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OPTIMAL COMBINATION OF CHEMICAL COMPOUND FERTILIZER AND HUMIC ACID TO IMPROVE SOIL AND LEAF PROPERTIES, YIELD AND QUALITY OF APPLE (*MALUS DOMESTICA*) IN THE LOESS PLATEAU OF CHINA

**LIXIN ZHANG[1*], JUN ZHOU[1], YONG GUI ZHAO[1], YOUYA ZHAI, KAI WANG,
ASHOK K. ALVA[2] AND SIVAPATHAM PARAMASIVAM[3]**

[1]College of Life Sciences, Northwest A&F University, 712100, Yangling, Shaanxi, P.R. China
[2]Vegetable and Forage Crops Research Unit, USDA-ARS, 99350, Prosser, WA, USA
[3]Deptment Of Engineering Technology and Mathematics,Savannah State University,GA,USA
[*]Corresponding author's e-mail address: zhanglixin@nwsuaf.edu.cn

Abstract

A two-year field experiment was conducted to investigate the effects of different application rates of chemical compound fertilizer (CF) (control, CF0; low, CF1; medium, CF1.5 and high, CF2 rates) without or with humic acid (no HA, HA0; with HA, HA) on soil chemical properties, growth of apple trees and their fruit yield and quality. With the increasing rates of CF, soil available nitrogen (N), phosphorus (P), and potassium (K) contents increased regardless of HA treatment. The soil organic matter (OM) content increased with the addition of HA. Most of the tree response parameters i.e. leaf N, P, K concentration, one-year-old shoot length, thickness and weight of 100 leaves, leaf chlorophyll index, fruit firmness, soluble solid and sugar, titratable acidity, and vitamin C contents, increased with increasing CF rates. Generally, the values of all these parameters as well as fruit yield peaked at CF1.5 and CF2 rates without and with HA, respectively. Addition of HA to CF increased the fruit yield by 12-35%. The results of this study demonstrated beneficial effects of HA addition to CF to improve soil properties, growth of apple trees, and finally fruit yield and quality. The CF combined with HA may be considered as a moderate and economical model of fertilizer regime for apple orchard in the Loess Plateau of China.

Introduction

Apple (*Malus domestica*) is a main economical crop grown in the Loess Plateau of China. Excessive use of mineral fertilizers and unbalanced ratios of different mineral nutrients occurring frequently in these apple production areas lead to hamper soil and fruit quality (Han, 2011; Asghar *et al.*, 2012). Addition of humic acid (HA), derived from lignitic coal, to chemical compound fertilizers (CF) could be a strategy to increase CF use efficiency, thereby suggesting reduction of CF application rate without compromising crops' yields (Mikkelsen, 2005; Han, 2011). Use of these HA fertilizers not only increases organic matter (OM) content and decreases soil pH (Mikkelsen, 2005; Daur & Bakhashwain, 2013), but influences transformation and availability of soil micronutrients (Mikkelsen, 2005). Nitrogen (N), phosphorus (P) and potassium (K) requirement for optimal crop production may be lower in a soil with adequate HA than that with insufficient HA (Mikkelsent, 2005; Memon *et al.*, 2012; Daur & Bakhashwain, 2013). The HA could supply carbon (C) as a food source for soil organisms which breaks the chemical bonds in the soil organic residues. The remaining by-products such as carboxyl, phenolic hydroxyl, alcoholic hydroxyl, ketone and quinoid, can help to retain the nutrients in the soil against leaching losses thereby increasing nutrient uptake efficiency (Mikkelsen, 2005; Fortun *et al.*, 2006; Daur & Bakhashwain, 2013). The stimulatory effects of HA on uptake of macro- and micro-nutrients have been reported by Clapp *et al.*, (2001) and Daur & Bakhashwain (2013). He *et al.*, (2010) and Milosevic & Milosevic (2009) reported the positive effects of combination of organic and inorganic fertilizers on soil fertility, and apple yield and quality.

Addition of HA augmented inorganic fertilizers has been applied in many crops as a better fertilization measure (Mikkelsen, 2005; Daur & Bakhashwain, 2013). However, investigations are seriously lacking on the influence of HA fertilizer on improvement of soil fertility and plant growth in apple orchard in the Loess Plateau of China, especially for the present dwarf rootstock trees (Nardi *et al.*, 2002; Liu *et al.*, 2008; Han *et al.*, 2011; Saruhan *et al.*, 2011). Thus, the objective of this study was to investigate different CF rates combined with HA on soil chemical properties, growth of apple tress with dwarf rootstock, fruit yield and quality in the Loess Plateau of China.

Materials and Methods

Site description: A two-year field experiment (2008-2010) was carried out using ten-year-old apple (*Malus domestica* Borkh.) trees, cv. Red Fuji on dwarf rootstocks, i.e., M26 (inter-stock) and *Malus sieversii* (base stock) at the Apple Experiment Farm in Fufeng area in the Loess Plateau of China (34º20''N, 108 º24''E) at an elevation of 900 m. The mean annual temperature in this location was 12.2ºC, while maximum and minimum mean monthly temperatures were 24.1ºC and 2.1ºC during July/August and January/February, respectively. The region has a temperate climate with cumulative rainfall of 590 and 750mm in 2009 and 2010, respectively. Trees were planted in the winter 2000 at 2 m×3 m spacing (1665 plant/ha). At the start of the experiment, the average tree height was 280-300cm, and crown diameter range was 200-250 cm. Soil in the orchard was an Inceptisols (Lou soil in Chinese soil taxonomy) (Gong & Chen, 1999).

In October 2008, soil samples were taken from 0-20, 20-40 and 40-60 cm depths, from unfertilized plots for the analysis of the following properties (Hesse, 1971) (data reported for the above 3 depths) : pH=8.31, 8.34, 8.41; OM=11.9, 10.2, 8.9 g/kg (Potassium Dichromate Volume Method); available N=88, 82 and 70 mg/kg [2 mol/L potassium chloride (KCl) Extraction, Automated Chemistry Analyzer]; available P = 51.2, 48.5, 44.5 mg/kg [0.5 mol/L sodium bicarbonate ($NaHCO_3$), molybdenum Blue method]; available K = 189.2, 172.6, 99.4 mg/kg [l mol/L ammonium acetate (NH_4OAc) Extraction, Flame photometry].

Experimental layout: The following treatments were evaluated: (1) main treatments: No HA (H0) or 495 kg/ha (HA); (2) sub-treatments: No N, P, K (CF0 i.e. control); N, P, K rates = 144.30, 23.07, 7.66 kg/ha, respectively (CF1); 217.14, 34.61, 11.47 kg/ha, respectively (CF1.5); 288.60, 46.14, 15.32 kg/ha, respectively (CF2). The eight treatment combinations were arranged in a complete randomized block design with four replicates. The fertilizer blend, as per treatment, was applied in 0.6 m wide bands on both sides of the tree-row (before fruit harvest) i.e. 15 October 2008 and 18 October 2009. Upon broadcasting, the fertilizers were incorporated into the soil to a depth of 30 cm by rototilling. Nitrogen and P were supplied as urea (46% N) and ammonium phosphate (20% N and 8% P), and K as potassium chloride (52% K) and HA as humic compound containing 85% HA (derived from lignitic coal using 0.1 M NaOH, produced in Yangling Lvdu Bioecology Technology Co., Ltd, Yangling) (Hai & Mir, 1998).

Sampling and measurement: Soil samples were taken at 0-20, 20-40 and 40-60 cm depths in mid-November 2010 i.e. end of the trial (2 years after initial fertilization on 15 October 2008). The available N, P, K and OM contents were analyzed following the procedure described by Cottenie *et al*., (1982).

The one-year-old shoot length, thickness and fresh weight of 100 leaves were measured. Chlorophyll meter (Zhengjiang Tuopu Instrument Co., Ltd., Hangzhou, China) was used to measure the index of leaf chlorophyll. The sampled leaves were dried at 70°C, ground using a grinder mill (Beijing SinoSec Micro Test Sci. & Tech. Co. Ltd., China) for particle size of <0.25mm and digested using concentrated H_2SO_4 and H_2O_2 for analysis of concentration of N, P and K (Cottenie *et al.*, 1982). Five one-year-old shoots and 20 leaves from the middle parts of the 10 tagged shoots were selected from each tree for the above measurements during the last week of September (Liu *et al.,* 2008; Han, 2011).

Fruit firmness and soluble solid content were measured using a portable firmness meter and refractometer, respectively. Soluble sugars content was estimated by the anthrone reagent method. Fruit acidity was assayed using the NaOH titrimetric method. Vitamin C content was determined using phosphomolybdate-blue spectrophotometry (Liu *et al.*, 2008).

The data for the above parameters and fruit yield were obtained from 16 trees in four replications (each replication represented by four trees) of each treatment. The given values are means of data in 2009 and 2010.

Statistical analysis: The analysis of variance (ANOVA) of response parameters was evaluated by *F*-test (SAS Institute Inc., Cary, NC, USA, 1996). Significant difference between the means was evaluated using the Duncan's multiple range test at 5% probability level.

Results and Discussion

Analysis of soil chemical properties: Improvement of soil fertility and maintenance of equilibrium among soil nutrients are important for enhancing soil productivity (Selim *et al.*, 2010; Demirsoy *et al.*, 2012; Memon *et al.*, 2012). Milosevic & Milosevic (2009) reported that combining organic amendments and CF could contribute to an increase in humus content, a partial increase in total N, and available P and K levels. Addition of HA increased soil N concentration significantly over control with no significant differences within the treatments of HA applied at different rates i.e. 50, 100, 150, 200, 250, and 300 mg/kg. The soil P concentration was improved only by the addition of 200 mg/kg HA in the above study (Sharifa *et al.*, 2002; Daur & Bakhashwain, 2013). Glisic *et al*., (2009) found that high rates of complex NPK fertilizers combined with organic fertilizer induced an increase in mineral nutrition, primarily for amounts of K. Sharifa *et al*., (2002) reported that soil OM content increased slightly (7 to 14%) by the HA treatments.

The current study demonstrated CF, HA and their interaction effects on the chemical properties at the end of the trial (Figs. 1 & 2). All fertilization treatments increased contents of available N, P and K above those of unfertilized treatment, regardless of HA application. At a given CF rate, addition of HA to CF increased the available P, K and OM contents at all soil depths, especially for available P in 20-40 and 40-60 cm depths, available K in 0-20 and 40-60 cm depths, and OM content at 0-20 cm depth (Figs. 1 & 2). Mikkelsen (2005) ascribed the main contributions to increase in available soil K and P due to the application of HA. Potassium is very easily released from K crystal structure into the soil solution by exchange of H^+ with K^+ when HA is applied to the soil (Mikkelsen, 2005). Humic acid application could decrease soil acidity resulting in an increased P release from the clay mineral fixed P by forming chelate compound, and increase activity of soil microorganisms contributing to transfer from organic P to inorganic P (Mikkelsen, 2005; Daur & Bakhashwain, 2013).

Analysis of leaf contents of N, P and K: Improvement in leaf contents of N, P and K may be beneficial for plant growth of many friut trees including apple (Han, 2011; Demirsoy *et al*., 2012). Addition of HA to CF can be a better measure to increase leaf P and K content regardless of CF rates, but leaf N content only at CF2 rate under no HA (Fig. 3). The results agree with those reported earlier by Sharifa *et al*., (2002). Nardi *et al*., (2002) reported that decreased percent inorganic N and an increased percent HA enhanced the leaf N, P and K concentrations. The effect of HA in enhancing soil fertility explains the increase in plant availability of soil nutrients.

Analysis of vegetative growth, leaf quality and fruit yield: Better supply of soil nutrients and OM contributes to improvement of vegetative growth, leaf quality and fruit yield of many friut trees including apple (Han, 2011; Asghar *et al*., 2012; Demirsoy *et al*., 2012). Du *et al*., (2011) reported that HA addition could enhance the efficacy of applied CF, resulting in increases in vegetative growth and fruit yields, especially for apple trees with M26 dwarf rootstock, due to its rapid response to all topsoil changes (Han, 2011). Differences in shoot growth based on one-year shoot length resulted from the applied nutrients (Han, 2011). Du *et al*., (2011) reported that CF with HA application increased thickness and fresh weight of 100 leaves, hastened stem growth and enhanced net photosynthetic rate as compared to the trees in unfertilized or only compound fertilizer treatments. One-year-old shoot length, thickness and fresh weight of one hundred leaves and chlorophyll index increased with increasing CF rates from CF0 to CF1.5, regardless of HA treatment in our study. Within a given CF rate, each of the above parameters was greater for HA amended than that for without HA treatment (Table 1).

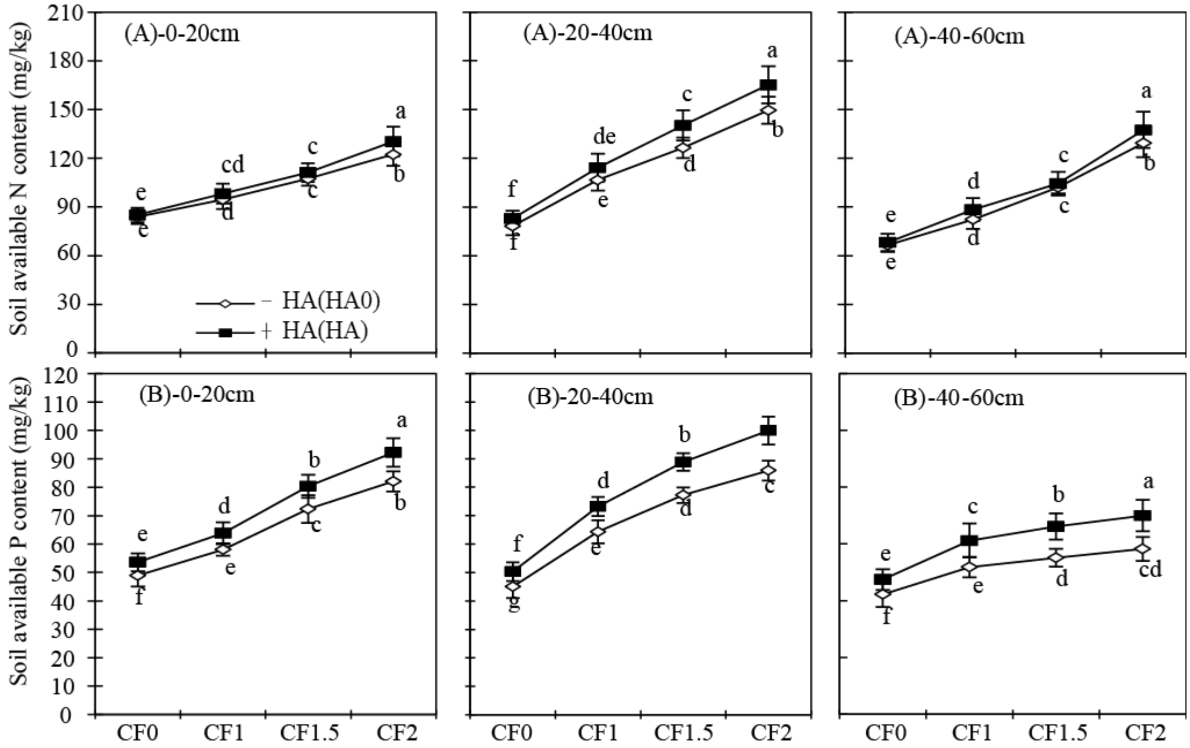

Fig. 1. Available soil-N (A) and P (B) contents (mg/kg soil) at different soil depths at the end of the trial (November 2010, i.e. after a two-year period) as affected by different rates of chemical compound fertilizer (CF) application (control, CF0; low, CF1; medium, CF1.5 and high, CF2 rates) without or with humic acid (no HA, HA0; with HA, HA). Vertical line at each data point represents standard error of mean (*n*=4). Means with different letters, within each parameter and each depth, indicate a significant difference at $p<0.05$.

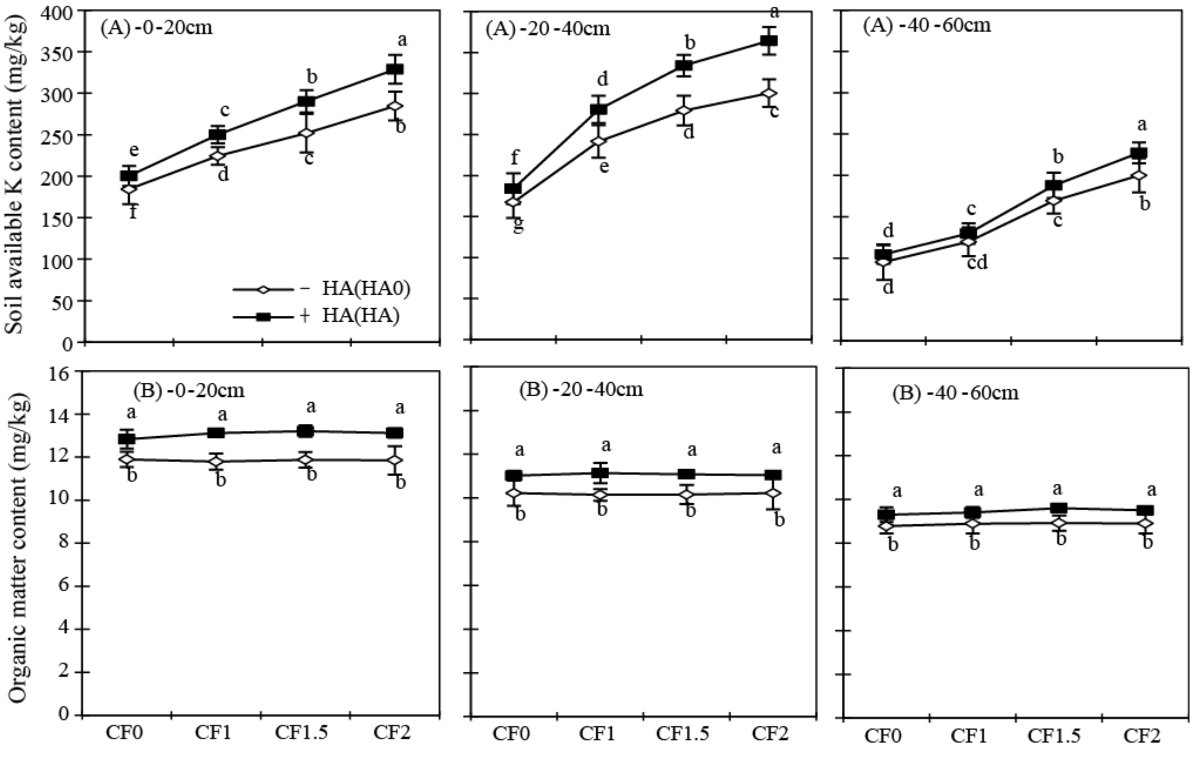

Fig. 2. Available soil-K (A) and organic matter (OM) contents (B) (mg/kg soil) at different soil depths at the end of the trial (November 2010, i.e. after a two-year period) as affected by different rates of chemical compound fertilizer (CF) application without or with humic acid. Details of treatments and data analysis method are given in Fig. 1.

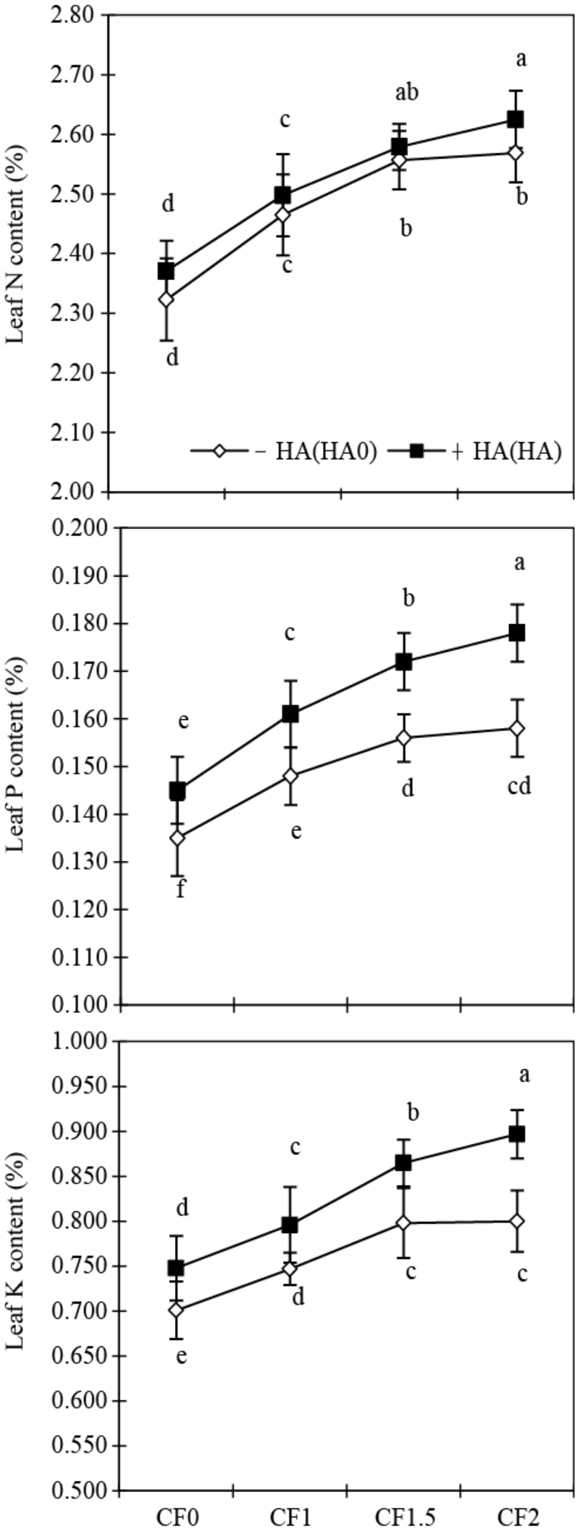

Fig. 3. Effects of different rates of chemical compound fertilizer (CF) application (control, CF0; low, CF1; medium, CF1.5 and high, CF2 rates) without or with humic acid (no HA, HA0; with HA, HA) on leaf N, P and K contents (Mean across two years i.e. 2009 and 2010). Vertical line at each data point represents standard error of mean (*n*=8). Means with different letters, within each parameter, indicate a significant difference at *p*<0.05.

Apple fruit yield generally depends on the tree genetics, growth environment and cultivation practices (Han, 2011; Asghar *et al.*, 2012). Improvement of soil OM, for example by addition of HA, could increase the yields of some field crops (Ulukan, 2008). Xue *et al.*, (1994) observed superior effects of CF augmented with HA for several crops including apple trees. The results of our study have shown highest fruit yield at CF2+HA treatment. However, without the addition of HA, the highest fruit yield was obtained at CF1.5 (Fig. 4). He *et al.*, (2010) found that the right amount of chemical and organic fertilizer increased apple yield by 24 to 54%, fruit weight by 4 to 19%. The combined effects of HA and composite NPK mineral fertilizer could improve soil water, air and temperature regimes in the shallow root zone of the M26 dwarf apple rootstock, and finally enhanced fruit yield (Butorac *et al.*, 2002; Mikkelsen, 2005). Humic acid may increase phosphate uptake, photosynthesis and respiration rates in plants, enhance protein synthesis, and promote plant cell elongation (Saruhan *et al.*, 2011).These results suggest the addition of HA can reduce the rate of CF without compromising apple tree growth and/or fruit yield.

Analysis of apple fruit quality: Most of the previous studies conducted on apple quality in different CF regimes (Neilsen *et al.*, 2004; Han, 2011; Asghar *et al.*, 2012). Du *et al.*, (2011) show that CF combined with HA can significantly improve fruit firmness and soluble sugar content. He *et al*., (2010) reported that the right amount of organic CF increased soluble sugars and soluble solids contents of apple fruit. In our study, contents of soluble solids, soluble sugars, titratable acidity, and vitamin C as well as fruit firmness increased with increasing CF rates from CF0 to CF1.5 rate, regardless of HA. Application HA to CF showed the greater positive effects on soluble solids, soluble sugar, and vitamin C contents and negative effects on titratable acidity content as well as no-significant effects on fruit firmness except CF2 treatment as compared to those in CF solely (Table 2). The stimulatory effects of HA have been directly correlated with enhanced uptake of P and K, which in turn, enhance the transport of photosynthate such as starch, sugar and organic acid from source to sink, and furthermore enhance the activities of some key enzymes such as acid invertase (AI), sucrose synthase (SS), sucrose phosphate synthase (SPS), malate dehydrogenase (MDH), and vitamin C synthesis enzymes (Clapp *et al.*, 2001; Mikkelsen, 2005; Han, 2011).

Conclusion

The results of the current study suggest that the application of HA to CF enhanced the soil chemical properties, leaf quality, vegetative growth, fruit yield and quality of apples. The main response parameters reached their maximums at CF1.5 and CF2 rates without and with HA, respectively. The HA application to soil may decrease CF application rate to reduce production cost, but without compromising apple tree growth, fruit yield and quality. The combined HA and CF application can be considered as a moderate and economical measure of fertilization for apple trees in the Loess Plateau of China.

Table 1. Effects of chemical compound fertilizer (CF) and humic acid (HA) interactions on shoot growth and leaf characteristics (Mean of two years i.e. 2009 and 2010). Means with different letters for each response parameter indicate a significant difference at *p*<0.05.

Treatment	CF0	CF1	CF1.5	CF2
One-year-old shoot length (cm)				
-HA (HA0)	38.1 d	41.5 c	44.4 b	43.6 bc
+HA (HA)	38.8 d	42.6 c	45.7 ab	47.4 a
Thickness of one hundred leaves (cm)				
-HA (HA0)	4.02 c	4.47 b	4.62 b	4.59 b
+HA (HA)	4.28 bc	4.71 b	5.21 a	5.27 a
Fresh mass of one hundred leaves (g)				
-HA (HA0)	71.3 c	75.5 bc	82.3 b	81.6 b
+HA (HA)	75.8 bc	81.2 b	89.4 a	92.2 a
Chlorophyll index				
-HA (HA0)	56.4 c	58.7 c	60.8 bc	61.6 b
+HA (HA)	58.8 c	62.6 bc	65.3 ab	66.1 a

Table 2. Effects of chemical compound fertilizer (CF) and humic acid (HA) interactions on fruit quality (Mean of two years i.e. 2009 and 2010). Means with different letters for each response parameter indicate a significant difference at *p*< 0.05.

Treatment	CF0	CF1	CF1.5	CF2
Firmness (kg/cm^2)				
-HA (HA0)	7.49 bc	7.64 b	7.88 a	7.72 ab
+HA (HA)	7.34 c	7.56 b	7.76 ab	7.38 c
Soluble solid content (%)				
-HA (HA0)	13.2 e	13.9d	15.6 b	14.9 c
+HA (HA)	14.1 cd	14.8 c	16.9 a	16.2 ab
Soluble sugar content (%)				
-HA (HA0)	9.8 e	10.4 de	11.2 cd	10.7 d
+HA (HA)	11.6 c	12.3 b	13.5 a	12.9 ab
Titratable acidity content (mg/kg)				
-HA (HA0)	0.0274 c	0.0287 bc	0.0329 a	0.0337 a
+HA (HA)	0.0262 d	0.0266 d	0.0286 bc	0.0292 b
Vitamin C content (mg/kg)				
-HA (HA0)	15.16 d	15.97 cd	16.35 c	15.67 cd
+HA (HA)	16.08 c	17.22 b	18.02 a	17.97 ab

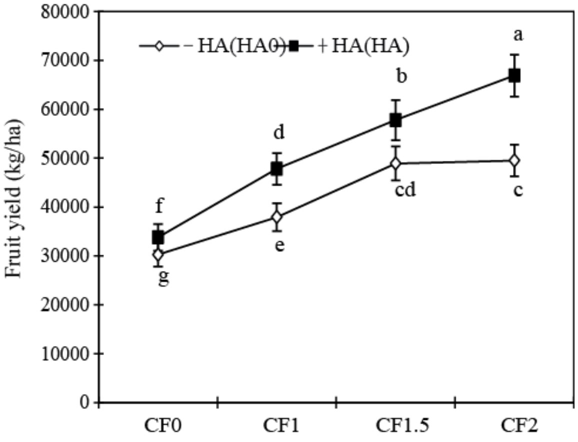

Fig. 4. Effects of different rates of chemical compound fertilizer (CF) application without or with humic acid on fruit yield (Mean of two years i.e., 2009 and 2010). Details of treatments and data analysis method are given in Fig. 3.

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