Mineral profiling of resistant and susceptible tomato varieties against *Alternaria* solani causing early blight

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The current research was conducted to investigate the alterations in the mineral status in the leaves of tomato plants against early blight (EB) caused by Alternaria solani. Six tomato varieties; viz. Riograndae, Roma and Basket (resistant) and T-88572, BHN-961 and BHN-1021(susceptible) were inoculated with a blend of five isolates of Alternaria solani, collected from different regions of Faisalabad District. These six varieties for mineral profiling were selected after two years screening from twenty-five varieties of tomatoes under field conditions. These varieties were sown in pots and artificial inoculation was performed to develop disease in inoculated type of tomato plants while distilled water was applied on un-inoculated type of plants. Newly infected leaves from upper, middle and lower parts of tomato plants from resistant and susceptible groups were used to prepare sample for mineral analysis at $p \le 0.05$ and variation in mineral profiling of resistant and susceptible groups of tomato plants was determined through Nested Structured Design. Significant variation was observed in inoculated (3.12, 0.48 %, 1.17, 0.14, 0.42, 0.21, 0.69 and 1.49 ppm and un-inoculated type (8.67, 1.61%, 10.45, 0.22, 1.75, 1.98, 3.09 and 3.39 ppm) while resistant group expressed 6.59, 1.19%, 8.13, 1.973, 1.69, 1.26, 1.36, 2.43 and 2.87ppm and susceptible group exhibited 5.19, 0.91%, 5.69, 1.693, 1.24, 0.91, 0.83, 1.35 and 2.22 ppm with respect to NPK, Ca, Mg, Na, Zn, Iron and copper. Resistant variety, Riograndae expressed maximum amount while T-88572 exhibited minimum amount of all mineral contents. Alterations in the mineral profiling in leaves of tomato plants can be used by researchers as biochemical markers for identification and development of resistant source against early blight of tomato and for the development of ecofriendly management strategy towards A. solani.

Keywords: Alternaria solani, lycopersicon esculentum, metabolic reactions, carotenes, lycopene.

INTRODUCTION

Tomato (Lycopersicon esculentum Mill) is the most imperative vegetable crop that is grown around the world (Mari and Lohano 2007) and belongs to family Solanaceae (Khan et al., 2014). It contains vitamins A, C, and E (Naik et al., 2010) and nutrients (27 mg phosphorus, 13 mg calcium, 0.5 mg iron, 3 mg sodium and 244 mg potassium) /100g of tomato (Sgherri et al., 2008). It contains anti-carcinogenic agents (carotenes and lycopene), thiamine, riboflavin, niacin and ascorbic acid Olaniyi et al., 2010). Worldwide, 4848 thousand hectares are cultivated with 182.3 million tons of tomato produce annually. In Pakistan, tomatoes are cultivated on 63.2 thousand hectares with 0.601 million tons production annually (FAOSTAT, 2017). Alternaria solani causes early blight (EB), or target spot disease of tomato (Momel and Pemezny 2006), which appears as small spots in yellow to dark-brown color in concentric rings on the leaves (Gleason and Edmunds 2006). These rings later on spread to entire foliage and stems, causing defoliation and death of plants (Chaerani et al., 2007), with 20 to 80% incidence of disease as observed by Akhtar et al., (2011) and Grigolli et al., (2011). Plant nutrients play a prime role in the defense system of plants, are classified into four groups based on biochemical and physiological behavior (Mitra 2015). The main elements of organic substances are C, H, O, N and S that are involved in oxidation-reduction and enzymatic reactions. The second group (P and B) regulates the energy transfer reactions. Elements of third group (K, Ca, Cl, Mn and Mg) works as catalysts, involved in osmotic and ion balance processes while fourth group minerals (Cu, Fe, Zn and Mo) are known as structural chelates (Mengel 2001; Mengel et al., 2001). In addition, to C, H and O, plants require many essential nutrients. Nitrogen is an essential component of all organic compounds, phosphorus regulates metabolism and potassium works as an activator of several enzymes through ionic regulation (Malvi 2011). Ca is involved in cell division and maintains the integrity of membranes. Mg works as co-factor

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in chlorophyll's enzymatic reactions, Sulfur maintains cell energetic process and iron is involved in key metabolic functions (Fageria and Stone 2006). Zinc is a core element of several proteinases, dehydrogenases and peptidases. Copper is a constituent of several important enzymes, including ascorbic acid oxidase, cytochrome oxidase and laccase. All of these play an important part in the defense mechanism of the plant (Datnoff *et al.*, 2007).

An increase or decrease in quantity of ionic contents of a plant, play an imperative role in the plant growth and disease severity as well as virulence of a pathogen. When the pathogen attacks, it competes with host plant for Na, Ca, Mg, K, NO₃, SO₄, H₂PO₄ and Cl to cause infection by deionizing them or by increasing or decreasing their concentration in the plant (Capula-Rodríguez et al., 2016). This change affects the plant's health and vigor. The transportation and utilization of nutrients in the plant and soil are also restricted by the pathogen. Causal agent of early blight is very destructive as it reduces the plant's uptake efficiency of minerals and the imbalance of plant nutrients causes physiological and metabolic problems, toxicities and enhances susceptibility in tomato plants towards pathogens (Huber and Jones 2013). Aim of the current study was to assess the mineral contents of healthy and diseased tomato leaves to determine the mineral concentration of N, P, K, Ca, Mg, Na, Cu, Fe and Zn to sustain the health of plant when caused by A. solani. These alterations in mineral contents can be used by researchers and scientist as biochemical markers for identification of resistant source in the available germplasm of tomato. These sources are helpful to developing eco-friendly management strategy towards early blight of tomato.

MATERIALS AND METHODS

Establishment and inoculation of tomato plants under greenhouse conditions: After two years' screening of twentyfive tomato varieties under CRD, six varieties were selected for mineral profiling. Three (Riograndae, Basket and Roma) out of six were resistant and three (T-88572, BHN-1021 and BHN-961) expressed susceptible response to A. solani. A nursery of these varieties was established in the experimental area of Plant Pathology Department, University of Agriculture Faisalabad (UAF). The plants were transferred in the pots (32×22cm) after 30 days that were filled with sterilized loamy soil (with formalin, 320:1) and kept on the bench in green house under 3 replications. After 25 days, young leaves of tomato plants were inoculated in the morning (when maximum stomata were opened) with 1×10^8 spores/mL of fungal suspension (mixture of five isolates of A. solani) by using hand sprayer. Fungal spore concentration was determined by using hemocytometer (Horshman PA. 19044). Symptoms of early blight appeared on leaves after seven days of inoculation and gradually spread to entire foliage. Newly infected 15 leaves from upper, middle and lower portion of tomato plants were collected from inoculated

and un-inoculated plants from both resistant and susceptible groups for the determination of ionic contents (Gomez and Gomez, 1984).

Determination of ionic status of leaves from inoculated and un-inoculated tomato plants: Plant samples were oven-dried in paper bags (Heraeus D 6450) for 48 h at 70°C, ground with sterilized mortar and pestle. A 100 mg quantity from dried, ground samples, was boiled in 10 mL of 1.4N HNO₃ on a hotplate (TH550, Adv. Tokyo) at 100 °C for 30 min. The suspension was cooled at room temperature and diluted in 250 mL distilled water. Then samples were analyzed through spectrophotometer (BEL: Model L.24) and flame photometer for determination of N, P, K, Ca, Mg, Na, Cu, Fe and Zn (Bhargava and Raghupathi 1993; Bhargava and Raghupathi 1995).

For determination of Phosphorus, an aliquot of the solution (0.1mL) was placed in a 100 mL volumetric flask then 8.6 mL distilled water and 1mL of Ammonium Molybdate were added in it. The flask was swirled to mix the solution after adding Amino-naphthol Sulphonic Acid (0.4 mL). Absorbance of this solution was measured at 720nm on a spectrophotometer (BEL: Model L.24) by using distilled water as blank. Phosphorus quantity was determined by comparing the absorbency to a previously prepared standard curve (Boltz and Mellon 1948). K and Na were measured by using flame photometer (PFP7/C). For the quantification of potassium (K) and sodium (Na), Potassium Chloride (KCl) and Sodium Chloride (NaCl) were used as standards. Standard curves for K and Na were prepared by using same concentrations (10, 20, 30 and 40 ppm) for both the elements. Fresh working standards were prepared immediately before use (Helrich 1990) while Mg, Ca, Fe, Cu and Zn were determined by using spectrophotometer (BEL: Model L.24). For the determination of these ions, Calcium Chloride (CaCl₂), Magnesium Sulphate (MgSO₄), Iron Sulphate (FeSO₄), Copper Sulphate (CuSO₄) and Zinc oxide (ZnO) were used as standards respectively.

Total nitrogen contents in each sample were determined by using Micro Kjeldahl Apparatus A dried sample of 100 mg was placed in a long neck of 250mL flask and 25mL conc. H_2SO_4 and 5mL CuSO₄ was added in it. The flask was heated in a sand bath until the solution was clear. Solution was then transferred into dissolution flask and treated with 10 mL of 40% NaOH. The liberated ammonia was distilled, and absorbance was determined for the volume of standard acid solution. Amount of unused acid was determined by back titration with standard NaOH solution (Jung *et al.*, 2003). The percentage of nitrogen was calculated by using the formula:

Nitrogen % = $\frac{\text{Volume of } 0.1\text{N H}_2\text{SO}_4 \times 0.0014 \times 250}{\text{Weight:}} \times 100$

Nitrogen $\% = \frac{1}{Weight of sample \times Vol. of sample} \times 100}$ Statistical Analysis: Tomato plant population entailed of two groups (inoculate and un-inoculated) and each group of tomato plants contained two types; resistant and susceptible. Susceptible type contained three varieties/ advanced lines of tomato namely T-88572, BHN-1021 and BHN-961 while resistant type contained Riograndae, Basket and Roma. For estimation of mineral contents, standard analytical methods via Nested Structured Design (Gomez and Gomez 1984) were used and data were statistically analyzed by Statistical Analysis System (Institute 2009). (p<0.05) of all of the elements than the susceptible ones, for both the un-inoculated and inoculated plants. Significant difference of nitrogen was observed between inoculated (3.12%) and un-inoculated (8.67%) group (Table 2).

RESULTS

The resistant plants had significantly higher concentrations

Table 1. Nested ANOVA of mineral concentrations (N, P, K, Na, Ca, Mg, Zn, Fe and Cu) of inoculated and un-inoculated
tomato plant leaves.

SOV	DF	SS SS	MS	F value	Pr>F	Variance component	% of total
				Nitrogen (%)			
Type (A)	1	834.444	834.444	0.033*	28.796	14.916	92.84
Group (B)	2	57.956	28.978	0.000*	28.433	1.036	6.45
Variety (C)	8	8.153	1.019	0.000*	899.444	0.113	0.70
Error	96	0.108	0.001			0.001	0.01
Total	107	900.663				16.066	
Phosphorus (%)							
Type (A)	1	34.612	34.612	0.030*	32.247	0.621	93.01
Group (B)	2	2.146	1.073	0.003*	12.774	0.037	5.49
Variety (C)	8	0.672	0.084	0.000*	111.551	0.009	1.39
Error	96	0.072	0.007			0.001	0.11
Total	107	37.503				0.668	
Potassium (ppm)							
Type (A)	1	1351.007	1351.007	0.050*	16.831	23.532	88.61
Group (B)	2	160.539	80.269	0.000*	113.824	2.947	11.10
Variety (C)	8	5.641	0.705	0.000*	598.997	0.078	0.29
Error	96	0.113	0.001			0.001	0.00
Total	107	1517.308				26.558	
Calcium (ppm)							
Type (A)	1	48.066	48.066	0.021*	45.204	0.870	92.83
Group (B)	2	2.126	1.063	0.025*	6.043	0.033	3.51
Variety (C)	8	1.407	0.176	0.000*	10.594	0.018	1.89
Error	96	1.594	0.016			0.017	1.77
Total	107	53.195				0.938	
Magnesium (ppm)							
Type (A)	1	110.697	110.697	0.033*	28.623	1.978	92.74
Group (B)	2	7.735	3.867	0.000*	25.766	0.138	6.45
Variety (C)	8	1.200	0.150	0.000*	234.340	0.017	0.78
Error	96	0.062	0.001			0.001	0.03
Total	107	119.693				2.133	
Sodium (ppm)		1- 00 - -	1- 000 -	0.0001		0.05	
Type (A)	1	47.893	47.893	0.033*	28.611	0.856	92.29
Group (B)	2	3.347	1.673	0.002*	13.924	0.058	6.21
Variety (C)	8	0.961	0.120	0.000*	187.282	0.013	1.43
Error	96	0.061	0.005			0.001	0.07
Total	107	52.264				0.927	
Zinc (ppm)	1	01 201	01 201	0.050*	14 077	1 452	96.06
Type (A) Group (B)	1	84.384	84.384	0.050*	14.277	1.453	86.06
Group (B)	2 8	11.821 1.736	5.910 0.217	0.000* 0.000*	27.235 478.084	0.211 0.024	12.49 1.43
Variety (C) Error	8 96	0.043	0.217 0.004	0.000**	4/8.084	0.024 0.000	0.03
Total	90 107	97.985	0.004			1.689	0.05
	107	71.705				1.009	
Iron (ppm) Type (A)	1	155.998	155.998	0.01*	7.233	2.489	70.46
Type (A) Group (B)	$\frac{1}{2}$	43.137	21.568	0.000*	40.611	0.779	22.05
Variety (C)	8	43.137 4.248	0.531	0.000*	2.297	0.033	0.94
Error	96	22.199	0.231	0.027	2.271	0.033	6.55
Total	107	225.584	0.231			3.533	0.55
Copper (ppm)	107	223.304				5.555	
Type (A)	1	97.261	97.261	0.021*	6.826	1.537	73.93
Group (B)	2	28.498	14.249	0.000*	78.623	0.521	25.06
Variety (C)	8	1.449	0.181	0.000*	191.523	0.020	0.96
Error	96	0.090	0.008	0.000	171.323	0.020	0.05
Total	107	127.300	0.000			2.079	0.05
$\frac{10tai}{*-Significant ns - n}$						2.017	

*= Significant, ns = non-significant

Nitrogen (%)												
Varieties (C)	Riograndae		Basket Roma			T-88572		BHN-1021		BHN-961		
Types (B)	8		Resistant							eptible		
Groups (A)	Inoc.	Unino	Inoc.	Unino	Inoc.	Unino	Inoc	Unino	Inoc	Unino	Inoc	Unino
Value of N in (C)	3.92	9.91	3.18	9.15	3.76	9.64	2.25	7.76	2.59	7.95	2.97	7.59
Av. val. of N in (C)		.92		.17		.70		.01		.27		.29
Av. val. of N in (B)					Resistar	nt = 6.59 S						
Av. val. of N in(A)				ι		lated $= 8.6$	-		2			
. ,				Phe	osphorus	(%)						
Value of P in (C)	0.62	1.77	0.56	1.64	0.64	1.87	0.25	1.35	0.35	1.47	0.46	1.56
Av. val. of Pin (C)	1	1.2	1.	.11	1	.26	0.	.80	0	.92	1	.01
Av. val. of P in (B)					Resistar	nt = 1.19 S	Susceptib	le = 0.91				
Av. val. of P in (A)				τ		lated $= 1.6$.8			
					assium (
Value of K in (C)	4.88	11.92	4.14	11.52	4.56	11.77	2.04	8.87	2.47	9.44	2.16	9.20
Av. val. of K in (C)	8	.40	7.	.83	8	.16	5.	.46	5	.96	5	.68
Av. val. of K in (B)	Resistant = 8.13 Susceptible = 5.69											
Av. val. of K in (A)				U		ated $= 10.4$			38			
					lcium (p							
Value of Ca in (C)	1.39	2.74	1.20	2.52	1.30	2.69	0.86	2.25	0.98	2.35	1.27	2.45
Av. val. of Ca in (C)	2	.06	1.	.86		.99		.56	1	.67	1	.86
Av. val. of Ca in (B)					Resistant	t = 1.973 S	Susceptib	le = 1.693	3			
Av. val. of Ca in (A)						lated $= 2.5$						
					nesium (
Value of Mg in (C)	0.54	2.86	0.45	2.73	0.64	2.96	0.25	1.93	0.36	2.14	0.50	2.27
Av. val. of Mg in (C)	1	.70	1.	.59	1	.79	1.	.09	1	.25	1	.39
Av. val. of Mg in (B)					Resista	nt = 1.69 S	Susceptib	le = 1.24				
Av. val. of Mg in (A)				J		lated $= 0.2$			4			
				Sc	dium (p	pm)						
Value of Na in (C)	0.76	2.05	0.42	1.85	0.52	1.96	0.24	1.45	0.35	1.65	0.24	1.55
Av. val. of Na in (C)	1	.40	1.	.14	1	.24	0.	.85		1.0	0	.89
Av. val. of Na in (B)					Resista	nt = 1.26 S	Susceptib	le = 0.91				
Av. val. of Na in (A)				ι	Jn-Inocu	lated $= 1.7$	75 Inocul	ated $= 0.4$	2			
					Zinc (ppr	n)						
Value of Zinc in (C)	0.30	2.67	0.26	2.23	0.29	2.43	0.12	1.34	0.14	1.46	0.16	1.76
Av. val. of Zinc in (C)	1	.48	1.	.25		.36		.73	0	.79	0	.96
Av. val. of Zinc in (B)					Resista	nt = 1.36	Susceptib	le = 0.83				
Av. val. of Zinc in (A)				ι	Jn-Inocu	lated $= 1.9$	98 Inocul	ated $= 0.2$	1			
]	fron (ppr	n)						
Value of Fe in (C)	0.94	3.93	0.85	3.55	0.89	4.39	0.43	1.99	0.49	2.25	0.51	2.43
Av. val. of Fe in (C)	2	.44	2.	.19	2	.65	1.	.21	1	.37	1	.47
Av. val. of Fe in (B)						nt = 2.43 §						
Av. val. of Fe in (A)				ι	Jn-Inocu	lated $= 3.0$	9 Inocul	ated $= 0.6$	9			
				C	upper (pj	om)						
Value of Cu in (C)	1.73	4.23	1.52	3.94	1.66	4.14	1.24	2.87	1.35	2.69	1.45	2.48
Av. val. of Cu in (C)	2	.98	2.	.73		.90		.68	2	02	1	.97
Av. val. of Cu in (B)					Resista	nt = 2.87 §	Susceptib	le = 2.22				
Av. val. of Cu in (A)				<u> </u>	Jn-Inocu	lated $= 3.3$	39 Inocul	ated $= 1.4$.9			

 Table 2. Amounts of N, P, K, Ca, Mg, Na, Zn, Fe and Cu in reaction groups (inoculated and un-inoculated), types (resistant and susceptible) varieties/lines of tomato plants.

Percent of total variance of Nitrogen was calculated through statistical analysis and found 6.45% for groups whereas 92.84% for types (Table 1). Maximum value of N concentration was exhibited by the resistant variety Riograndae (6.92%) and minimum (5.01%) by susceptible variety T-88572 (Table 2).

Difference in phosphorus concentration between 2 groups (inoculated 0.48% and un-inoculated 1.61%) was observed significant in tomato leaves (Table. 2) whereas percent of

total variance of Phosphorus was 5.49% (Table 1). Similarly, average amount of P in resistant varieties (Riograndae, basket and Roma) is 1.19% while average amount of phosphorus in susceptible varieties (T-88572, BHN-1021 and BHN-961) is 0.91%. Maximum value of P concentration was exhibited by resistant variety "Roma" (1.26%) and minimum (0.80%) by T-88572 susceptible variety (Table 2). In case of potassium, significant variation was exhibited by inoculated (3.38ppm) and un-inoculated plant leaves 10.45ppm (Table 2). The

percent of total variance (88.61) was exhibited by type (resistant vs susceptible) while 11.10% by group (inoculated vs un-inoculated) (Table 1). High concentration of potassium was shown by resistant variety i.e., Roma (8.13ppm) as compared to susceptible tomato variety namely T-88572 exhibited 5.69ppm (Table 2).

Varieties with 1.89 % of total variance, group (resistant vs susceptible) with 3.51% while type (inoculated vs uninoculated) expressed 92.83% of the total variance of Calcium (Table 1). Significant variation was exhibited by calcium (inoculated = 1.17ppm and un-inoculated = 2.50ppm). Resistant group expressed 1.973 ppm while susceptible group exhibited 1.693ppm of calcium concentration (Table 2). Maximum amount of Ca was expressed by resistant variety Riograndae (2.06ppm) while minimum by susceptible variety T-88572 (1.56 ppm). Significant difference in Mg concentration was indicated by Nested ANOVA types, groups and variety with 92.74, 6.45 and 0.78 % of the total variance (Table 1). Resistant and susceptible type exhibited 1.69 and 1.24 ppm of Mg while un-inoculated varieties expressed 2.50 ppm as compared to inoculated (1.17ppm) during disease attack (Table 2).

Inoculated (0.42ppm) and un-inoculated leaves (1.75ppm) of tomato plants exhibited significant variation in concentration of sodium (Table 2) with 92.29 % of the total variance (Table 1) while resistant group expressed 1.26 and susceptible one showed 0.91ppm of sodium. Maximum amount of Na was determined in variety Riograndae (1.40ppm) as compared to other varieties (Table 2). Similarly, in case of Zinc concentration, resistant group of tomato leaves expressed 1.36ppm and susceptible one exhibited 0.83 ppm concentration of Zn (Table 2) with 12.49 % of the total variance while inoculated type of tomato plants expressed 0.21 and un-inoculated 1.98 ppm of Zinc concentration with 86.06 % of the total variance (Table 1). Minimum amount of Zinc was noted in T-88572 (0.79 ppm) while maximum in Riograndae with 1.48 ppm of Zinc concentration with 1.43% of the total variance (Table 1 & 2). Resistant and susceptible group of tomato leaves exhibited significant variation in Fe contents (2.43, 1.35 ppm respectively) with 22.05 % of the total variance while in case of varieties Riograndae exhibited 2.44 ppm and T-88572 = 0.79 ppm contents of Fe with 0.94 % of the total variance. Significant variation in contents of Cu was noted in un-inoculated and inoculated type of tomato leaves i.e., 3.39 and 1.49 ppm with 73.93 % of the total variance. Similar results were noted in group (resistant vs susceptible) and varieties with 25.06 and 0.96 % of the total variance (Table 1 & 2).

DISCUSSION

Minerals expressed a pronounced effect on the vigor, physiology, resistance, and biochemical reactions taking place within plants. Pathogens attack on the plants to snatch minerals to perform their activities and excessiveness or reduction in these minerals may increase resistance/ susceptibility of host plant or enhance/ reduce aggressiveness of pathogens. Balanced amount of nutrients in host plant can minimize its fragility towards fungal pathogens and can induce resistance in host plants against different microbes. All physiological, biochemical and metabolic processes were observed more efficient than those host plants which received deficient or excessive quantity of nutrients (Curci *et al.*, 2017). That is why, the current study was designed to evaluate the impact of *A. solani* causing early blight of tomato as well as to observe alterations in minerals contents of resistant and susceptible cultivars. In contemporary studies significant variation in concentrations of N, P, K, Ca, Mg, Fe, Zn and copper was observed.

Nitrogen is a key component of amino acids, purines and pyrimidine rings of nucleic acids, proteins, enzymes and chlorophyll of the plants (Miller et al. 2007). Its deficiency reduces the ability of plants to uptake other nutrients (Curci et al., 2017). A low concentration of nitrogen in a growth medium favors some diseases in plants. Plants show nitrogen deficiency which are attacked by necrotrophic fungi. Nitrogen expresses statistically different effects depending upon type of the pathogen (Benard et al., 2009). High N application decreases the severity of the infection against facultative parasites like Alternaria species (Dordas 2008). In present study, Nitrogen (%) was significantly higher in resistant than susceptible and un-inoculated than inoculated leaves of tomato plants. These results are supported by the work of Veverka et al., (2007) who reported that N contents are reduced after the attack of fungal pathogen. Phosphorus plays an important role in the formation of phospholipids, nucleic acids, ADP, ATP, coenzymes (NAD and NADP) and other high energy compounds (Jones et al., 2012). In current study, Phosphorus concentration was observed higher in leaves of resistant and un-inoculated plants than susceptible and inoculated plants. These outcomes are in agreement with the results of Babu et al., (2015) who reported reduction in amount of P after attack of ealy blight of tomato. Potassium activates the enzymes involved in carbohydrate metabolism (Akhtar et al., 2010). The reduction of K concentration in leaves affects the photosynthetic process because it plays a prime role in stomatal opening. In the present study, K was observed in both susceptible and resistant varieties. There was considerably low concentration in the susceptible plant. Reduction in K concentration enhances the severity of early blight in susceptible cultivars (El-mougy and Abdel-Kader 2009) because plants deficit in K are vulnerable to attack of early blight of tomato.

Calcium is an essential element of the plant cell which is absorbed by plants in the form of Ca^+ (Aghofack-Nguemezi*et al.*, 2014). Calcium foliar treatments are known to enhance resistance against many fungal pathogens by improving the structural integrity of the cell wall. In current study, low concentration of calcium was observed in inoculated leaves of both resistant and susceptible cultivars. These results are in line with the finding of Dordas, (2008) who observed the low calcium contents in plants affected with fungal pathogens. Magnesium also plays an important role in the overall mechanism of plant growth and is a key constituent of chlorophyll. The Mg concentration of resistant varieties was significantly higher than susceptible ones which was also witnessed by Aghofack-Nguemezi, (2014) who reported higher contents Mg in resistant cultivars of tomato which strengthened resistance of tomato plants towards early blight disease. Na has a role in chlorophyll synthesis and helps in metabolism of plants. In contemporary study, sodium (Na) contents were observed higher in un-inoculated resistant/ susceptible cultivars and lower in inoculated plants. El-Mougy& Abdel-Kader (2009) reported that the application of sodium against Alternaria solani resulted in the fungal growth inhibition in the *in-vitro* experiments.

Zinc plays a key role among plant's minerals as it is an important component of many enzymes and proteins which triggers the growth and production hormones along with internode elongation. Its deficiency can upset different physiological and biochemical processes in the host plants. In the present study, Zinc contents in resistant tomato cultivars were higher than susceptible tomato varieties. Findings of the present study are supported by the work of Machado *et al.*,(2018) who reported that Zn improved the defense system of tomato plants towards early blight infection in potato by providing a positive impact on plant growth and the severity of disease.

Iron plays an important role in chlorophyll production, nucleic acid metabolism, protein synthesis, acts as oxygen carrier and required for N fixation. Its deficiency causes different maladies in plants. Similarly, Cu plays an imperative role in photosynthetic process and in respiration process to control electron transport chain. It involves in metabolism of cell wall, provides protection against oxidative stress and also it has strong antifungal and antibacterial properties. So, its deficiency alters different physiological functions of the plants and makes the plants vulnerable to the attack of pathogens (Yuerla., 2009). In current studies, higher concentration of copper and iron was noted in resistant and un-inoculated plants as compared to susceptible and inoculated plants. These findings are supported by the work of Noulas et al., (2018) who reported higer concentration of copper and iron in resistant plants towards diseases.

Conclusion: All resistant and un-inoculated tomato varieties have higher values of Nitrogen, Phosphorus, Potassium, Calcium, Magnesium, Sodium, Iron, Copper and Zinc than susceptible and inoculated ones. Application of balanced nutrients is the most suitable strategy to manage the early blight disease of tomato.

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