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COMBINE EFFECT OF NITROGEN AND HUMIC ACID ON CARBOHYDRATE-NITROGEN RATIO, PHOTOSYNTHETIC ACTIVITIES, FRUIT YIELD AND QUALITY OF 'KINNOW' MANDARIN

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The ability of plants to produce high fruit yield and quality depends on the utilization of reserved carbohydrates and photosynthetic activity of plants at initial stages of bud sprouting and flowering. Optimum dose of N is essential for this mechanism. Therefore, different doses of N (600, 900 and 1200g per plant) along with 120 mL of 8% humic acid (HA) per plant were applied in three equal installments (before flowering, after fruit setting and fruit development stages). Growth parameters, C:N ratio, photosynthetic activities and their effects on yield and quality of 'Kinnow' fruit of these plants were compared with the control plants receiving recommended dose of N (1200g) applied in two equal installments (before flowering and at fruit setting). Source of fertilizers for nitrogen, phosphorus and potassium and method of their application was kept same in all treatments. Results revealed that plants receiving N dose of 900g with 120 ml of 8% humic acid showed the best utilization of carbohydrates which was also found directly correlated with photosynthesis. This treatment depicted increase in photosynthesis and maintenance in C:N ratio of plant at fruit setting stage. It was noted that maintenance of carbohydrates at flowering time, during fruit setting and harvesting stages (13.51, 13.44 and 13.39%, respectively) showed promising results on fruit development (956 fruit per plant) and quality of Kinnow. Maximum TSS (12.20 °Brix), ascorbic acid (44.96mg100g^{-1}), total sugars (13.69%), phenolic compounds (398.5mg GAE100g^{-1}) and antioxidants (90.13 Ic µgL^{-1}) were also recorded in the fruits of plant treated with 900g N and 120 mL HA. The dose of N (900g and 120mL HA) in three equal splits (before flowering, fruit setting and fruit maturation) along with recommended doses of P and K (each 600g per plant) were found effective to build synergism between photosynthetic and C:N ratio which resulted in enhancing fruit yield and quality of 'Kinnow' mandarin.
Keywords: Citrus, ascorbic acid, antioxidants, humic acid, carbohydrates, photosynthetic, phenolics

INTRODUCTION

In citrus, regulation of fruit growth is an intricate phenomenon involving both the external and internal factors which sometimes operate sequentially and simultaneously. Among many environmental factors, carbon (C) and nitrogen (N) are crucial for citrus plants to perform the routine and fundamental cellular activities. Both C and N nutrients are essential for various cellular functions, and therefore adequate supply of these two nutrients are critical for plant growth, development and response to a wide array of stresses and ultimately for the completion of life cycle and the production of harvestable organs (Zheng, 2009; Mehmood *et al.*, 2016). Plants accumulate the carbohydrate and nitrogen and use the reserve to support initial growth after dormancy period (Bushway and Pritts, 2001) and it has a direct effect on plant growth and production. Fruit set and further vegetative and fruit development in Citrus are supported mainly by actual photosynthetic rates because carbohydrate reserves in the tree are depleted after the initial stages of bud sprouting and flowering (Khan *et al.*, 2006; Syvertsen and Lloyd, 1994). Carbohydrate reserve can be either increased or decreased by increasing N availability. This phenomenon is related to the photosynthetic activity of the plants. Photosynthesis triggering enzyme Rubisco determines the fate of available nitrogen. When Rubisco is limiting, an increase in N can lead to increased CO_2 assimilation because more Rubisco will be manufactured. In contrast, if Rubisco is not limiting, further increase in N availability can decrease carbohydrate status because decarboxylation is required for N assimilation (Cheng and Fuchigami, 2002). The endogenous and environmental elements which affect the growth and production are being interpreted and this knowledge may provide tools leading to the ultimate goal of citrus industry i.e; optimize the production and enhance the nutritional value of the fruit (Iglesias *et al.*, 2007; Naz *et al.*, 2007; Naqvi *et al.*, 2011).

Plant growth is related to the good vegetative growth and photosynthetic activity. Role of fertilizers containing macro nutrients, are well documented. Nitrogen is among the major nutrient essential for plant growth but its excessive application affect yield and fruit quality (Moreno *et al.*, 2014). More studies have suggested that high dose of nitrogen impose negative effects on product quality, taste and acidity of fruits (Krueskopf *et al*., 2002). To gain a better insight in the optimal use of N fertilizers, one must correctly evaluate the plants responses to possible deficiencies (Boussadia *et al.,* 2006). C:N ratio of plant directly control the flower formation (Goldschmidt and Golomb, 1982; Goldschmidt *et al*., 1997), fruit setting (Guardiola, 1997; Mehouachi *et al*., 1995) and photosynthesis of the plants (Iglesias *et al.,* 2002; Quilot *et al*., 2004; Urban *et al*., 2004).

Effect of humic acid (HA) on physiological attributes of plants such as strawberry, grapevine and apple were increased in photosynthetic activity, increment in total leaf chlorophyll contents and facilitated opening of stomata, respectively (Neri *et al*., 2002; Cimrin and Yilmaz, 2005; Wu *et al*., 2008; Lopez, 1993). Other beneficial effects of HA are increasing in stomatal conductance and net photosynthesis (Liu, 1998; Azcona *et al*., 2011), leaves nutritional status (Barakat *et al*., 2012), increase in chlorophyll contents which, in turn, could affect photosynthesis (Nardi *et al*., 2002). The combined effect of mineral nitrogen fertilizer and humic acid resulted with increased berry size (Eman *et al*., 2008) and fruit weight, yield and soluble solid contents (Li *et al.,* 1999). Humic acid reduces the fertilizer requirement and significantly increase the yield (Freeman, 1970; Syabryai *et al*., 1965).

MATERIALS AND METHODS

Experiment was conducted at Experimental Fruit Orchard, Square No.9, (Latitude 31°-26´ N, Longitude 73°-06´ E and Altitude 184.4 m), Institute of Horticultural Sciences, and University of Agriculture Faisalabad, Pakistan. The experiment treatment plan was laid out according to randomized complete block design. 14 years old trees were randomly selected for allocation of treatments. Each treatment was replicated three times by keeping one tree per replication. Trees were fertilized with Urea (46% Nitrogen), Sulphate of potash (50% potassium) and single super phosphate (18% P_2O_5) as a source of Nitrogen (N), potassium (K) and phosphorus (P), respectively. Different doses of N (600, 900 and 1200g per plant) along with 120 mL aqueous solution of 8% humic acid were applied in three equal installments i.e.in February (before flowering), April (after fruit set) and August (at fruit development stage). A total of 600g P_2O_5 and 600g K_2O per plant were applied as basal dose at once in February in each treatment. Control trees were fertilized with recommended dose of N, P and K (600g N + 600g P + 600g K in February and 600g N in April). Physiological attributes such as photosynthesis rate (A), stomatal conductance (g_s) and transpiration rate (E) was measured using Infra-Red gas analyzer (IRGA) (Analytical Development Company, Hoddesdon, England). Leaf chlorophyll contents were counted on fully grown leaves by using chlorophyll meter (Chlorophyll Contents Index) by following the methods of Zekri (1991), Moya *et al*. (2004) and Blackmer and Schepers (2013). Leaf nitrogen (N %) and total carbohydrates (%) were estimated to calculate the C:N ratio by the method described by Hedge and Hofreiter (1962). Fruit quality parameters including juice contents (%), TSS (°Brix), ascorbic acid (mg/100g), glucose, fructose, sucrose, total sugars, total antioxidant (% DPPH inhibition) and total phenolic contents (mg GAE/100g) were calculated as recommended by Hortwitz (1960), Ghafoor (2014), Anisworth and Gillespie (2007) to study the effect of nitrogen dose and humic acid on quality of fruits. The data was statistically analyzed using MINITAB 17.0 and SPSS 21. Analysis of variance was used to test significance of variance. While difference among the treatment means were compared using Tukey HSD test (P=0.05) (Steel *et al*., 1997).

RESULTS

Photosynthesis rate**:** The plants where N was applied @ 900g + 120 ml HA showed maximum photosynthesis (4.06 µmol m^{-2} s^{-1}) as compare to control plants where it was (3.97µmol m^{-2} s^{-1}) (Table 1). Mean values of photosynthetic rate at phonological stages indicated lower rate of photosynthesis at flowering time (3.28 µmol m^{-2} s^{-1}), while increase in photosynthesis was occurred at fruit setting stage (4.35 µmol m^{-2} s^{-1}), this is because utilization of carbohydrate reserves allow the plants for more photosynthesis. Fruit development and growth stages of plant showed decreasing pattern in photosynthesis (4.16µmol m^{-2} s^{-1}) and this was preceded till harvesting stage with the lowest value of photosynthesis (4.11 µmol m^{-2} s^{-1}) in plants.

Stomatal conductance**:** Among the treatment means higher stomatal conductance of 0.33 mmol $m^{-2}s^{-1}$ was recorded in the plants receiving 900g N + 120 ml HA as compared to control plants (0.18 mmol $m^{-2}s^{-1}$). Stomatal conductance values at different growth stages of 'Kinnow' plants showed decreasing pattern from flowering to harvesting. At flowering and fruit set stages, stomatal conductance of 0.29 and 0.31 mmol $m^{-2}s^{-1}$ were recorded, followed by 0.23 mmol $m^{-2}s^{-1}$ at fruit development stage. This value remained lowest till the time of harvesting (Table 1).

Transpiration rate**:** Treatment means showed more transpiration rates in the plants receiving low dose of N as compare to the plants receiving higher N doses applied in three splits. Increase in transpiration rate was found in plants treated with 600g N + 120 ml (5.14 mmol $m^{-2}s^{-1}$) and 900g N + 120 ml HA (5.43 mmol $m^{-2}s^{-1}$), while it was 4.20 mmol $m^{-2}s^{-1}$ in plants treated with 1200g N, while transpiration rate of 3.68 mmol $m^{-2}s^{-1}$ was noted in the leaves of control plants. Higher value of transpiration rate (4.89 mmol $m^{-2}s^{-1}$) was

Table 1. Interactive effect of nitrogen+hunic acid and phenological stages of 'Kinnow' mandarin x Treatment interaction (mean ± SE) for photosynthesis.

Treatments x Gas exchange characteristics	Phenological Stages				Mean
	Flowering	Fruit set	Development and growth	Harvest	
Control	3.32±0.041f	4.23±0.043bcd	4.05±0.031de	4.00±0.059e	3.90±0.106C
1200g N+120 ml HA	3.10±0.046g	4.40±0.051ab	4.23±0.026bc	4.17±0.040cde	3.97±0.155B
900g N+120 ml HA	3.36±0.051f	4.47±0.054a	4.22±0.054bcd	4.18±0.014cde	4.06±0.128A
600g N+120 ml HA	3.33±0.055f	4.29±0.063abc	4.14±0.032cde	4.09±0.054cde	3.96±0.115BC
Mean photosynthesis	3.28±0.037C	4.35±0.036A	4.16±0.027B	4.11±0.029B	
Control	4.10±0.046ef	3.96±0.047fg	3.40±0.052h	3.27±0.145h	3.68±0.112D
1200g N+120 ml HA	4.53±0.046d	3.65±0.042gh	4.37±0.039de	4.23±0.145def	4.20±0.106C
900g N+120 ml HA	5.59±0.074a	5.50±0.041ab	5.37±0.033abc	5.25±0.156abc	5.43±0.055A
600g N+120 ml HA	5.33±0.064abc	5.00±0.074c	5.13±0.042bc	5.09±0.148c	5.14±0.053B
Mean transpiration rate	4.89±0.182A	4.53±0.228B	4.57±0.232B	4.46±0.245B	
Control	0.21±0.009	0.23±0.009	0.15±0.009	0.13±0.009	0.18±0.013C
1200g N+120 ml HA	0.28±0.029	0.29±0.024	0.26±0.023	0.22±0.017	0.26±0.013B
900g N+120 ml HA	0.33±0.009	0.36±0.009	0.34±0.015	0.29±0.012	0.33±0.009A
600g N+120 ml HA	0.32±0.015	0.35±0.014	0.31±0.012	0.29±0.006	0.32±0.008A
Mean stomatal conductance	0.29±0.016AB	0.31±0.017A	0.27±0.022B	0.23±0.020C	
Control	32.55±1.25bcd	34.85±0.96abc	31.26±1.02b-e	27.01±1.01de	31.42±0.97B
1200g N+120 ml HA	32.69±2.15bcd	35.69±0.68ab	33.42±0.54abc	29.56±1.26cde	32.84±0.87AB
900g N+120 ml HA	33.14±2.26abc	38.78±0.68a	29.82±0.72cde	26.32±0.43e	32.02±1.48B
600g N+120 ml HA	34.25±1.47abc	35.75±0.53ab	34.57±0.85abc	33.59±1.29abc	34.54±0.52A
Mean chlorophyll contents	33.16±0.81B	36.27±0.55A	32.27±0.65B	29.12±0.97C	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

recorded at flowering time, which followed decreasing trend till harvesting stage which was 4.46 mmol $m^{-2}s^{-1}$ (Table 1).

Chlorophyll contents index (CCI): Plants fertilized with 900g N and HA (120 mL) showed higher CCI (38.78) at the time of fruit set followed by plants treated with 600g N and 1200g (35.75 and 35.69, respectively) (Table 1). Plants treated with 900g N + 120 mL HA showed maximum activity which is obvious from chlorophyll contents (26.32) at harvesting stage compared to chlorophyll contents (33.59 and 29.56) in plants applied with N @ 600 and 1200g, respectively.

Total nitrogen (%): A decreasing trend of nitrogen (%) in leaves from flowering till harvesting was recorded in each treatment. Maximum N (2.54%) was noted at flowering, followed by fruit set (2.40%), development stage (2.32%) and at harvesting stage (2.29%). Comparison among the treatments indicated that plants treated with 1200g N + 120 ml HA showed maximum nitrogen concentration (2.47%) in leaves, followed by the plants treated with 600g N +120 mL (2.40%) and 900g N +120 mL HA. A significant difference was noted in N concentration at flowering and harvesting stage (Table 2).

Total carbohydrates (%): Trees applied with 900g N + 120 ml HA showed same carbohydrate contents (13.44%) as

Table 2. Effects of Nitrogen and HA on total N (%) in leaves of 'Kinnow' plant at different phenological stages.

Treatments	Phenological stages				Mean
	Flowering	Fruit set	Development and growth	Harvesting	
Control	2.50±0.125	2.30±0.190	2.20±0.144	2.15±0.140	2.29±0.076A
1200g N+120 ml HA	2.56±0.136	2.50±0.180	2.40±0.147	2.42±0.130	2.47±0.067A
900g N+120 ml HA	2.53±0.120	2.41±0.190	2.33±0.170	2.28±0.140	2.39±0.073A
600g N+120 ml HA	2.57±0.100	2.40±0.190	2.34±0.145	2.30±0.130	2.40±0.069A
Mean	2.54±0.052A	2.40±0.083AB	2.32±0.068AB	2.29±0.064B	

Means sharing similar case letter for main effects and interaction, do not differ significantly at 5% probability level.

Table 3. Effects of Nitrogen and HA on total carbohydrate (%) in leaves of 'Kinnow' plant at different phenological stages.

Treatment	Phenological Stages				Mean
	Flowering	Fruit set	Development and growth	Harvest	
Control	13.50±0.007ab	13.45±0.006cde	13.44±0.006def	13.43±0.006efg	13.46±0.009A
1200g N+120 ml HA	13.50±0.006ab	13.47±0.008bcd	13.43±0.007efg	13.42±0.007eh	13.46±0.010A
900g N+120 ml HA	13.51±0.007a	13.44±0.008def	13.41±0.009fgh	13.39±0.008h	13.44±0.014B
600g N+120 ml HA	13.50±0.006ab	13.48±0.008abc	13.43±0.008efg	13.40±0.009gh	13.45±0.012A
Mean	13.50±0.003A	13.46±0.006B	13.43±0.005C	13.41±0.006D	

Means sharing similar case letter for main effects and interaction, do not differ significantly at 5% probability level.

Table 4. Effects of Nitrogen and HA on Carbohydrate-Nitrogen ratio in leaves of 'Kinnow' plant at different phenological stages.

Treatments	Phenological Stages				Mean
	Flowering	Fruit set	Development and growth	Harvesting	
Control	5.40±0.080d	5.85±0.070bc	6.11±0.070ab	6.25±0.080a	5.90±0.102A
1200gN+120 ml HA	4.98±0.080e	5.39±0.070d	5.60±0.080cd	5.55±0.070cd	5.38±0.079C
900g N+120 ml HA	4.67±0.070f	4.78±0.080ef	4.97±0.080ef	5.00±0.070e	4.86±0.051D
600g N+120 ml HA	5.00±0.080e	5.62±0.070cd	5.74±0.070c	5.83±0.070bc	5.55±0.102B
Mean	5.01±0.084C	5.41±0.123B	5.60±0.128A	5.65±0.140A	

Means sharing similar case letter for main effects and interaction, do not differ significantly at 5% probability level.

compared to untreated plants (13.46%) (Table 3). A different trend for carbohydrates was observed regarding the phenological stages and total carbohydrate showed decreasing trend from flowering till harvesting. Maximum carbohydrates (13.50%) were found in leaves at flowering stage, followed by the fruit setting stage (13.46%), fruit development stage (13.43%) and at harvesting stage (13.41%).

Carbohydrate-nitrogen ratio (C:N): Minimum C:N ratio (4.86) was recorded in plants where 900g N + 120 mL HA was applied. Plant received 1200g N + 120 mL HA and 600g N +120 mL HA showed 5.38 and 5.55 C:N ratios, respectively. Regarding the phenological stages, increasing trend was found from flowering to harvesting stage. The highest C:N ratio (5.65) was observed at harvesting time, followed by fruit development stage (5.60), fruit setting (5.41) and flowering stage (5.01). Interaction between treatments and phenological stages showed maximum C:N ratio (6.25) at the time of harvesting and minimum ratio (4.67) at flowering stage in plants treated with 900g N with 120mL HA (Table 4).

Number of fruit/plant: Effect of nitrogen dose was significantly increased the total no. of fruit/plant. Maximum 956 fruits were counted from the tree where N was applied @ 900g along with 120mL HA, while 927 fruits were counted in control plant (1200g N in two installments). Application of N @ 600 g+ 120mL HA also showed good result where 952 fruits were counted in plants (Fig. 1).

Total soluble solids (TSS) (°Brix): TSS value of 12.40°Brix was recorded in fruit when trees were fertilized with N @ 900 g +120mL HA in three equal splits, while minimum of 11.07 °Brix were found in fruit of control plants (Fig.2).

Ascorbic acid (mg 100/g): Maximum ascorbic acid (44.96 mg $100g^{-1}$) were noted in fruit of plants were N was applied @ 900 g along with 120 mL HA, followed by the fruit of plants (45.41 mg $100g^{-1}$) were N was applied @ 600 g+ 120 mL HA, while, minimum ascorbic acid of 29.46 mg $100g^{-1}$ were noted in control tree fruits (Fig. 3).

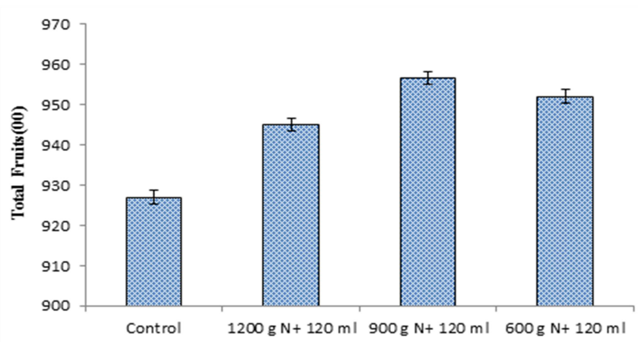

Figure 1. Effect of nitrogen and HA on total number of fruits in 'Kinnow' plants ± S.E.

Total sugars (%): The analyzed data showed highly significant results (P<0.01) regarding the effects of treatments (nitrogen split dose and humic acid). Fruits from the tree those were fertilized with N @ 900 g + 120 ml HA showed higher levels (13.69%) of total sugars contents in juice, while plants treated with N @ 1200 g + 120 ml HA yielded fruits with 9.67% total sugars. Plants with N @ 600 g + 120 ml HA showed 11.39% total sugar contents in their fruit juice, followed by (10.72%) sugar contents in controlled plant fruits (Fig. 4).

***Total antioxidant (Ic µg/L)*:** Higher antioxidants activities of 90.13 Ic µg/L were noted in fruits from the plants treated with N @ 900 g + 120 ml HA, followed by the plants treated with N @ 1200 g + 120 ml HA where antioxidant in fruits was 87.32 Ic µg/L. Plants treated with N @ 600 g + 120 ml showed 85.97 Ic µg/L antioxidant activities and these were at par with the controlled plants where 84.72 Ic µg/L antioxidants were recorded (Fig. 5).

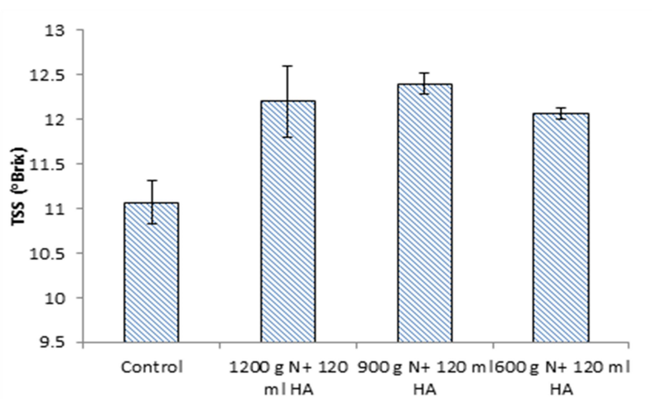

Figure 2. Effect of nitrogen and HA on total soluble solids (°Brix) of 'Kinnow' fruits ± S.E.

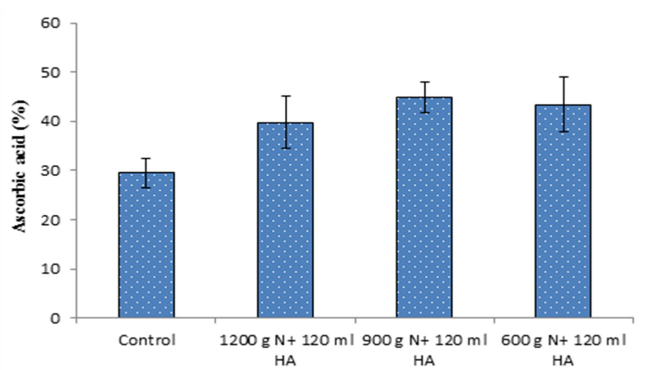

Figure 3. Effect of nitrogen and HA on ascorbic acid (%) of 'Kinnow' fruits ± S.E.

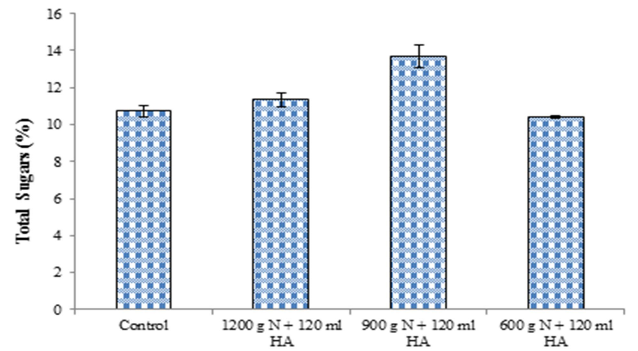

Figure 4. Effect of nitrogen and HA on total sugars (%) in 'Kinnow' fruits ± S.E.

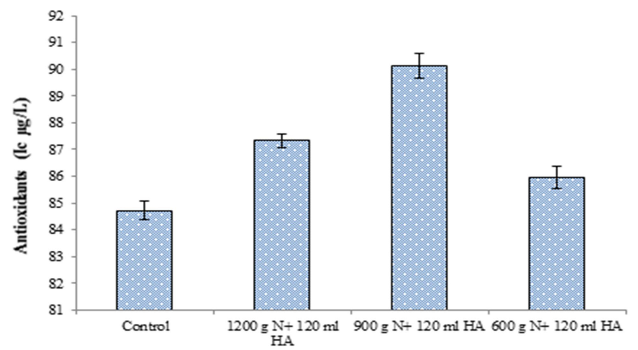

Figure 5. Effect of nitrogen and HA on antioxidant (Ic µg/L) in 'Kinnow' fruits ±S.E.

***Total phenolic contents (mg GAE/100g)*:** Plants treated with N @ 900 g and 600 g along with 120mL HA showed maximum phenolic contents of 398.55 and 376.39 mg GAE/100 g in fruit juices respectively, followed by 358.26 mg GAE/100 g contents in the plants treated with N @ 1200 g + 120 ml HA and these were at par with each other. Fruit juice of control plants showed 327.64 mg GAE/100 g of total phenolic contents (Fig.6).

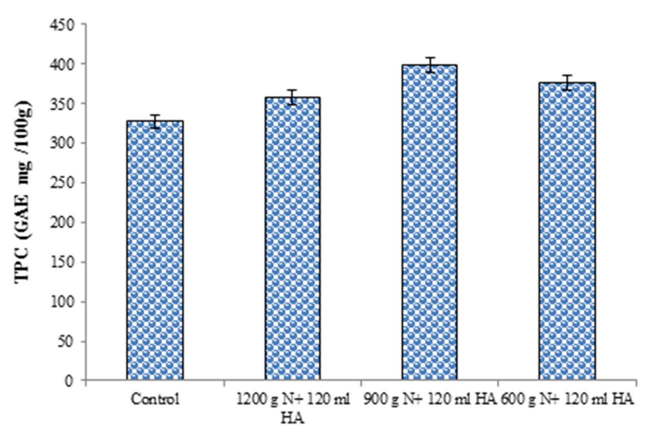

Figure 6. Effect of nitrogen and HA on TPC (mg GAE 100/g) in 'Kinnow' fruits ± S.E.

DISCUSSION

Maintenance of appropriate carbohydrate-nitrogen ratio depends on photosynthetic activity and adequate nitrogen supply. Imbalance use of nutrients particularly over and under use of nitrogen can upset smooth running of photosynthesis mechanism which provide substrate for growth and development of plant. Imbalance in C:N ratio influences the growth by less supply of energy source for further plant growth on one hand and by attracting disease and insect attack on the other hand. In the present study, physiological parameters such as photosynthesis, stomatal conductance and transpiration rate were significantly positively affected by combined application of N with HA. Generally, plant showed low rate of photosynthesis at the time of flowering due to accumulation of carbohydrates in plant, which has suppressing effect on photosynthesis (Araya *et al*., 2006). Increase photosynthesis with onset of fruit set stage was due to the utilization of reserved carbohydrates. The findings of Iglesias *et al*., (2003) supported present results who reported that fruit set was highly dependent on carbohydrate availability in citrus which ultimately increased the number of fruits. Plants fertilized with 900g N + 120 mL HA produced maximum fruit set and highest yield (956 fruits) per plant. These plants showed relatively more photosynthesis (4.47 µmol m^{-2} s^{-1}) and less carbohydrates accumulation (13.44%) at fruit set stage. Documented results of Nebauer *et al*. (2011) strengthened present findings that there is a down regulation of photosynthesis if source and sink (fruit) are imbalance in Salustiana sweet orange plants. Iglesias *et al*. (2002) further confirmed that lack of sink activity leads to the inhibition of photosynthesis in Satsuma mandarin (*Citrus unshiu).*

Results of current study revealed that plants treated with 900 g N + 120 mL HA showed more carbohydrates contents at the time of flowering and after fruit setting, carbohydrates contents were less that further reduced at harvesting stage. This trend indicated that carbohydrates were actively utilized during fruit development stage. Results of previously studies confirmed that presence of sink (fruits) causes decrease in the carbohydrate level in all vegetative parts (Lenz and Küntzel, 1974; Ryugo *et al*., 1977). Syvertsen and Lloyd (1994) found that carbohydrate level in citrus plants depleted after initial flowering and fruit set.

Application of nitrogen in three splits in combination with HA gave better results as compared to control plants (receiving two splits). Among all the treatments, application of 900g N + 120 mL HA in three splits significantly increased the fruit setting and decreased the fruit drop (41.7%). Saleem *et al*. (2005) reported that application of nitrogen fertilizer according to plant needs resulted in more number of fruit set per branch with minimum fruit drop in 'Kinnow' mandarin. Chaudhry and Farrakh (1992) reported that split dose of nitrogen secured more fruit set percentage and led to minimum fruit drop percentage in sweet lime citrus cultivar. A great variation in response to application of 1200g N in two and three splits as compared to lower dose of N (900g) in three splits for fruit production indicated better use of plant resources/reserves. Low dose of N (900g) performed better among all the treatments because this low dose of nitrogen application in three splits facilitated the plant with maximum utilization of its sources, avoiding the leach down of nitrogen fertilizer, and hence resulted in more number of fruit set and less fruit drop percentage.

Maximum total soluble solid contents were found in fruits of treated plants. It is because the starch and carbohydrates hydrolyzed into sugars during the progression of ripening and higher sugar contents were noted in fruit juice after harvesting. Plants treated with 900g N + 120 mL HA showed higher TSS contents. This increase in TSS may be due to the activity of enzymes in response to nitrogen application which ultimately increased the total soluble solids in the fruits. The significantly highest ascorbic acid contents (44.96 mg100 g^{-1}) and sugars contents (total and non-reducing) were found in fruits of plant treated with 900g N along with 120mL HA. This is because of the catalytic activity of several enzymes which take part in biosynthesis of ascorbic acid and its precursor as well as due to the participation of nitrogen in different energy sources such as amino acids and amino sugars. Application of 1200g nitrogen decreased the TSS, ascorbic acid and reducing and total sugars. Application of more nitrogen than plant need reached to toxic level; as a result it decreased other enzymes and nutrient molecules which were necessary for synthesis of these quality attributes. This mechanism is also supported by the findings of previous researcher in different fruit such as Prasad and Mali (2000); Kashyap *et al*. (2012) in pomegranate and Kaul and Bhatanagar (2006) in 'Kinnow'. Increased acidity at higher dose of nitrogen (1200g) might be due to the more production and translocation of organic acids in the fruits as reported by Sharma *et al.* (2013) in guava and Prasad and Mali (2000) in pomegranate. Higher amounts of phenolic compound and antioxidants at 900 g N +120mL HA showed the optimization of this dose. It is because was found better balance application of nitrogen significantly increased the phenolic compounds and antioxidants in the fruit juice that protect plant from many biotic or abiotic stresses (Kefeli *et al*., 2003; Zapprometov, 1989). Therefore, balance use of nitrogen (900g) and its utilization synthesized more phenolic compound and triggered the plant to operate photosynthesis and respiration mechanisms smoothly to maintain balance C:N ratio, which is necessary for regulation of growth and phenological cycles to maintain plant health, production of high quality fruit and reserve for future flowering and fruiting. The results of this study can provide guidelines to produce value added fruit quality in 'Kinnow' plant.

***Conclusion*:** N @ 900 g and 120 ml humic acid per plants in three installments (before flowering, fruit set, fruit maturation) were found most effective to build better photosynthesis, chlorophyll contents and C:N ratio for good health of plants also to produce maximum yield with good nutritional status of fruit. These finding are also important and can improve the economic status of farmers by reducing the cost of production and increasing the yield of good quality fruits.

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