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Growth Stimulation, Metabolic Activities and Fruit Yield of Tomato as Influenced by Fulvic Acid

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ABSTRACT
A pot experiment was conducted at Land Resources Research Institute, National Agricultural Research Centre (NARC), Islamabad during spring 2012 to study the effect of foliar applied fulvic acid (FA) on tomato grown under greenhouse conditions. Six kg sandy loam soil (*Nabipur series mixed hyperthermic Udic Ustochrept*) filled in plastic pot and grown with tomato plants (*Riogrande*). Basal dose of N, P, K, along with Zn, Fe, and B were applied @ 200, 150, 200, 10, 2 and 1 mg/kg soil FA was applied as foliar @ 0, 150, 300, 450 and 600 mg/l respectively. Results showed a significant increase in fruit yield, harvest index and other agronomic parameters with FA application. Besides macronutrients plant micronutrient concentration also increased with FA application which could be ascribed to stimulating effect of FA on enhanced biomass. Protein and chlorophyll contents of leaves also significantly changed with applied FA. Based on the results of the study it could be concluded that FA spray at 150 and 300 mg/ l were appropriate dose for maximum nutrient uptake by plant for optimum growth and maximize yield.

Keywords: Fulvic acid, tomato, mineral composition, biomass weight, FTIR

1. INTRODUCTION
Humic substances (HSs) are naturally occurring, biogenic, heterogeneous mixture of organic material derived from decayed plant and animal residues, and mainly composed of -COOH, and –C=O, -OH and other functional groups. Primarily, HSs are composed of humic acid, FA and humin[1], as major component of ecosystem, HSs made up to 50-80% of the organic matter in water, soil and sediments[2]. The HSs can play significant role in increasing nutrients concentration in soil and plant. The polycarboxylic nature HA and FA may be absorbed by Fe and Al oxides in acidic soil so that phosphate sorption to these oxides are affected[3,4]

Different techniques such as ultraviolet spectroscopy (UV-VIS), high pressure liquid chromatography (HPLC) and Fourier Transforms infrared spectroscopy (FTIR) are used to ascertain chemical composition and structural arrangement of different functional group present in this macromolecule. The presence of hydrophilic and hydrophobic ends have significant role in nutrients acquisition and availability in soil[5].

Earlier research found that foliar application of FA caused increased stem length, fresh and dry wt[5,6,7]. While comparing coal extracted FA applied as foliar to wheat @ 0.05 or 0.1% it was found that FA transport from roots to shoots was greater than that of HA[8]. Application of FA to tomato had significant effects on roots and stem weight[9] ascribed to seedling quality.

Tomato is major vegetable grown in Pakistan on about 53.4 thousand ha with an average yield of about 10.52 t/ha[10] less than world average tomato production (28.34 t/ha), almost one-fourth less than tomato yield in USA(42.1 t/ha)and far below of that in Netherland (146 t/ha)[11]. Imbalanced fertilizer application such as N, P and K @ 250, 125 with virtual no or low use of K and micronutrient[12, 13] is one of the main reasons of low tomato yield. It is estimated that on average, a tomato crop producing 30 t/ha require 280 kg N, 55 kg P_2O_5 and 540 kg K_2O/ ha[14, 15]

Keeping into account the low nutrients availability in alkaline calcariious soil, the current study was conducted to evaluate the effect of foliar applied FA in presence of basal of N, P and K on the growth, yield and nutrient uptake of tomato variety *Riogrande* under greenhouse conditions.

2. EXPERIMENTAL

2.1 *Soil Description*
This study was conducted at headhouse of Land Resources Research Institute (LRRI), National Agricultural Research centre (NARC), Islamabad (latitude 33^o.43' N, longitude 73^o 5'E), on *Nabipur* soil series collected from top 15 cm mainly sandy loam and moderately calcareous, very deep, well drained and developed from rocks of Murree formation and transported by water during the sub-recent epoch. The soil was air dried; sieved (2 mm) and analyzed for pH, EC, organic matter, NO_3- N, P, K, Na and micronutrients (Zn, Fe, Cu, B and Mn as listed (Table 1). All the laboratory analyses were carried following standard operation procedures[16]. The AB-DTPA was used to extract nutrients from soil[17] and thereafter NO_3-N and P in the extract were determined by spectrophotometer (S-200 D) and Na and K by flame photometer (Jenway PFP-7). The Zn, Fe, Cu, and Mn were determined by using atomic absorption spectrophotometer[18].

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2.2 *Extraction of FA from Coal*
Coal samples were ground; sieved (2 mm), treated with KOH (0.5N) and NaOH (0.5N) separately; filtered, centrifuged and dried to get HA as reported earlier[19] (Fig. 1), following grinding and sieving, extraction was acidified (10% HNO_3) as (1:4; coal: acid); filtered and dried (65 ^{0}C) to FA, a yellow color substances. Purity of FA and HA was determined by spectrophotometer. The chemical composition of coal derived FA was determined as described[20].

Table-1: Physicochemical properties of soil

Soil Characteristic	Values	Soil Characteristic	Values
pH	8.10	K (mg kg^{-1})	48.00
ECe(ds/m)	0.16	Zn (mg kg^{-1})	0.34
$CaCO_3$ (%)	4.00	Cu (mg kg^{-1})	0.46
O.M (%)	1.20	Fe (mg/kg)	6.60
Olsen P (mg kg^{-1})	4.90	Mn (mg kg^{-1})	6.20

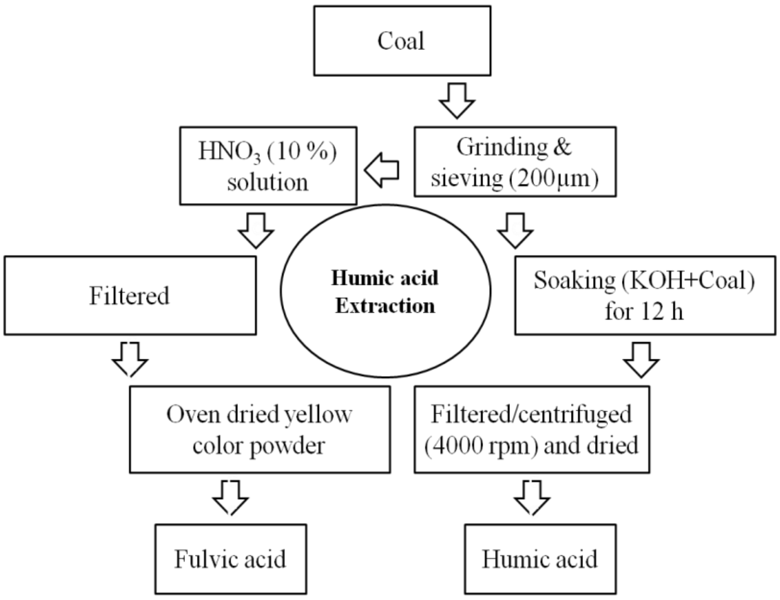


Fig-1: Extraction of HA and FA from coal (Khan et al. 2014)

2.3 *Soil and Plant Sampling, Preparation and Analysis*
Previously air dried, sieved (2 mm) air was filled into 7 kg plastic container applied with basal dose of N, P, K, Zn, along with Fe, and B @ 200, 150, 200, and 10, 2 and 1 mg/kg soil respectively. FA were applied @ 0 (no FA), 150, 300, 450 and 600 mg/l as FA_0, FA_1, FA_2, FA_3, and FA_4, respectively. Twenty five days old tomato seedlings were transplanted to pots. The FA was applied as foliar sprays in three stages; flower initiation, fruit setting and post 1st fruit pick. The treatments were triplicate under completely randomized design (CRD). The data regarding plant fresh and dry biomass, biological yield and fruit weight were recorded. The diagnostic leaf and whole shoot samples were collected for determination of N, P, K and micronutrient. The plant samples were washed with distilled water, air and then oven dried at 65 ^{0}C and then ground finely. Total N was estimated by Kjeldahl method[21]. For other nutrients, plant material was wet digested in mixed acid solution (HNO_3: $HClO_4$) in 2:1 ratio[22]. Aliquot was used for the determination of P, K, Zn, Fe, Cu and Mn using Atomic absorption photometer (Perkin Elmer, USA). The Na and K concentration in digest was determined by flame photometer and Zn, Fe, Cu, and Mn were determined by atomic absorption spectroscopy. Chlorophyll content was measured using chlorophyll meter SPAD 502 (Konica Minolta Sensing Inc. Japan). Soil pH, EC, organic matter, macronutrients (NO_3-N, P and K) and micronutrients (Zn, Fe, Cu, B and Mn) were determined following standard procedures.

2.4 *Statistical Analysis*
The data obtained were analyzed using the MSTATC 5. Difference in means was compared using least significant difference (LSD) test.

3. RESULTS AND DISCUSSION

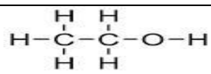
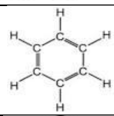
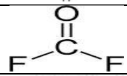
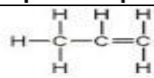
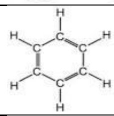
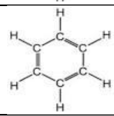
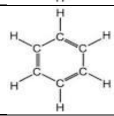
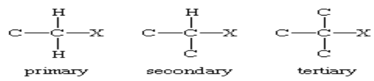
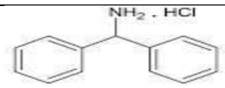
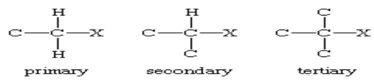
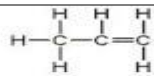
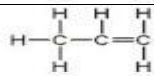
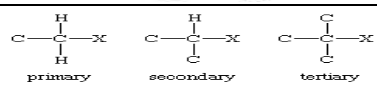
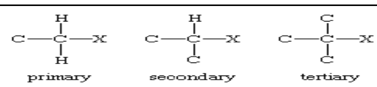
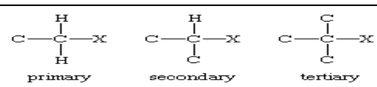
3.1 *Nutrients Composition and FTIR Analysis of FA*
The detail nutrients composition of coal derived FA extracted with HNO_3 (Table 2) showing presence of macro micro and some other heavy metal such as As, Pb.

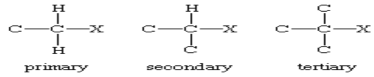
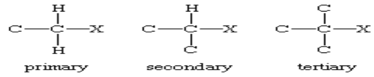
The (FTIR) spectroscopy of FA showed peaks of different altitude and heights (Fig. 2) ascribed to the presence of various functional groups (Table 3). The data revealed that extracted FA contains alcohol, carboxyl, alkyl halide, amine, alkenes and aromatic compounds. The presence alkyl halide in the product was predominated by Br, F and Cl. The aromatic compounds are conjugated with -C=C in 1507-1558 cm^{-1} wave number range while in 3029 cm^{-1} range it was dominated by -C-H group. Amines were also detected that were conjugated with aromatic compounds. From the type of vibration found, it can be concluded that extracted coal FA contains variety of functional groups including hydroxyl, phenolic O-H, alkyl halide, alkene (C=C), Amine NH_2 and aromatic compound with -C=O.

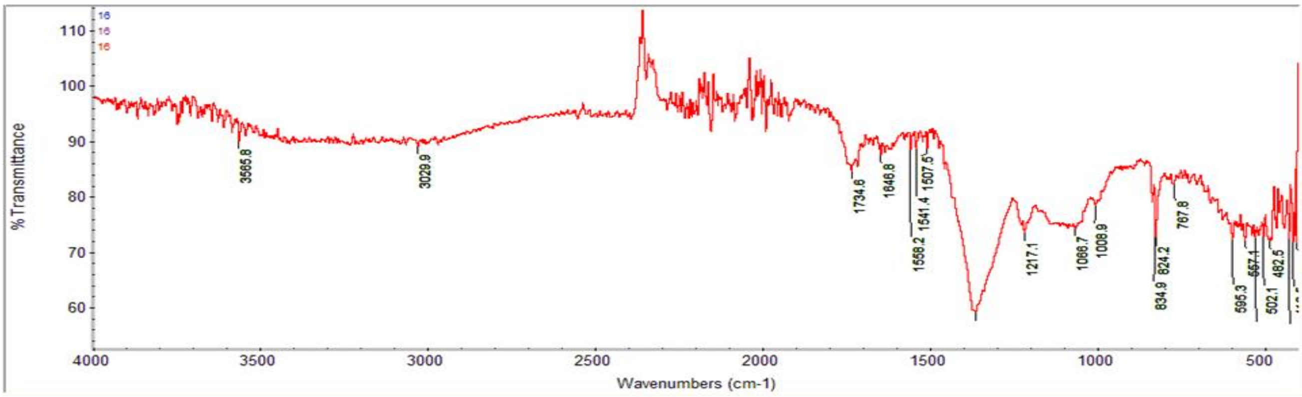
Table-2: Nutrient Composition of coal derived FA

	P	K	Cu	Zn	Fe	Na	Cd
	mg kg^{-1}	**%**	**---------------mg kg^{-1}**	**------------**		**%**	**mg kg^{-1}**
Black coal	12.0	0.047	17.8	9.1	3123	2.2	1.58
Brown coal	15.1	0.060	18.1	13.0	3669	1.8	2.81
Half white coal	75.8	0.113	825.9	506.1	5079	2.1	1.25

Table-3: Characterization of coal derived FA

Functional Group	Vibration	Absorption(cm^{-1}) peak	Intensity	Structural formula
-O-H	(stretch, free)	3565.8	strong, sharp	
Aromatic (C-H)	stretch	3029.9	medium	
-C=O	stretch	1734.6	strong	
-C=C	stretch	1646.8	variable	
Aromatic (C=C)	stretch	1558.2	medium-weak, multiple bands	
Aromatic (C=C)	stretch	1541.4	medium-weak, multiple bands	
Aromatic (C=C)	stretch	1507.5	medium-weak, multiple bands	
-C-F	stretch	1363.7	strong	
-C-N	stretch	1217.1	medium-weak	
-C-F	stretch	1066.7	strong	
-C-F	stretch	1008.9	strong	
=C-H	bending	834.9	strong	
=C-H	bending	824.2	strong	
-C-Cl	stretch	767.8	strong	
-C-Br	stretch	595.3	strong	
-C-Br	stretch	557.1	strong	

-C-Br	stretch	527.1	strong	
-C-Br	stretch	502.1	strong	



Index	Match	Compound name Library
1	21.72	ACETOCHLORO-A-D-GLUCOSAMINE Sigma Biological Sample Library
435	19.67	Ammonium nitrate HR Thermo Nicolet Sampler Library
129	19.55	SODIUM HYDROXIDE IN KBR Georgia State Crime Lab Sample Library
79	18.87	2-PYRIDINECARBOXALDEHYDE, 99% Aldrich Vapor Phase Sample Library
198	18.48	SALICYLIC ACID IN KBR Georgia State Crime Lab Sample Library
40	18.45	LYSERGIC ACID IN KBR Georgia State Crime Lab Sample Library
8	18.38	CELLOPHANE Hummel Polymer Sample Library
56	17.67	L-LEUCINE-B-NAPHTHYLAMIDE FREE BA Sigma Biological Sample Library
39	16.98	POLYGALACTURONIC ACID SODIUM Sigma Biological Sample Library
32	16.76	RISTOCETIN SULFATE Sigma Biological Sample Library

Fig-2: FTIR spectra of coal dried FA

3.2 *Agronomic Parameters*

3.2.1 *Effect of FA on Plant height, Biological yield, Plant dry weight and Chlorophyll contents*

The FA application increased tomato plant height because of its stimulating effect on plant growth. Maximum plant height was recorded in FA_3 as compared to control (Fig. 3). It was almost at par statistically with plant height in FA_1, FA_2 and FA_3. However, the difference among the height of plants at different FA applied levels was non-significant; but highly significant between the FA treatment and control (FA_0).

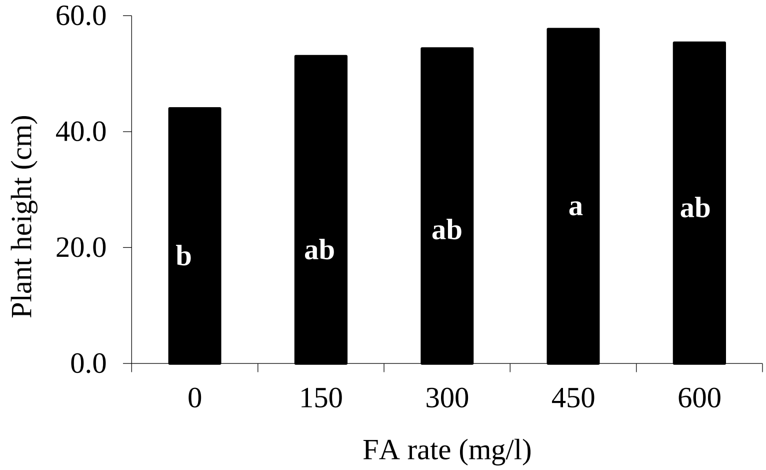


Fig-3: Effect of FA on plant height

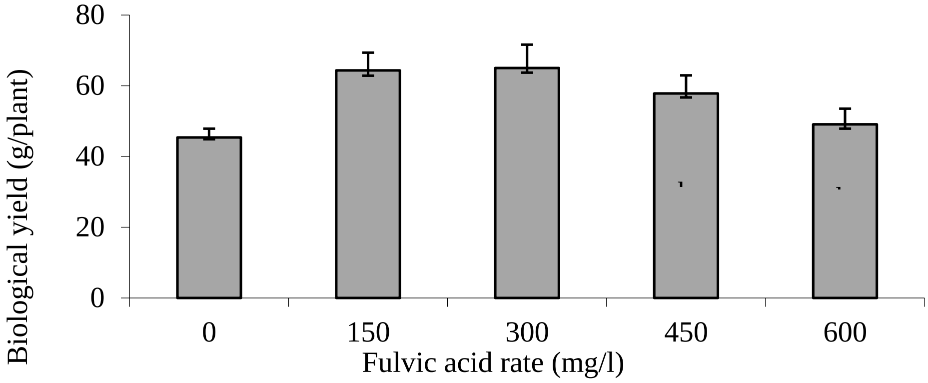


Fig-4: Effect of FA on biological yield

The biological yield was also affected with application of FA. Applied FA @ 150 mg/l gave the maximum biological yield which was at par with FA spray at 300 mg/l. The biological yield declined as FA application rate increased (FA_3 and FA_4). The dry weight of plant increased with FA application as compared to control (Fig. 4). Dry matter produced by FA_1 and FA_2 was at par statistically with each other. These results showed that FA application at higher rates tends to decrease the plant dry weight.

The chlorophyll content of a plant indicates its health as photosynthetic and metabolic activities taking place in the leaves. The chlorophyll contents positively affected with foliar application of FA (Fig. 5). The maximum concentration of chlorophyll contents was recorded in plants treated with FA_1 and FA_2 and then decreased at higher applied rates of FA.

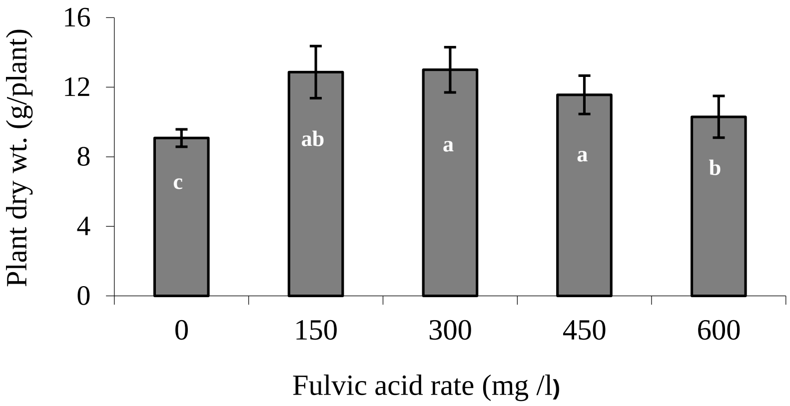


Fig-5: Effect FA on plant dry weight of tomato

3.2.2 *Effect of FA on macronutrients, micronutrients and plant protein contents*

Nitrogen (N), phosphorous (P) and potassium (K) concentration in tomato leaves increased with FA application as compared to control (Table 4). The maximum N concentration was recorded in FA_3 over control. The increase in leaf N concentration was 8.5% in FA_1 and FA_2 foliar treatments. However, its application at higher rate (FA_3, FA_4) decreased nitrogen concentration. The N concentration in the shoots decreased with increasing rates of FA application. The maximum shoots N was recorded in control treatments where no FA was sprayed which decreased gradually with increasing rate of applied FA.

Table-4: Effect of FA on leaf and whole plant N, P and K composition of tomato

Treatments	Leaf			Whole plant		
	N	P	K	N	P	K
	------------------------------(g 100 g^{-1})------------------------------------					
Control FA_0	3.36 C	0.173 C	1.13 C	2.50	0.039	0.73
Foliar FA_1	3.60 B	0.241 B	1.14 B	2.37	0.055	0.62
Foliar FA_2	3.61 B	0.222 A	1.17 A	2.30	0.063	0.64
Foliar FA_3	4.28 A	0.130 D	1.03 D	2.25	0.079	0.63
Foliar FA_4	3.52 B	0.100 E	1.00 A	2.15	0.094	0.62
CV (%)	10.34	12.23	13.2	10.34	12.23	13.2
LSD at $P \leq 0.05$	*0.134*	*0.004*	*0.009*	*NS*	*NS*	*NS*

The maximum P in leaves was recorded in FA_1 foliar spray compared with control. The P concentration in leaves with FA_2 was at par to that of FA_1 which depressed at higher rate of applied FA. Similarly, shoot P concentration increased with application of FA as compared to control. The maximum P was recorded in FA_4 treatment as compared to all other treatments including control. The P concentration increased substantially with applied rates of FA. In case of K uptake, the maximum was recorded at FA_2 foliar spray compared to control. The concentration of K improved in tomato leaves with the use of FA up to FA_2 however, application of FA at higher rate tends to decrease concentration. The K concentration in shoot decreased with FA_1 and FA_2 treatments but it increased at higher rates of FA which was again less than that of control treatment.

The Zn content of diagnostic leaves range from 40 to 48.8 mg/kg in different treatments of FA application (Table 5). The maximum Zn contents were recorded in plants grown in control treatment (FA_0). The Zn content decreased significantly with increasing rate of FA and the minimum concentration was recorded in FA_4 treatment. Contrary to plant leaves Zn content of plant shoots increased with the application of FA as compared to control. It varied in different treatment of FA and the maximum was at FA_2 treatment. The minimum value was recorded in FA_4 treatment which was even more than control treatment. The Cu content of leaves increased with the application of FA with respect to control treatment. The highest Cu concentration (7.6 and 8.3 mg/kg) was recorded in plants treated with FA_3 and FA_4 and was almost at par with FA_1, FA_2 and FA_3, which was 7.2, 7.1 and 7.6 mg/kg respectively. On over all leaf Cu content increased by 18% as compared to control. The Cu content of whole plant increased with application of FA compared to control treatment. The maximum Cu content was recorded at FA_4 which was 56.2% more as compared to control. The increase in Cu concentration in Plant shoot was statistically at par with FA_1 and FA_2 treatments. On over all bases shoot Cu content increased by 43% over the control. The Fe content of leaf decreased with the application of FA and the minimum content was recorded FA_3 and FA_4 treatments. Contrarily, the shoot Fe contents increased with the application of FA as compared to control. The maximum Fe was recorded in the treatment FA_3 as compared to control which decreased with FA_4 application.

Table-5: Effect of FA on leaf and shoot micro nutrient composition of tomato

Treatments	**Leaf**				**Shoot**			
	Zn	**Cu**	**Fe**	**Mn**	**Zn**	**Cu**	**Fe**	**Mn**
	(mg kg^{-1})							
Control FA_0	42.3 A	6.4 C	660 A	91.8 A	22.20	2.90	238	45.83
Foliar FA_1	40.1 B	7.2 B	645 B	81.9 B	31.83	4.27	457	70.93
Foliar FA_2	40.1 B	7.1 B	625 C	76.5 C	28.93	4.10	542	64.43
Foliar FA_3	28.4 C	7.6 B	483 D	76.2 C	26.60	3.70	564	68.57
Foliar FA_4	24.4 D	8.3 A	482 D	36.9 D	24.20	4.53	457	88.30
CV (%)	12.56	8.45	13.45	14.08	10.13	9.23	12.13	10.70
LSD at $P \leq 0.05$	0.244	0.092	14.00	0.84	NS	NS	NS	NS

The Mn concentration of diagnostic leaves decreased with the application of FA and it was the minimum at FA_4, hence decreased antagonistically with increased FA rate. In contrary to leaf concentration the Mn content of shoot increased with increase in FA rate and the maximum Mn was recorded in with FA_4 treatment as compared to control.

Leaf protein contents increased with the use of FA and the maximum were recorded at FA_3 and declined in FA_4 (Figure 5). Activation of several biochemical processes results in an increase in enzyme synthesis and consequently protein contents. During these metabolic changes increases in the concentration of several important enzymes like catalases, peroxidases, diphenoloxidase, polyphenoloxidases, and invertase that activate the formation of both carrier and structural proteins[23].

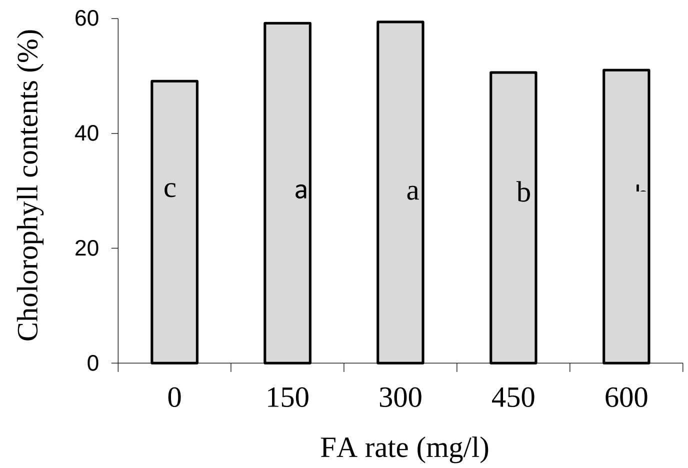


Fig-6: Effect of FA on chlorophyll

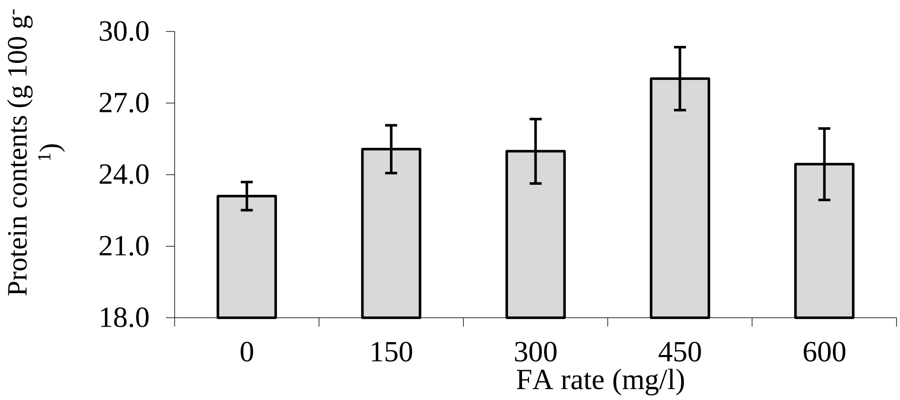


Fig-7: Effect of FA on protein content of tomato plant

3.3 *Discussions*

3.3.1 *Effect of FA on Yield and its Components of Tomato*

FA application increased the height of tomato plant positively due to its stimulating effect on plant growth. The biological yield of tomato increased positively by the foliar application of FA but only up to FA_2. Higher rates of FA stimulated growth and plant grew obnoxiously which effect fruit formation. This could be ascribed to ligand effect that FA application at higher rate complexed metal ions hence making it less available to plant roots and reduced plant uptake. These findings are supported by earlier research on the ligand potential of FA on micronutrients. The trend of tomato response to applied FA regarding dry weight and harvest index was more or less similar to that of biological yield. This is because that FA has relatively smaller molecular size and it readily entered in plant system and traveled from leaves to other plant parts[24], FA is key ingredient in high quality foliar fertilizers. Foliar spray of FA at specific plant growth stages entered into the plant system by absorption, chelated mineral elements and carried them to the metabolic sites in the plant cells where metabolic activities were in progress, it took part in the photosynthesis as well as in translocation of photosynthates to the sink.

Similarly, the effect of FA on chlorophyll content and plant height were also positively affected and its application at the higher rates depressed chlorophyll contents which indicated that at 300 mg/l would be adequate for optimum chlorophyll contents. This in turn accelerated energy metabolism and photosynthetic activities. Chlorophyll within plant leaves was more pronounced when FA was applied as foliar FA_1 and FA_2 such as at lower concentration as were reported by Sladky[25]. The plant geometry were also affected and plants applied with FA as $FA_1$and FA_2 gained symmetrical geometry whereas the plants grown in pots applied with FA_3 and FA_4 were of odd in geometry, indicating that the appropriate rate for optimizing the growth stimulation is FA_1 and FA_2. The polynomial equation showed that 1 mg/l applied foliar FA increased corresponding biomass by 1.2 g, with intercept of 472; dry weight by 123 mg with intercept of 44.6; fruit weight by 2.0 g with intercept of 92 (Table 6). Similarly the harvest index increased by 0.11% with intercept of 11.51 with applied one mg FA. Unfractionated HAs are the most effective in regulating plant growth hormones. HSs also influence other enzymes involved in growth regulation. When the activity of growth regulators is maintained within plant tissues, plant metabolism remains functional and normal growth processes continue to occur[5].

Table-6: Regression equation and coefficient of determination showing quantified effect of FA on yield components of tomato

Parameter	Regression equation	R^2
Tomato plant fresh weight	-45.14x2 + 271.71x + 244.62	0.909
Fruit weight	-5.5119x2 + 35.092x - 20.662	0.914
Tomato plant dry weight	-0.8342x2 + 5.1187x + 5.1804	0.870
Harvest index	-7.3696x2 + 44.698x - 11.13	0.940

3.3.2 *Effect of FA on Nutrient Composition of Tomato*

Decreasing trend of shoot N was due to translocation of N from root towards leaves and even tomato fruits as it was obvious from higher concentration in FA treated leaves (Table 4). This effect was different in case of P which increased both in the leaves and shoot at lower solublization and uptake at high concentration. Similar might be the case for K uptake and translocation. The presence of HSs substantially increased effective assimilation of all mineral nutrient elements. Humate was tested on barley along with NPK; it improved crop growth and decreased the use of mineral fertilizer[27]. The tests on wheat showed that one-way use of N fertilizer on wheat crop did not have positive effect on the crop, while its use along with humates and super phosphate achieved an expected positive effect[28]. The positive process of N assimilation occurs due to an intensification of the ion-exchange processes, while the negative processes of NO_3 formulation decelerates. Potassium assimilation accelerates due to a selective increase in the penetrability of cell membranes. As for P, humates bind ions of Ca, Mg, Fe and Al first, which prevents the formation of insoluble PO_4. That is why the increase of humate content leads to an increase of the plant's P consumption[24]. It shows that micronutrients such as Mn, Cu and Zn uptake increased in the presence of FA mainly through chelating effects.

4. CONCLUSION

Based on the results of study it is concluded that use of FA as foliar spray with low concentration (150-300 mg/l) will be helpful for nutrient absorption, stimulation of plant growth, biomass production, reduce the nutrient losses and ultimately improved crop productivity.

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