.94 bc	9.50 cd	29.28 ab	14.67 ab	93.67 a	7.05 b
Cu_2SO_4	69.67 b	9.05 d	30.78 ab	9.11 e	81.50 e	5.50 c
H_3BO_3	68.00 c	8.33 d	19.89 c	7.16 f	76.28 f	4.94 d
$ZnSO_4 + Cu_2SO_4$	72.22 a	11.94 a	32.11 a	15.22 a	91.61 ab	7.55 b
$ZnSO_4 + H_3BO_3$	71.78 a	11.50 ab	28.06 b	13.61 bc	89.78 bc	7.55 b
$Cu_2SO_4 + H_3BO_3$	69.56 b	10.56 bc	31.00 ab	12.44 cd	85.00 de	8.11 a
$ZnSO_4 + Cu_2SO_4 + H_3BO_3$	69.56 b	10.78 ab	29.39 ab	11.56 d	87.22 cd	7.33 b
LSD value	*1.45*	*1.25*	*3.53*	*0.73*	*3.87*	*0.41*

*Mean values sharing similar letter(s) in a column separately for cultivars and micronutrient treatments are statistically non-significant at $p = 0.05$ (LSD test)

Yield related traits: Days to first pod formations were not affected by the cultivars studied. The maximum days to first pod formation was counted in $ZnSO_4 + Cu_2SO_4$ and $ZnSO_4 + H_3BO_3$ foliar sprays. However, days to first pod formation was the minimum in control (Table 3). The higher number of days to first pod formation was counted in Pencil × $ZnSO_4 + Cu_2SO_4$ and Meteor × $ZnSO_4 + H_3BO_3$, while lower number of days to first pod formation was in Pencil × control, followed by Pencil × H_3BO_3 (Fig. 3A). The cultivar Pencil had the greater number of pods per plant than Meteor. The highest number of pods per plant was recorded in $ZnSO_4 + Cu_2SO_4$, followed by $ZnSO_4 + H_3BO_3$, while the lowest number of pods per plant was observed in control treatment (Table 3). The highest number of pods per plant was recorded from combined effect of Pencil × $ZnSO_4 + Cu_2SO_4$, followed by Pencil × $ZnSO_4 + H_3BO_3$, while the lowest number of pods per plant was noted in both Meteor and Pencil cultivars under control treatment (Fig. 3B). Cultivar Pencil had the greater fresh and dry weights of pods per plant, while lower fresh as well as dry weights were observed in Meteor (Table 3). Fresh weight of pods/ plant was recorded more from $ZnSO_4 + Cu_2SO_4$, followed by $Cu_2SO_4 + H_3BO_3$, Cu_2SO_4, $ZnSO_4 + Cu_2SO_4 + H_3BO_3$ and $ZnSO_4$ treatments, while significantly less fresh weight of pods/ plant was noted in control (Table 3). The greater dry weight of pods per plant was estimated from application of $ZnSO_4 + Cu_2SO_4$, followed by $ZnSO_4$, while lesser dry weight of pods/ plant was found in control plants and H_3BO_3 treated ones (Table 3). The higher fresh pod weight per plant was found in cultivar Pencil × $ZnSO_4$, followed by Meteor × $ZnSO_4 + Cu_2SO_4$ and Meteor × $Cu_2SO_4 + H_3BO_3$, while the lower fresh pod weight/ plant was recorded in cultivar Meteor under control (Fig. 3C). Dry pod weight/ plant was more in Pencil × $ZnSO_4$, followed by both Pencil and Meteor cultivars under $ZnSO_4 + Cu_2SO_4$ treatment, while lesser dry pod weight/ plant was recorded in cultivar Meteor under control treatment and Meteor × H_3BO_3, followed by Meteor × Cu_2SO_4

(Fig. 3D). Pod length was found to be almost similar in both pea cultivars and was statistically non-significant. The larger pod length was recorded in $ZnSO_4$, followed by $ZnSO_4$ + Cu_2SO_4 sprayed plants, while shorter pod length was measured in control plants (Table 3). The largest pod length was recorded from combined effect of Pencil × $ZnSO_4$ and Meteor × $ZnSO_4$ + Cu_2SO_4, Meteor × $ZnSO_4$ + H_3BO_3 and Meteor × $ZnSO_4$ + H_3BO_3, while the shortest pod length was measured in Meteor under control treatment (Fig. 3E). The cultivar Pencil had greater number of seeds per pod than cultivar Meteor. The highest number of seeds per plant was counted from Cu_2SO_4 + H_3BO_3 treated plants, while the lowest number of seeds per plant was counted in control plants (Table 3). The higher number of seeds per pod was calculated in Pencil × $ZnSO_4$ + Cu_2SO_4 and Meteor × Cu_2SO_4 + H_3BO_3, followed by Pencil × $ZnSO_4$, while lower number of seeds per pod was recorded in Meteor × control and Pencil × control, followed by Meteor × H_3BO_3 (Fig. 3F).

Quality traits: 100 seed weight was not significantly affected between the studied pea cultivars. Higher 100 seeds weight was recorded in $ZnSO_4$ + Cu_2SO_4 and $ZnSO_4$, whereas lower 100 seeds weight was observed in control (Table 4). The maximum 100 seed weight was in Pencil × Cu_2SO_4, followed by Meteor × $ZnSO_4$ + Cu_2SO_4 and Pencil × $ZnSO_4$ + Cu_2SO_4, while lower 100 seed weight was in Pencil × control, followed by Meteor × control and Pencil × H_3BO_3 (Fig. 4A). Moisture content was non-significant between the studied pea cultivars. The greater moisture content in seeds was estimated in $ZnSO_4$ + H_3BO_3, followed by $ZnSO_4$ + Cu_2SO_4, while lesser moisture content in seeds was measured in control plants (Table 4). The maximum moisture content was recorded from combined effect of Meteor × $ZnSO_4$ + H_3BO_3, and Pencil × Cu_2SO_4, followed by Pencil × $ZnSO_4$ + Cu_2SO_4, while the minimum moisture content was measured in Meteor and Pencil cultivars from control treatment (Fig. 4B). Ash content was not significantly affected between the studied pea cultivars. The higher ash content was in $ZnSO_4$ + H_3BO_3, followed by $ZnSO_4$ + Cu_2SO_4 and Cu_2SO_4 + H_3BO_3, whereas the lower ash content was in control, followed by H_3BO_3 (Table 4). The higher ash content was recorded from combined effect of Meteor × $ZnSO_4$ + H_3BO_3, followed by Pencil × $ZnSO_4$ + Cu_2SO_4, Pencil × $ZnSO_4$ + H_3BO_3 and Pencil × Cu_2SO_4 + H_3BO_3, whereas lower ash content was in both Meteor and Pencil cultivars from control treatment (Fig. 4C). Vitamin C content was not significantly affected between the studied pea cultivars. The maximum vitamin C content was estimated from application of $ZnSO_4$ + H_3BO_3, $ZnSO_4$ + Cu_2SO_4, while vitamin C was the minimum in control treatment and H_3BO_3 (Table 4). The highest vitamin C content was estimated from combined effect of Pencil × $ZnSO_4$ + Cu_2SO_4, Meteor × $ZnSO_4$ + H_3BO_3, followed by Pencil × $ZnSO_4$ + H_3BO_3, while the minimum vitamin C was determined in Pencil × H_3BO_3, followed by Meteor × control, Pencil × control and Meteor × H_3BO_3 (Fig. 4D). Phenolics were not significantly affected between the studied pea cultivars. The highest phenolics content was recorded from $ZnSO_4$ + H_3BO_3, followed by $ZnSO_4$, $ZnSO_4$ + Cu_2SO_4 and Cu_2SO_4 + H_3BO_3, while the lowest content was measured in control, followed by $ZnSO_4$ + Cu_2SO_4 + H_3BO_3 (Table 4). The highest phenolics content was recorded from interaction of Pencil × $ZnSO_4$ + Cu_2SO_4, followed by Pencil × Cu_2SO_4, Pencil × $ZnSO_4$, Meteor × $ZnSO_4$ + H_3BO_3 and Pencil × $ZnSO_4$ + H_3BO_3, Pencil × Cu_2SO_4 + H_3BO_3, while the lowest content was measured in Meteor × control (Fig. 4E). Meteor and Pencil did not show any variation in carotenoids of seeds. The greater carotenoids content was recorded from $ZnSO_4$ + H_3BO_3, followed by $ZnSO_4$ and $ZnSO_4$ + Cu_2SO_4, while the lesser content was determined in control (Table 4). The higher carotenoids content was recorded from interaction of Pencil × $ZnSO_4$ + Cu_2SO_4, followed by Meteor and Pencil × $ZnSO_4$ + H_3BO_3, Pencil × Cu_2SO_4 and Pencil × $ZnSO_4$, while the lower carotenoids content was measured in Meteor × control treatment (Fig. 4F).

Table 4. Quality traits and nutraceutical properties of pea as affected by cultivars and micronutrients

Cultivar/ Treatment	100 seeds weight (g)	Moisture content of seed (%)	Ash content of seed (%)	Vitamin C (mg 100 mL^{-1})	Phenolics (µg GE mL^{-1})	Carotenoids (µg g^{-1})
Meteor	40.19 a	76.01 a	4.04 a	42.62 a	20.25 a	13.29 a
Pencil	40.55 a	76.51 a	4.16 a	43.20 a	21.12 a	14.12 a
LSD value	*0.69*	*0.64*	*0.19*	*0.72*	*0.92*	*0.85*
Control	36.00 f	67.33 f	3.44 f	38.81 e	17.67 d	10.50 d
$ZnSO_4$	44.11 a	79.61 b	3.92 de	42.48 cd	21.83 ab	15.00 ab
Cu_2SO_4	40.28 c	72.67 d	4.03 cd	41.21 d	20.17 bc	13.50 bc
H_3BO_3	37.72 e	70.28 e	3.57 ef	39.13 e	20.50 bc	13.50 bc
$ZnSO_4$ + Cu_2SO_4	44.22 a	80.78 ab	4.47 ab	46.12 a	21.83 ab	14.67 ab
$ZnSO_4$ + H_3BO_3	41.39 b	81.94 a	4.70 a	47.43 a	23.00 a	16.00 a
Cu_2SO_4 + H_3BO_3	39.11 d	80.11 b	4.40 abc	43.48 bc	21.17 abc	14.17 b
$ZnSO_4$ + Cu_2SO_4 + H_3BO_3	40.17 c	77.39 c	4.27 bcd	44.65 b	19.33 cd	12.33 c
LSD value	*1.41*	*1.28*	*0.38*	*1.44*	*1.81*	*1.71*

*Mean values sharing similar letter(s) in a column separately for cultivars and micronutrient treatments are statistically non-significant at $p = 0.05$ (LSD test)

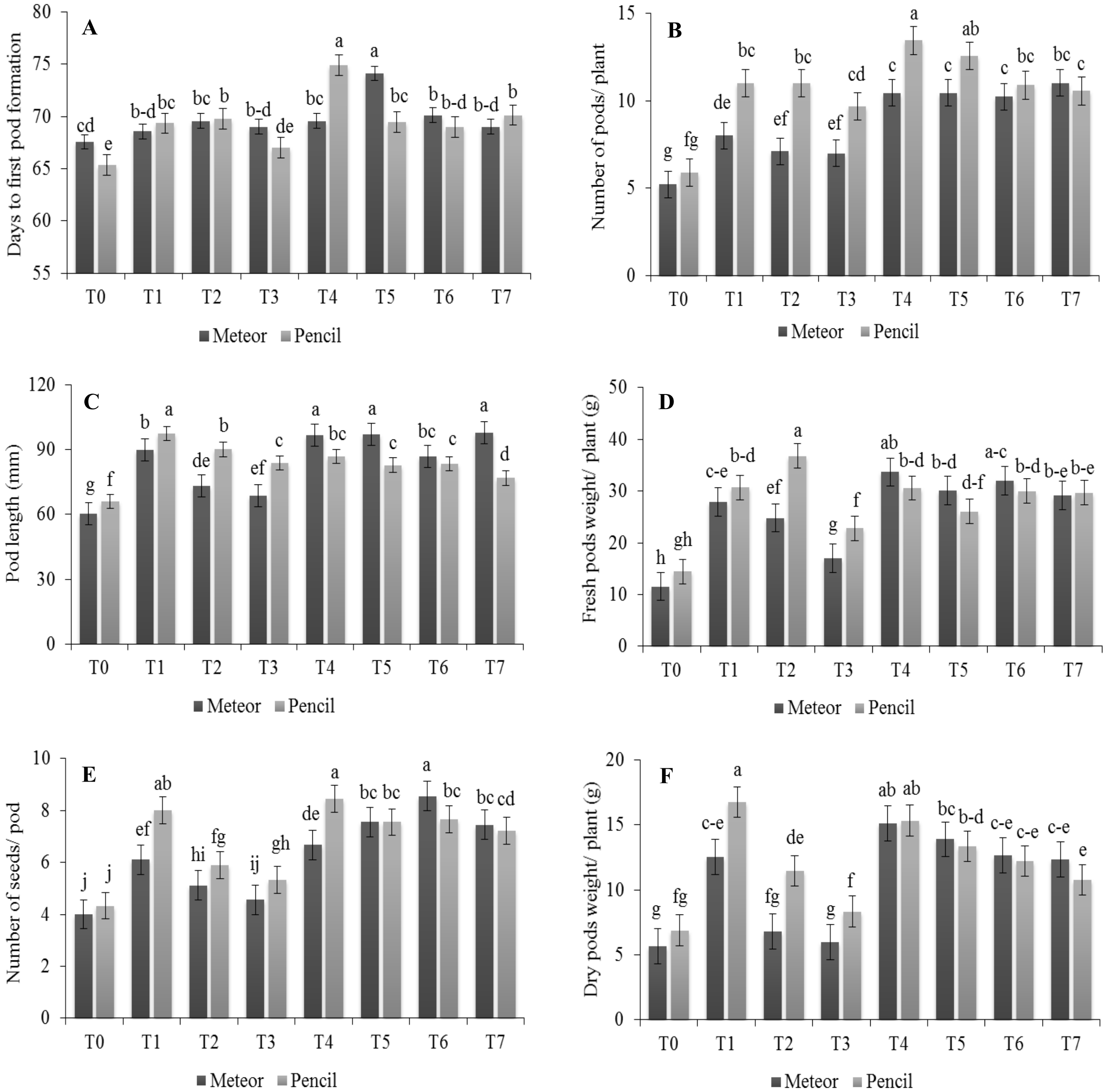

Fig. 3. Yield related traits of pea as affected by cultivars and micronutrients. Vertical bars indicate standard errors of the means and data are mean of three biological replicates. Different letters indicate significant differences among the means according to LSD test $p \leq 0.05$. T_0 = control, T_1 = $ZnSO_4$, T_2 = Cu_2SO_4, T_3 = H_3BO_3, T_4 = $ZnSO_4$ + Cu_2SO_4, T_5 = $ZnSO_4$ + H_3BO_3, T_6 = Cu_2SO_4 + H_3BO_3 and T_7 = $ZnSO_4$ + Cu_2SO_4 + H_3BO_3

Trait association matrix: Plant height has significant association with fresh plant biomass (0.93***), dry plant biomass (0.89**), number of branches/ plant (0.77*), number of leaves/ plant (0.85**), leaf chlorophyll content (0.87**), days to first pod formation (0.94**), number of pods per plant (0.89**), fresh weight of pods per plant (0.80*), dry weight of pods per plant (0.89**), pod length (0.84**) and number of seeds per pod (0.84**). Detailed description of growth and yield related traits and their association analyses are presented in Figure 5. 100 seed weight significantly linked with moisture content of seeds (0.90**), vitamin C content (0.67*), phenolics (0.73*) and carotenoids (0.74*). However, ash content of seeds did not exhibit any association with 100 seed weight (0.59) (Fig. 6).

Biplot analyses: Significant trait association was recorded through biplot analysis because angle among all the studied traits was less than 90°. Similar trait association was recorded through biplot analysis as in correlation matrix. Fresh plant biomass had significant association with dry plant biomass, plant height, fresh and dry weight of pods, leaf chlorophyll content, pod length, days to first pod formation, number of pods and number of seeds per pod because angle was less than 90°. Moreover, biplot analysis showed the performance of different treatments of micronutrients. Five different treatments i.e. $ZnSO_4$, $ZnSO_4$ + Cu_2SO_4, $ZnSO_4$ + H_3BO_3, Cu_2SO_4 + H_3BO_3 and $ZnSO_4$ + Cu_2SO_4 + H_3BO_3 improved the growth, yield and quality traits and performed better than control, H_3BO_3 as well as Cu_2SO_4 treatments (Figs. 7 & 8).

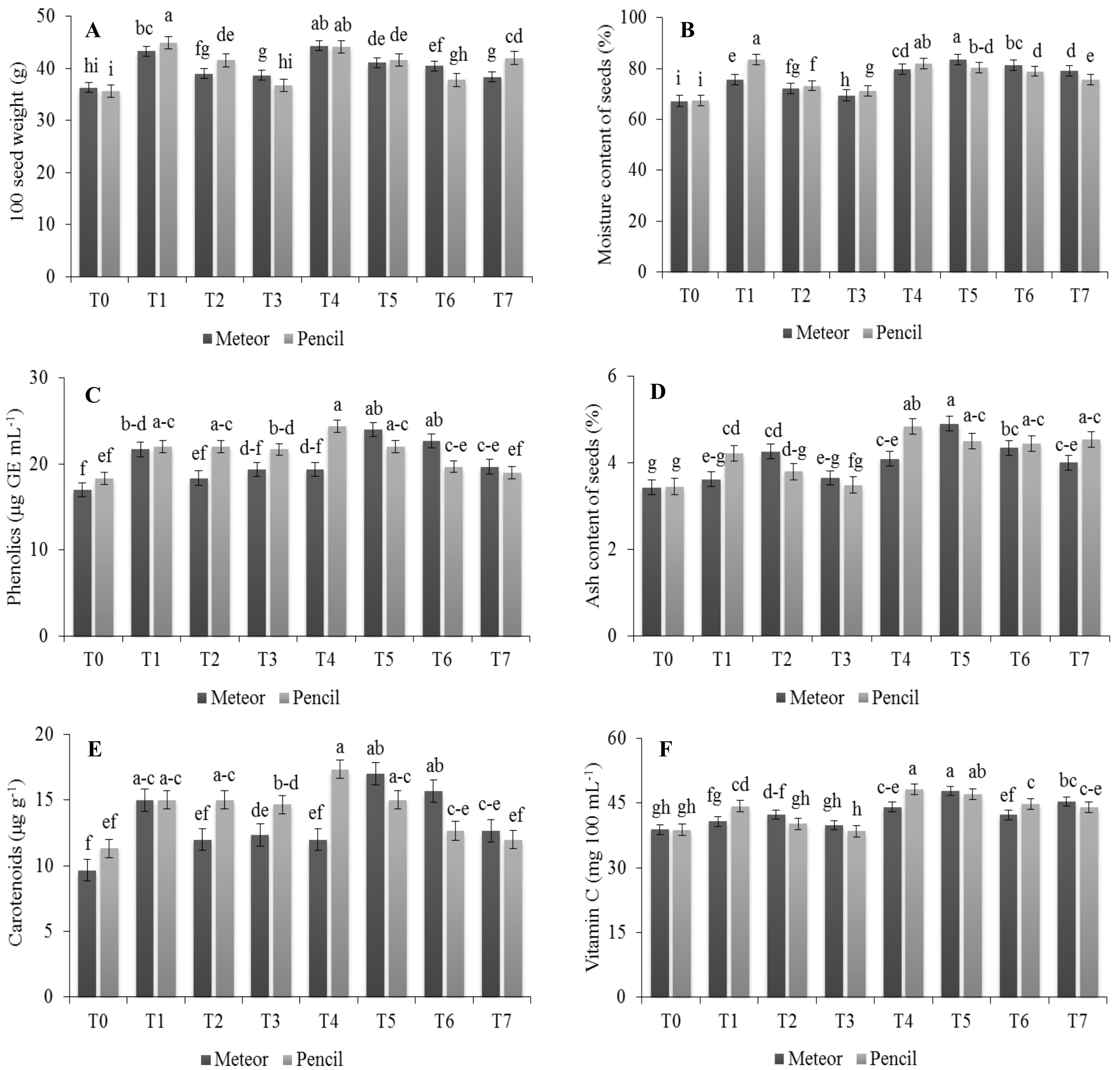

Fig. 4. Quality and nutraceutical properties of pea as affected by cultivars and micronutrients. Vertical bars indicate standard errors of the means and data are mean of three biological replicates. Different letters indicate significant differences among the means according to LSD test $p \leq 0.05$. T_0 = control, T_1 = $ZnSO_4$, T_2 = Cu_2SO_4, T_3 = H_3BO_3, T_4 = $ZnSO_4$ + Cu_2SO_4, T_5 = $ZnSO_4$ + H_3BO_3, T_6 = Cu_2SO_4 + H_3BO_3 and T_7 = $ZnSO_4$ + Cu_2SO_4 + H_3BO_3

Discussion

In the present study, the cultivars Pencil and Meteor differed in some of the growth and yield traits. Therefore, present work is in line with earlier findings (Moghazy *et al.*, 2014) which reported that the performance of cultivars vary from one cultivar to other because of variability in genetic make-up and adaptation under different climatic conditions. Different cultural practices, nutritional management, irrigation requirements and numerous other factors are major causes of variation for the growth and yield parameters between cultivars (Achakzai & Bangulzai, 2006).

Different growth parameters i.e. plant height, fresh as well as dry plant biomass, number of branches/ plant, number of leaves/ plant and leaf chlorophyll content were increased because of foliar application of $ZnSO_4$ + Cu_2SO_4, and $ZnSO_4$ + H_3BO_3. Cu and Zn are important micronutrients involved in the maintenance of photosynthesis mechanism, regulation of carbohydrates and multiplication of proteins (Quartacci *et al.*, 2001; Akay, 2011). B plays a vital role in increase of plant growth. It is involved in root elongation, cell wall formation and metabolism of RNA (Moghazy *et al.*, 2014). Hence, present work is in agreement with earlier work because use of $ZnSO_4$, Cu_2SO_4 and H_3BO_3 significantly increased the plant height, fresh as well as dry plant biomass, number of branches/ plant as well as number of leaves/ plant (Mary and Dale, 1990). Similarly, previous study confirmed that mixture of different micronutrients had excellent potential to improve crop growth (Kumari *et al.*, 2009).

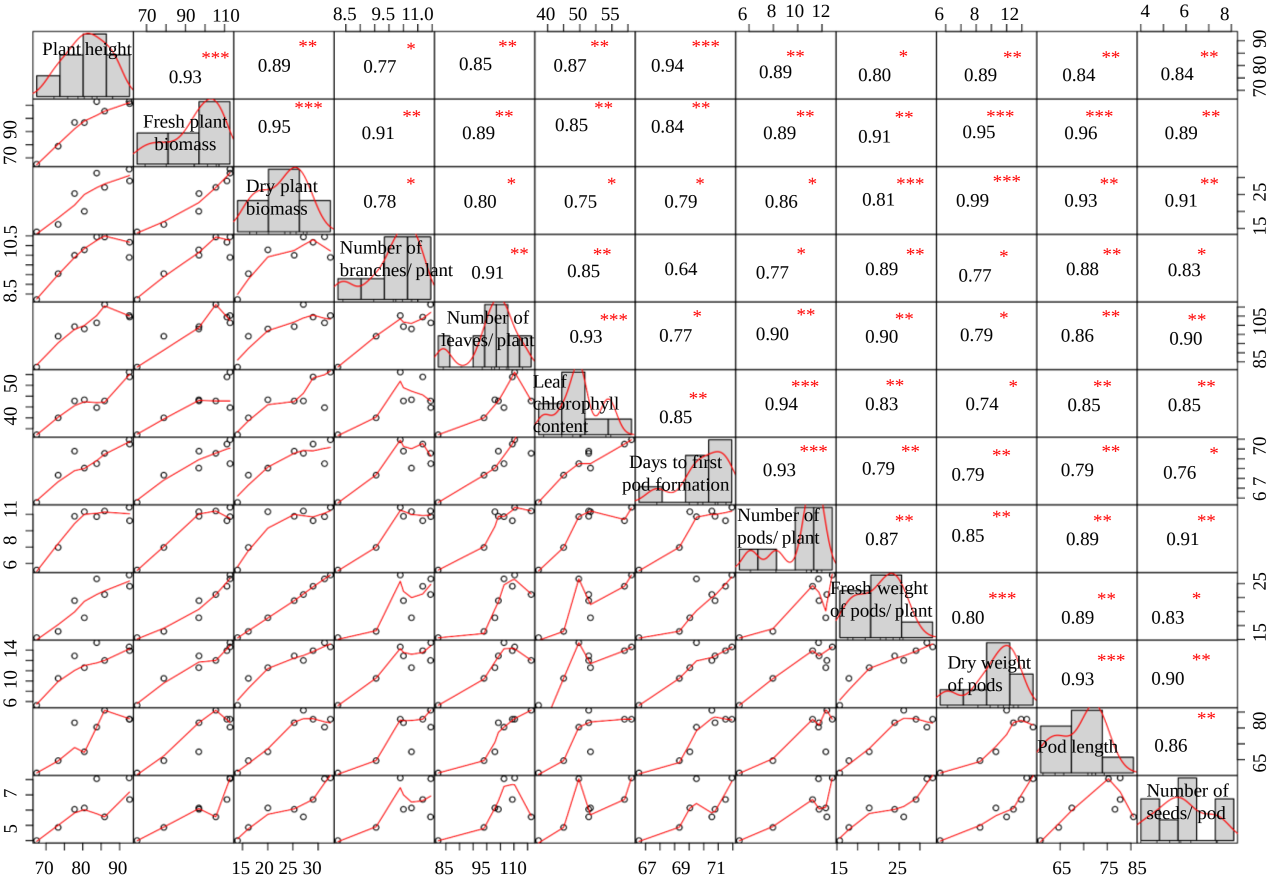

Fig. 5. Correlation matrix among growth and yield related traits of pea cultivars.

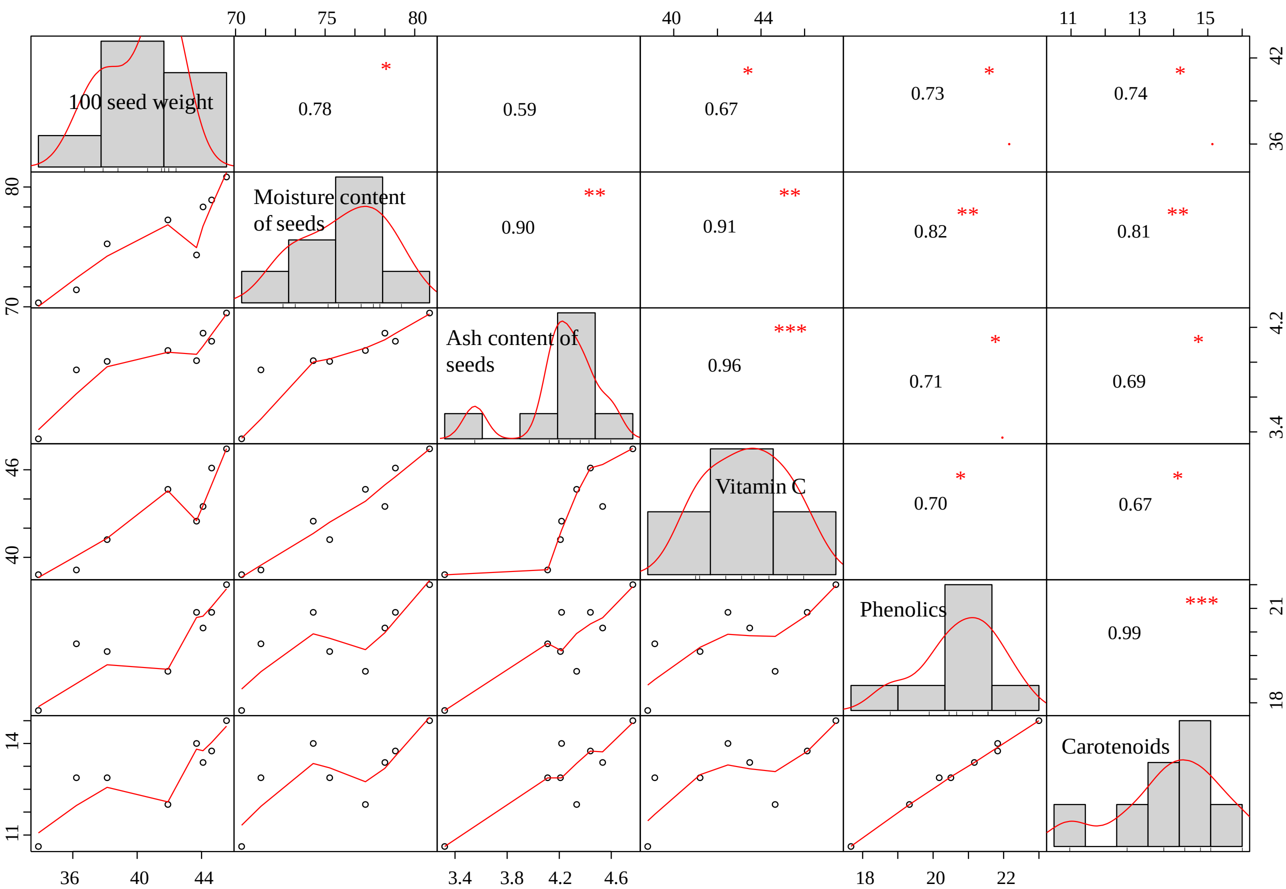

Fig. 6. Correlation matrix among quality and nutraceutical traits of pea cultivars.

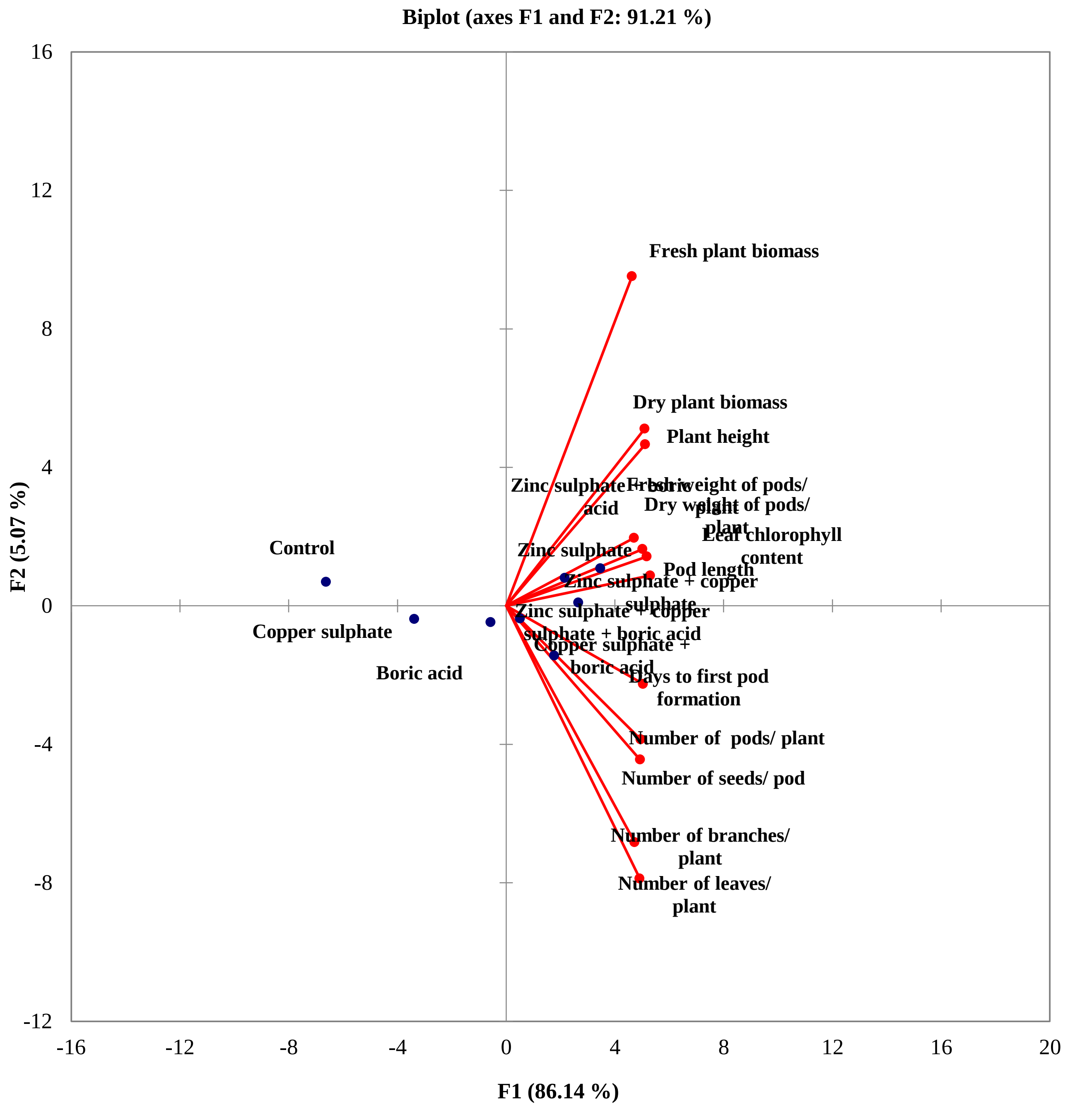

Fig. 7. Biplot analysis of growth and yield related traits of pea cultivars.

Numerous yield parameters i.e. days to first pod formation, fresh as well as dry weight of pods per plant, pod length, number of seeds per pod were improved by foliar spray of the micronutrients. Hence, in current study a mixture of three different micronutrients increased the yield of pea cultivars. Previous findings concluded that combination of different micronutrients is more effective for pea crop because these nutrients are required for different processes in small quantities and also resulted in higher yield of chilli (Kumari *et al*., 2009). Earlier work agreed with the present findings because foliar spray of $ZnSO_4$, Cu_2SO_4 and H_3BO_3 improved the yield related traits i.e. number of pods per plant, fresh as well as dry weight of pods per plant and number of seeds per pod (Dutta *et al*., 2000; Montenegro *et al*., 2010). Recently, Sultana *et al*., (2016) also confirmed that combination of different doses of micronutrients i.e. $ZnSO_4$ and Cu_2SO_4 and H_3BO_3 are very suitable for increased crop production.

The mixture of nutrients $ZnSO_4$ and H_3BO_3 enhanced the quality and nutraceutical related traits i.e. moisture content of seeds, ash content of seeds, vitamin C, phenolics and carotenoids contents in the present research. Current study indicated that foliar spray of different micronutrients enhanced the quality and nutraceutical related traits of peas. Previous studies confirmed the current study results because mixture of different micronutrients increased quality traits i.e. 100 seeds weight, moisture and ash content of seeds, vitamin C, phenolics and carotenoid in different crops including tomato (Broadley *et al*., 2007; Sbartai *et al*., 2011).

Trait association is an imperative way to determine the relationship among different traits. Many plant breeders showed vital interest in trait relationship analysis for development of higher yielding cultivars. Correlation studies have also been used in different crops (Anjum *et al*., 2018; Anjum *et al*., 2019). Regarding the growth and yield related traits, the increase of plant height significantly enhanced the fresh as well as dry plant biomass, days to first pod formation, number of pods per plant, fresh and dry weight of pods per plant, pod length and number of seeds per pod. These are dependent on each other because increase of one trait significantly increased the other. Hence, current work is line to an earlier finding (Anjum *et al*., 2019). Regarding the quality traits i.e. moisture content of seeds is linked with ash content of seeds, vitamin C, phenolics and carotenoids contents. However, ash content of seeds did not exhibit any association with 100 seed weight and remained independent in the present work. Similar correlation among quality traits was recorded in an earlier work (Anjum *et al*., 2018).

Biplot analyses detected that five different treatments i.e. $ZnSO_4$, $ZnSO_4$ + Cu_2SO_4, $ZnSO_4$ + H_3BO_3, Cu_2SO_4 + H_3BO_3 and $ZnSO_4$ + Cu_2SO_4 + H_3BO_3 performed comparatively better. These treatments increased the growth, yield, quality or nutraceutical trait of pea cultivars. Hence, these treatments remained near the trait vector. However, three different treatments i.e. control, H_3BO_3 and Cu_2SO_4 did not performed better and were not involved in increase of any growth, yield or quality trait. Hence, these treatments stood far away from the traits vector. Biplot analyses are considered as multivariate approach that can be used for evaluation of treatment performance and trait association (Anjum *et al*., 2018).

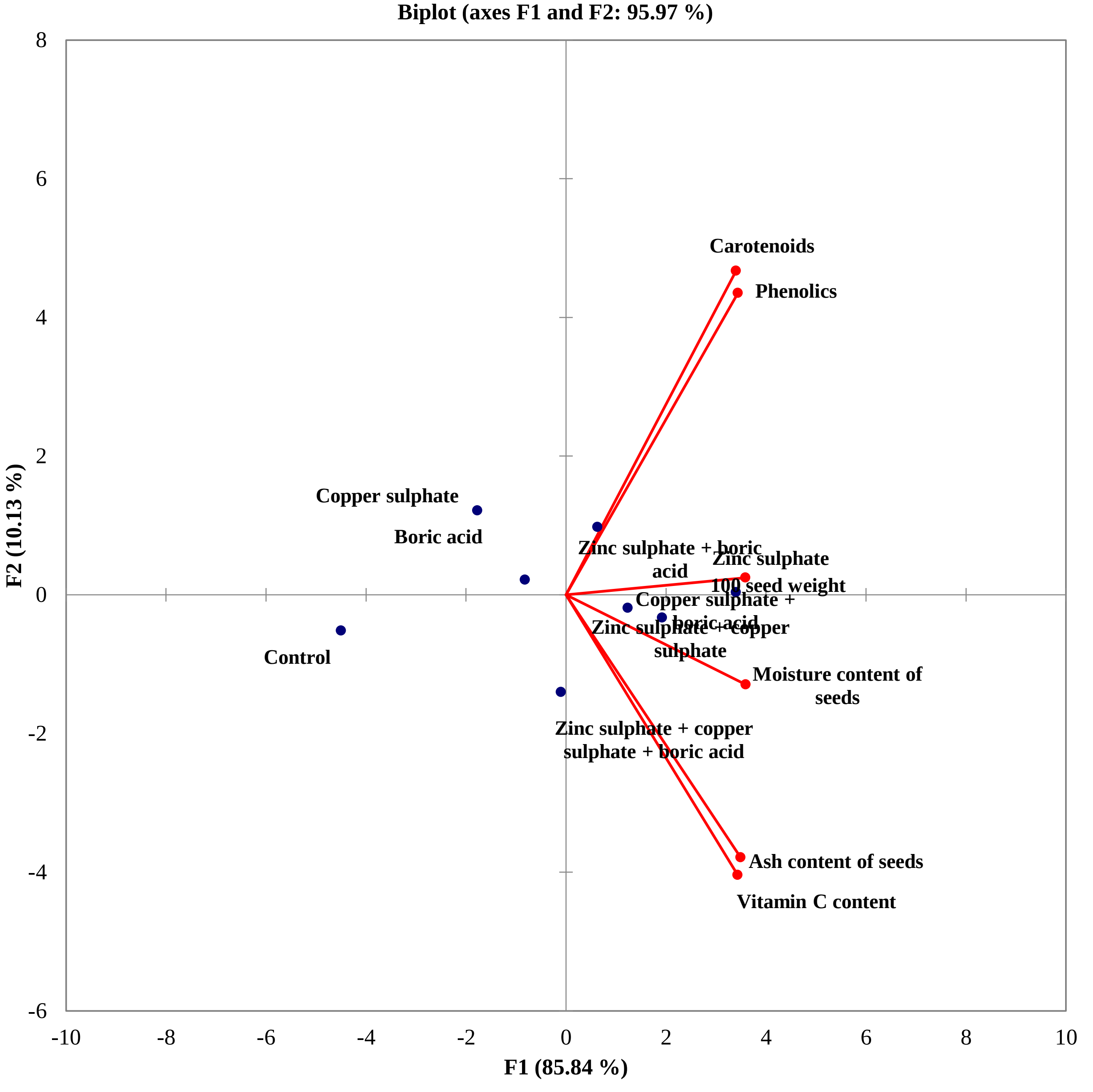

Fig. 8. Biplot analysis of quality and nutraceutical related traits of pea cultivars. The current study is novel work as very little work is done on foliar application of nutrients in this crop. I reviewed this article and strongly recommend for publication with some minor correction.

Conclusion

Pea is one of the most valuable vegetables worldwide. It is imperative to work on improvement of yield related traits of this crop. Foliar application is an effective approach for application of micronutrients. Leaves are excellent and rapid source of nutrient absorbance in plants. Conclusively, cultivar Pencil performed better as compared to cultivar Meteor under the climatic conditions of Multan. Moreover, foliar application of mixture of different micronutrients is effective for higher yield of peas. Therefore, it is recommended that application of mix micronutrients are suitable and cultivation of Pencil cultivar be encouraged to increase farmer production for higher return.

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