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Physiological Impacts of Potassium Citrate and Folic Acid on Growth, Yield and some Viral Diseases of Potato Plants

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ABSTRACT

Two field experiments were conducted during the seasons of 2013 and 2014 to study the effect of foliar application of potassium citrate (KC) at 0, 1000 and 2000 ppm and folic acid (FA) at 0, 50 and 100 $\mu g.L^{-1}$ individually and in combination on some growth aspects, biochemical constituents, enzyme activities, tolerance to viral diseases, tuber yield and quality of potato plants (*Solanum tuberosum* L) cv. Cara. The results indicated that the treatments of KC at 2000 ppm or FA at 100 $\mu g.L^{-1}$ as individuals or in combination improved significantly ($P \leq 0.05$) most studied growth parameters including plant length, leaf area, haulm dry weight and chlorophyll reading of SPAD compared to the controls in both seasons. Additionally, total soluble carbohydrates, phenolic compounds and total soluble protein were increased significantly ($P \leq 0.05$) in the leaves by both examined concentrations of KC, whereas, FA had significant positive effects on phenolic compounds, total soluble protein and free amino acids compared to the controls in the two seasons. Generally, the combination of KC at 2000 ppm with FA at 50 or 100 $\mu g.L^{-1}$ achieved the highest increments for most studied biochemical constituents. The same tendency was observed in regard to the activity of phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO). These findings led to reduce the viral symptoms and percentage of infected plants. On the other hand, the examination by DAS-ELISA revealed that the treatment of KC at 2000 ppm plus FA at 100 $\mu g.L^{-1}$ had only negative reactions with PVX and PLRV whereas, PVY showed positive reaction in both seasons. Also, the application of 2000 ppm KC combined with or without foliar application of FA was the most favorable treatments for increasing number of tuber, yield per plant and Feddan as well as marketable yield in the two tested seasons. The interaction treatments clarify that the combination of KC at 2000 ppm plus FA at 100 $\mu g.L^{-1}$ gave the highest results in both seasons. Hence, it could be recommended that KC at 2000 ppm with FA at 50 or 100 $\mu g.L^{-1}$ foliar applications can be used to increase the final yield and tuber quality of potato plants.

Key words: Biochemical constituents, folic acid, potato, potassium citrate, viral diseases, yield

Introduction

Potato (*Solanum tuberosum* L.) is a crop of major significance in human nutrition, ranking fourth in food production, after wheat, corn (maize), and rice. It is the first in energy production, and second in carbohydrate and protein production (Nieder, 1992; Fabeiro *et al.,* 2001). Their tubers are a good source of vitamins and minerals (Blagoeva *et al.,* 2004). The quality of potato tubers and their chemical composition are influenced by genetics, soil fertility, weather conditions and the applied chemical treatments (Rytel *et al.,* 2013).

Potassium is a major plant nutrient, which is essential for a variety of physiological processes i.e. photosynthesis, protein synthesis, enzyme activation and maintenance of water status in plant tissues (Marschner, 2012). Also, it influences synthesis, location, transformation and storage of carbohydrates, tuber quality and processing characteristics as well as plant resistance to stresses and diseases (Ebert, 2009). Potassium citrate (KC) increase leaf area, improves leaf mineral content, enhancing yield and improved fruit quality as well as physical and chemical properties of mango trees (Taha *et al*., 2014). In this respect, Ebeed and Abd El-Migeed (2005) found that spraying mango trees with KC was very effective in improving yield as number of fruits or weight (kg)/tree and increased fruit weight. Moreover, spraying onion plants with potassium markedly increased vegetative growth, yield, bulb quality and bulb chemical composition (Behairy *et al*., 2015). Potassium citrate is potassium salt of citric acid which considered one of the most important organic acids in the respiratory pathways into plant cell. The mitochondrial citric acid cycle provides the energy for ATP synthesis which is essential for different biochemical and physiological processes (Taiz and Zeiger, 2002). Additionally, citric acid plays an important role in plant metabolism, it's as non-enzymatic antioxidant in chelating free

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radicals and protecting plant from injury could result in prolonging the shelf life of plant cells and improving growth characters (Sadak and Orabi, 2015). Other authors, emphasized the importance of citric acid for improving quality of fruits (such as tomato, carrot and bean) (Nahar and Gretzmacher, 2002; Abou El-Nasr and Ibrahim, 2011; El-Tohamy *et al.,* 2013).

Folic acid (FA) is a central cofactor for one-carbon transfer reactions which are involved in many cellular reactions such as synthesis of purines, metabolism of amino acids, a glycine to serine conversion, synthesis of methionine and the formation of lignin, chlorophyll and choline and also in the photorespirations cycle (Hanson and Roje, 2001; Jabrin *et al.,* 2003). It can be used as a new, convenient, and affordable organic fertilizer to increase the efficiency of the plant and preserve its nutrients (Poudineh *et al*., 2015). On the other hand, FA has become the most prominent of B complex vitamins despite its essential biochemical function in amino acid metabolism and nucleic acid synthesis (Andrew *et al*., 2000). In this respect, Stakhova *et al.* (2000) reported that exogenous FA increased weight of pea seeds and yield. Amino acid analysis revealed a notable increase in the content of folate-dependent amino acids. In these words, treatment of the plants with exogenous FA increased both the content of chlorophyll in the leaves and their continuance function. In strawberry, Li *et al.* (2015) reported that folate is one of the most important micronutrients and has many forms, but only FA form has cofactor activity. In this respect, a few literatures reported that exogenous FA has positive effect on growth, yield and quality of some plants such as flax (Emam *et al.,* 2011), faba bean (Zewail *et al.,* 2011) and winter wheat (Vician and Kovacik, 2013).

The viral diseases are considered one of the most limiting factors affecting potato production in all over the world. More than 35 different viruses are known to affect potatoes. Worldwide; potato leaf roll virus (PLRV), potato virus Y (PVY), potato virus A (PVA), potato virus X (PVX), potato virus M (PVM) and potato virus S (PVS) are the most important widespread and cause severe damages to yield when combined (Salazar, 1996).

The objective of this study was to evaluate the physiological roles of potassium citrate and folic acid as foliar applications individually and in combination on the growth, biochemical constituents, yield and the tolerance to three of most limiting viruses for potato production under Egyptian conditions (PLRV, PVX and PVY).

Materials and Methods

In this study, the field experiment was carried out during the two growing seasons of 2013 and 2014, at the experimental farm, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Egypt, in order to investigate the effect of foliar application of potassium citrate (KC) and folic acid (FA) on yield, tuber quality and tolerance to viruses of potato plants. The chemical and physical analyses of the experimental soil are shown in Table (1).

Table 1: *Some chemical and physical analysis of the experimental soil

pH	EC µS cm^{-1}	$CaCO_3$ %	Cation meq / L			Anion meq / L		
			Ca^{++}	Mg^{++}	Na^{+}	HCO_3^-	CL^-	$SO4^{-2}$
8.71	0.65	1.27	7.54	2.47	1.78	3.10	3.25	1.54
N	P	K	Sand %		Silte %	Clay %	Soil texture	
(ppm)								
61	85	387	49.72		11.47	38.81	Sandy clay	

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Tubers of potato cv. Cara were sown on 25th of January during 2013 and 2014 seasons. The experimental plot area was 12.25 m^2 involved five rows; each row was 3.5 X 0.7 meter. The plant distance was 25 cm apart on one side of row.

Calcium super-phosphate (15 % P_2O_5) at 400 kg / feddan was banded on rows at two times, the first (200 kg) was added during the soil preparation and the second one (200 kg) was carried out after one month from transplanting. Ammonium nitrate (33% N), at 300 kg / feddan, was applied as soil application in three portions, the first addition (100 Kg) was added during the soil preparation, the second one (100 kg) was carried out after one month from planting and the third one (100 kg) was added one month later. Potassium sulphate (48 % K_2O) was applied at a rate of 150 kg per Feddan at two times. The first portion took place one month from planting, whereas, the second part was added one month later. Cultural management, disease and pest control programs were followed according to the recommendations of the Egyptian Ministry of Agriculture.

The experimental design and treatments:

Potassium citrate (38.3% K_2O), produced by U.A.D. Company, was used at three concentrations, i.e., 0 (control, sprayed with distilled water), 1000 and 2000 ppm, applied after 30 and 50 days from planting as foliar application. Folic acid produced by Mepaco Medifood Company Egypt, was used at three levels, namely 0 (control, sprayed with distilled water), 50 and 100 $\mu g.L^{-1}$, applied after 40 and 60 days from planting as foliar application. The experiment was laid out in a split plot design with three replicates. Potassium citrate foliar application was assigned in the main plots and foliar application of folic acid was distributed in the sub-plots.

Studied characteristics:

Vegetative growth characteristics:

Plant samples in the two seasons were randomly taken from each treatment after 75 days from planting. The growth parameters of potato included plant length, leaf area, number of branches, haulm dry weight and total chlorophyll reading was recorded. Leaf area was determined using the recently full expanded fourth leaf from the plant top as relation between unit area and leaf fresh weight (Koller, 1972). Chlorophyll reading was recorded by Minolta Chlorophyll Meter SPAD – 502.

Biochemical constituents and enzyme assays

Total soluble carbohydrates (TSC) of 0.1 g dry weight in leaves were extracted according to A.O.A.C. (1990) and estimated by phenol and sulfuric acid method as described by Chow and Landhausser (2004) by reading the yellow developed color at the wavelength 490 nm. The extraction of dry leaves in cold MeOH 85% was used to determine the phenolic compounds as catechol according to the method of Folin-Denis as described by Shahidi and Naczk (1995) by reading the developed blue color at 725 nm. To prepare the extraction of total soluble proteins (TSP), free amino acids (FAA) and enzyme assays: phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO), fresh leaves were homogenized in 5 mL phosphate buffer (0.1 mol/L, pH 7.8), centrifuged at 10,000 ×g for 20 min at 4 °C and then the supernatant was used for assays. Total soluble protein was evaluated by the method of Bradford (1976). Free amino acids were determined according to Hamilton and Van Slyke (1943). One ml of each sample extract was treated with 1ml of 10% pyridine and 1 ml of 2% ninhydrine solution. The optical densities of these colored solutions were then read at 570 nm. Glycine solution were prepared as standard. The activity of phenylalanine ammonia-lyase, PAL (E.C 4.3.1.5) was assayed as described by Lister *et al.* (1996). The PAL assay reaction consisted of 100 µL crude extract and 900µL of 6 µmol phenylalanine in 500 mM tris-HCl buffer (pH 8.5). The mixture was incubated at 37 ºC for 1 h and measured spectrophotometrically at 290 nm. Polyphenol oxidase (PPO) (EC 1.14.18.1) activity was measured according to Oktay *et al.* (1995). The reaction mixture consisted of 100 µl crude enzyme, 600 µl catechol and 2.3 ml phosphate buffer (0.1 M, pH 6.5). The absorbance at 420 nm was recorded at zero time and after 1 min.

Yield components:

Each experimental plot was harvested individually after 110 days from planting, number of tubers/plant, tuber yield / plant and feddan as well as marketable tuber yield/feddan were recorded.

Estimation of tuber quality indicators:

After 110 days from cultivation, a random sample of three plants from each plot was chosen to estimate some of quality indicators including the weight, volume, specific gravity, starch and the dry matter of the produced tubers. The volume of tubers was calculated by the water displaced by each one in a graduated cylinder. The specific gravity (SG) was estimated by the ratio of tuber density to water. The starch content was calculated according to the formula of Burton (1948): Starch (%) = 17.546+199.07 (SG – 1.0988). Dry matter (DM) ratio was determined by the expression described by Pereira *et al.* (2008) as follows: DM = 24.182 + 211.04 * [SG – 1.0988].

Symptoms and ratio of disease incidence:

Symptoms expected to virus infection were recorded after 45 and 65 days after planting. Ratio of disease incidence depending on developed symptoms was estimated after 65 days from planting in all treatments.

Percentage of virus infection

Percentage of virus infection was expressed as the disease percentage (DP) of plants which developed symptoms, 65 days after planting infected potato plants were divided on total plants in each treated area.

$$DP = \frac{\text{Number of plants developed symptoms}}{\text{Total number of plants in each treated area}} \times 100$$

Serological detection:

Sample collection: during first and second seasons, field samples were conducted to estimate the incidence of viruses in potato cv. Cara that developed symptoms expected to virus infection. For each treatment, leaf samples (top, middle and bottom) were collected from symptomatic plants for serological assay after 65 days from planting.

Serological detection: viruses were detected by double antibody sandwich (DAS) enzyme-linked immunosorbent assay (ELISA) on polystyrene microtitre plats (Clark and Adams, 1977). Leaf samples were tested for the presence of PVY, PVX and PLRV using ELISA kit. Absorbance values were determined using ELISA reader (BIE & BERNTSEN A.S) at 405 nm [1 hr. after addition of the substrate].

Statistical analysis:

Data of the two seasons were arranged and statistically analyzed using Mstatic (M.S.). The comparison among means of the different treatments was determined by using Duncn test, as illustrated by Snedecor and Cochran (1982).

Results and Discussion

Growth parameters:

The plant length, leaf area, number of branches and haulm dry weight and chlorophyll reading of potato plants were increased significantly ($P \leq 0.05$) by foliar application of KC at 2000 ppm in both season, but the effect on number of branches was insignificant in the two seasons (Table 2). The enhancing effect of potassium on plant growth might be attributed to its association with the efficiency of leaf as an assimilator to CO_2 (Rai *et al.,* 2002), activating phyto-hormone, regulation of cellular pH, enhancing N uptake, and acting as an activator to enzymatic systems (Marschner, 2012). In this respect, Zewail *et al.* (2011) found that foliar application of KC increased plant height, number of branches, total leaf area and dry weight of leaf and stems of faba bean plants. Also, the increasing in chlorophyll reading might be due to the potassium affects photosynthesis at various levels (Abou El-Yazied and Mady, 2012). On the other hand, these results may be attributed to the positive effect of citric acid as antioxidant which has an auxin action that improved vegetative growth parameters. These results are in somewhat in harmony with the findings of Hagag *et al.* (2000) who stated that antioxidant such as citric acid have auxin action. Recently, antioxidants are used instead of auxins and other chemicals for enhancing growth and fruiting of olive seedlings. In this regard, Sun and Hong (2011), when citric acid was exogenously applied it significantly improved the plant growth by increasing the activities of antioxidant enzymes. Although the mechanism behind this effect is not clearly explained, probably the stomatal penetration by aqueous solutions plays here a role, bean plants sprayed with citric acid showed higher total chlorophyll content compared to control (El-Tohamy *et al.,* 2013). On the other hand, Nour *et al.* (2012) found that spraying snap bean plants with citric acid gave the highest values of plant growth and total dry weight of snap bean pods. The foliar spray with citric acid increased vegetative growth of faba bean (Nosser, 2011).

Data indicated that the vegetative growth were significantly ($P \leq 0.05$) improved and higher by using foliar application of FA in both seasons. It clearly shown that, foliar application of FA at 50 or 100 $\mu g.L^{-1}$ in the first season and 100 $\mu g.L^{-1}$ in the second season induced a significant increase in plant length and leaf area as compared to control treatment. The foliar application of FA insignificantly affected the number of branches in both seasons. Haulm dry weight of plants treated with the foliar application of FA at 100 $\mu g.L^{-1}$ was significantly increased as compared with the other treatments. In this respect, Emam *et al.* (2011) showed that foliar application of FA significantly increased the growth parameters of flax. Burguieres *et al*. (2007) indicated that some seedling properties such as shoot weight, shoot and root length were increased with FA treated plants. The maximum increase in chlorophyll reading of potato leaves was measured in FA treated plants in the two seasons. In this respect, Stakhova *et al*. (2000) reported that pea and barely treated with exogenous FA showed increase in plant dry weight. The FA increased the chlorophyll content in lower leaves and this may be due to that FA induced activation of the biosynthesis of glycin, which is involved in the synthesis of porphyrins and chlorophyll in chloroplast membranes. Moreover, Sladky (1959) observed that application of FA could enhance chlorophyll content of leaves.

Concerning the interactions, the studied combination between foliar application of KC and FA indicated that plants subjected to foliar application of 2000 ppm KC with 50 or 100 $\mu g.L^{-1}$ FA s showed the highest plant length and chlorophyll reading than the other combination treatments. Moreover, leaf area produced with foliar application of 2000 ppm KC and 100 $\mu g.L^{-1}$ FA showed the highest values in the two seasons. The highest values of number of branches were produced with 2000 ppm KC with spray or without FA in the first season. These effects were insignificant in the second tested season. These results were in harmony with Esfandiari *et al*. (2012) who found that dry weights of shoots were increased in wheat by exogenous application of FA.

Table 2: Effect of foliar application of potassium citrate (KC) and folic acid (FA) on some vegetative growth of potato plants in the two seasons (2013 and 2014).

Treatments		Plant length (cm)	Leaf area (cm^2)	Number of branches	Haulm dry weight (g)	Chlorophyll reading (SPAD)
2013 season						
Potassium citrate (ppm)						
0		81.7 b	30.4 c	6.8 a	39.0 c	38.4 c
1000		85.4 b	33.4 b	7.3 a	44.2 b	42.2 b
2000		93.0 a	37.0 a	8.0 a	52.5 a	45.5 a
Folic acid (µg/l)						
0		81.7 b	31.7 b	7.1 a	43.7 b	40.3 b
50		87.5 a	33.9 ab	7.4 a	44.1 b	42.6 a
100		90.9 a	35.3 a	7.6 a	47.9 a	43.2 a
KC	FA	Interaction treatments between potassium citrate and folic acid				
0	0	77.9 f	28.6 f	6.3 c	36.9 d	36.8 e
	50	82.7 de	30.8 ef	7.0 bc	38.0 cd	38.6 de
	100	84.5 cd	32.0 de	7.0 bc	42.0 b	39.9 c-e
1000	0	79.0 ef	31.5 ef	7.0 bc	41.8 bc	40.7 c-e
	50	85.3 cd	33.5 c-e	7.7 ab	41.3 bc	42.1 b-d
	100	91.9 ab	35.3 bc	7.3 a-c	49.6 a	43.8 a-c
2000	0	88.2 bc	35.1 b-d	8.0 ab	52.4 a	43.4 a-c
	50	94.4 a	37.3 ab	7.6 ab	53.0 a	47.2 a
	100	96.3 a	38.7 a	8.3 a	52.1 a	46.0 ab
2014 season						
Potassium citrate (ppm)						
0		70.1 b	29.0 c	7.3 a	42.7 c	36.0 c
1000		75.8 b	32.6 b	7.7 a	48.2 b	39.6 b
2000		88.0 a	36.8 a	8.3 a	55.1 a	45.3 a
Folic acid (µg/l)						
0		74.8 b	31.4 b	7.6 a	46.3 b	39.0 b
50		77.8 ab	32.3 b	7.9 a	47.7 b	40.0 ab
100		81.3 a	34.9 a	7.9 a	52.0 a	41.9 a
KC	FA	Interaction treatments between potassium citrate and folic acid				
0	0	69.0 e	27.4 e	7.0 a	38.3 f	35.0 e
	50	69.0 e	28.8 de	7.7 a	43.9 ef	35.5 e
	100	72.3 e	30.9 cd	7.3 a	46.1 c-e	37.5 de
1000	0	71.6 e	31.0 cd	7.3 a	47.7 b-e	39.0 d
	50	75.3 de	32.0 c	7.7 a	45.1 d-f	38.9 d
	100	80.4 cd	34.9 b	8.0 a	51.8 a-d	40.8 cd
2000	0	83.6 bc	35.7 b	8.3 a	53.0 a-c	43.0 bc
	50	89.2 ab	36.0 b	8.3 a	54.2 ab	45.7 ab
	100	91.0 a	38.8 a	8.3 a	58.2 a	47.3 a

Mean within a column and their interaction following with the same latter are not significantly different according to Duncan multiple range test at the probability of 0.05 levels.

Biochemical constituents

Data presented in Table (3) show that foliar application of KC improved significantly ($P\leq 0.05$) all the investigated biochemical constituents except free amino acids (FAA) in potato leaves. The maximum increments were achieved by the high concentration of KC at 2000 ppm in the two seasons. The positive influences of KC on total soluble carbohydrates, phenolic compounds and total soluble protein may be attributed to the importance of potassium as a cation (+) and/or citrate anion (-) in the different physiological processes: Potassium plays a major role in the transport of water and nutrients throughout the xylem (Malvi, 2011). These processes are required in enhancing most biochemical pathways into the plant; also, citric acid is considered one of the most important organic acids in the respiratory pathways into plant cell. The mitochondrial citric acid cycle provides the energy for ATP synthesis which is essential for different biochemical and physiological processes (Taiz and Zeiger, 2002). On the other hand, the negligible reductions in the FAA by the foliar application of KC could be explained by that the incomplete protein such as amino acids, amides and nitrate may not accumulate in the cell with adequate supply of potassium. Two reasons could be interpreted (1) potassium help to increase the rate of complete protein synthesis and enzyme activation (2) potassium may facilitate the translocation of amino acids from leaves to another organs.

Concerning, the effect of FA, the foliar application of FA had positive effects on the leaf content from phenolic compounds (PC), total soluble protein (TSP) and free amino acids (FAA) in both seasons. The highest significant ($P\leq 0.05$) increases were obtained by the treatment of FA at 100 $\mu g.L^{-1}$ for PC, TSP and FAA compared to the controls in the two seasons. Improving the biochemical constituents by the foliar application of FA was emphasized earlier by many researchers (Emam *et al.,* 2011; Esfandiari *et al*., 2012). Folic acid is considered the central cofactor for one-carbon transfer reactions which are involved in many cellular reactions such as synthesis of purines, nucleic acids, metabolism of amino acids, a glycine to serine conversion, synthesis of methionine and the formation of lignin, chlorophyll and choline and also in the photorespirations cycle (Andrew *et al.,* 2000; Hanson and Roje, 2001; Jabrin *et al.,* 2003).

As for the interaction treatments, it was clear that the combinations of KC with FA were more effective in increasing the biochemical constituents in potato leaves than the individual ones and controls in both seasons. The highest values were obtained by the treatment of KC at 2000 ppm with FA at 100 µg.L^{-1}. These findings may be related to the synergistic effect of the two studied substances on the different biochemical pathways in plant cell.

Table 3: Effect of foliar application of potassium citrate (KC) and folic acid (FA) on Total soluble carbohydrates (TSC), phenolic compounds (PC), total soluble protein (TSP) and free amino acids (FAA) in the leaves of potato plants in the two seasons (2013 and 2014).

Treatments		TSC (mg/g d.wt)	PC (mg/g d.wt)	TSP (mg/g F.wt)	FAA (mg/g F.wt)
2013 season					
Potassium citrate (ppm)					
0		158.4 c	1.94 b	2.35 b	2.42 a
1000		199.9 b	2.52 a	2.50 a	2.30 a
2000		216.5 a	2.76 a	2.54 a	2.28 a
Folic acid (µg/l)					
0		176.0 a	2.10 b	2.34 b	1.73 c
50		201.7 a	2.50 ab	2.50 a	2.49 b
100		197.1 a	2.62 a	2.55 a	2.78 a
KC	FA	Interaction treatments between potassium citrate and folic acid			
0	0	146.4 f	1.73 e	2.23 d	1.90 d
	50	164.3 ef	1.93 de	2.37 c	2.39 c
	100	164.6 ef	2.17 c-e	2.44 bc	2.54 bc
1000	0	184.4 de	2.20 c-e	2.38 c	1.87 d
	50	210.8 a-c	2.63 a-c	2.54 ab	2.56 b
	100	204.4 b-d	2.73 ab	2.59 a	2.83 a
2000	0	197.3 cd	2.37 b-d	2.42 c	1.41e
	50	229.9 a	2.93 a	2.59 a	2.52 bc
	100	222.4 ab	2.97 a	2.62 a	2.96 a
2014 season					
Potassium citrate (ppm)					
0		181.8 c	2.08 b	2.44 b	2.41 a
1000		201.8 b	2.76 a	2.52 ab	2.32 a
2000		217.1 a	2.98 a	2.59 a	2.18 a
Folic acid (µg/l)					
0		192.4 a	2.21 b	2.38 b	1.77 c
50		201.4 a	2.68 ab	2.57 a	2.46 b
100		206.9 a	2.92 a	2.60 a	2.68 a
KC	FA	Interaction treatments between potassium citrate and folic acid			
0	0	171.4 f	2.00 d	2.26 e	1.88 f
	50	185.1 ef	1.93 d	2.52 c	2.25 e
	100	188.8 d-f	2.30 cd	2.53 c	2.41 d
1000	0	195.5 c-e	2.07 d	2.41 d	1.91 f
	50	203.0 b-e	3.03 ab	2.55 bc	2.58 c
	100	206.9 a-d	3.17 a	2.62 ab	2.74 b
2000	0	210.1a-c	2.57 bc	2.48 c	1.54 g
	50	216.1ab	3.07 a	2.64 a	2.55 c
	100	225.0 a	3.30 a	2.64 a	2.88 a

Mean within a column and their interaction following with the same latter are not significantly different according to Duncan multiple range test at the probability of 0.05 levels.

Enzyme activities

Data presented in Table (4) show that the two studied concentrations of KC had significant ($P\leq 0.05$) positive effects on the activity of both enzymes, except KC at 1000 ppm on PAL in the first season compared to the controls in both seasons. These increases reached 47, 35.6% for PAL and 30.7, 30.9% for PPO over the controls in both seasons, respectively.

Concerning the effect of FA, it can be noticed that the activity of PAL and PPO were increased significantly ($P\leq 0.05$) by the foliar application of FA compared to the controls in the two seasons. The maximum increases in the PAL (43.7, 42.9 %) and PPO (49, 70.3 %) activities were obtained by the treatment of FA at 100 µg.L^{-1} over than the controls in both seasons. Although the remarkable superiority of the high concentration of FA in this respect, no significant differences were noticed with the other investigated concentration of FA on the activity of both enzymes in the two seasons. The interaction between all treatments indicated that the combination of KC at 2000 ppm with FA at 100 µg.L^{-1} gave the highest activity for PAL and PPO under studied conditions. The increases in the activities of PAL and PPO could be attributed to increase the concentration of phenolic compounds under previous conditions (Table, 3). This result may be explained by increase the rate of photosynthesis which is considered the main source for phenolic compounds by shikimic acid pathway (Taiz and Zeiger, 2002) as affected by the application of KC and /or FA.

The increase in enzyme activities under high potassium application could be explained by that potassium neutralizes various organic anions and other compounds within the plant, helping to stabilize pH between 7 and 8 the optimum for most enzyme reactions. Also, potassium plays essential roles in enzyme activation, protein synthesis (Marschner, 2012). Emam *et al.* (2011) showed that foliar application of FA activated the antioxidative properties of flax seeds in terms of increasing the endogenous contents of glutathione, ascorbic acid and total phenols.

Table 4: Effect of foliar application of potassium citrate (KC) and folic acid (FA) on the activity of phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO) in the leaves of potato plants in the two seasons (2013 and 2014).

Treatments		PAL (based on ΔA/h)	PPO (based on ΔA/min)	PAL (based on ΔA/h)	PPO (based on ΔA/min)
		2013 season		2014 season	
Potassium citrate (ppm)					
0		0.275 b	0.0394 b	0.296 c	0.0434 b
1000		0.322 ab	0.0493 a	0.342 b	0.0523 a
2000		0.368 a	0.0515 a	0.401 a	0.0568 a
Folic acid (µg/l)					
0		0.248 b	0.0345 b	0.260 b	0.0333 b
50		0.360 a	0.0517 a	0.366 a	0.0544 a
100		0.357 a	0.0541 a	0.372 a	0.0567 a
KC	FA	Interaction treatments between potassium citrate and folic acid			
0	0	0.214 g	0.0275 f	0.234 f	0.0233 d
	50	0.296 de	0.0448 cd	0.312 d	0.0485 b
	100	0.316 de	0.0460 c	0.322 d	0.0473 b
1000	0	0.260 f	0.0403 de	0.272 e	0.0368 c
	50	0.369 b	0.0519 b	0.395 b	0.0533 ab
	100	0.336 c	0.0558 ab	0.371 c	0.0586 a
2000	0	0.271 ef	0.0357 e	0.274 e	0.0402 c
	50	0.415 a	0.0584 a	0.390 bc	0.0589 a
	100	0.419 a	0.0604 a	0.422 a	0.0618 a

Mean within a column and their interaction following with the same latter are not significantly different according to Duncan multiple range test at the probability of 0.05 levels.

Tuber yield

Data in Table (5) show that there were statistically significant (P≤ 0.05) effects for foliar application of KC on number of tubers, yield per plant and Feddan as well as marketable yield in the two tested season. Plants foliar application of 2000 ppm KC showed the highest values of yield characteristics than the other treatments in the two seasons, except number of tubers in the first season. In this respect, Zewail *et al.* (2011) found that foliar application of KC increased yield and yield components of faba bean plants. On the other hand, Nour *et al.* (2012) found that spraying snap bean plants with citric acid gave the highest values of total dry weight, yield and its components of snap bean pods. The foliar spray with citric acid increased total green yield and weight of faba bean (Nosser, 2011).

Foliar application of FA at 50 or 100 µg.L^{-1} increased significantly number of tubers, yield per plant and Feddan as well as marketable yield, except number of tubers in the second season. In this respect, Stakhova *et al.* (2000) reported that application of FA in form of exogenous treatment increases the productivity through improving the yield of the seed in both of pea and barley. Also, the effect of FA on paddy yield and thousand kernel weight was significant and the maximum averages (Rezaee *et al.*, 2012). Emam *et al.* (2011) showed that foliar application of FA enhanced yield of flax. Moreover, application of FA, increased yield of white button mushroom (Dahmardeh *et al.,* 2015).

Regarding the interaction between foliar application of KC and FA, the application of 2000 ppm KC combined with or without foliar application of FA was the most favorable treatment for increasing number of tubers, yield per plant and Feddan as well as marketable yield in the two tested season. These results could be attributed to the stimulation effect of foliar application of KC and FA on vegetative growth (Table 2) and leaf chemical constituents and enzyme activities (Tables 3 and 4).

Table 5: Effect of foliar application of potassium citrate (KC) and folic acid (FA) on yield and its components of potato plants in the two seasons (2013 and 2014).

Treatments		Number of tubers/plant	Tuber yield/plant (g)	Tuber yield/ Fed. (Ton)	Marketable yield/Fed. (Ton)
2013 season					
Potassium citrate (ppm)					
0		12.0 a	483.3 c	10.49 c	8.19 c
1000		12.4 a	528.9 b	11.48 b	9.07 b
2000		13.0 a	582.0 a	12.64 a	10.26 a
Folic acid (µg/l)					
0		11.8 b	503.2 b	10.93 b	8.61 b
50		12.6 a	526.3 ab	11.43 ab	9.15 ab
100		13.1 a	564.8 a	12.26 a	9.76 a
KC	FA	Interaction treatments between potassium citrate and folic acid			
0	0	11.0 d	455.0 d	9.9 d	7.65 d
	50	12.7 bc	487.8 cd	10.6 cd	8.43 c
	100	12.3 bc	507.1 c	11.0 c	8.48 c
1000	0	12.0 c	494.6 cd	10.74 cd	8.35 cd
	50	12.3 bc	514.3 bc	11.17 bc	8.93 c
	100	13.0 b	577.8 a	12.5 a	9.93 b
2000	0	12.3 bc	560.0 ab	12.16 ab	9.82 b
	50	12.7 bc	576.7 a	12.52 a	10.10 b
	100	14.0 a	609.4 a	13.2 a	10.87 a
2014 season					
Potassium citrate (ppm)					
0		11.8 b	502.6 b	10.91 c	8.98 b
1000		12.0 b	546.2 b	11.86 b	9.68 b
2000		13.7 a	634.1 a	13.77 a	11.70 a
Folic acid (µg/l)					
0		12.0 a	526.7 b	11.44 b	9.42 c
50		12.6 a	559.2 ab	12.14 ab	10.08 b
100		12.9 a	597.0 a	12.96 a	10.86 a
KC	FA	Interaction treatments between potassium citrate and folic acid			
0	0	10.3 d	442.3 e	9.60 e	7.87 e
	50	12.7 bc	506.5 de	11.00 cd	9.13 cd
	100	12.3 c	559.2 b-d	12.14 bc	9.94 bc
1000	0	11.0 d	493.5 de	10.72 de	8.78 de
	50	12.3 c	548.1 cd	11.90 b-d	9.38 cd
	100	12.7 bc	597.0 a-c	12.96 ab	10.88 ab
2000	0	14.7 a	644.3 a	13.99 a	11.60 a
	50	12.7 bc	623.1 ab	13.53 a	11.74 a
	100	13.7 ab	634.9 a	13.79 a	11.77 a

Mean within a column and their interaction following with the same latter are not significantly different according to Duncan multiple range test at the probability of 0.05 levels.

Tuber quality

Data presented in Table (6) show that, generally, the foliar application of KC increased significantly ($P \leq 0.05$) the mean weight and volume of tuber comparing with controls. The highest increments were achieved at 2000 ppm in both seasons. A similar trend was observed in respect to FA but these increases did not reach the level of significance at 50 $\mu g.L^{-1}$ in the second season comparing with the control. The interaction treatments revealed that the combination of KC plus FA had positive significant ($P \leq 0.05$) effects on both traits. The highest results were recorded by the combination of KC at 2000 ppm and FA at 50 or 100$\mu g.L^{-1}$ in both seasons. On the other hand, no significant differences were detected in the specific gravity among all FA application in both seasons, but KC application increased it.

Concerning the tuber content of starch and dry matter, it is clear that KC had improved significantly ($P \leq 0.05$) both of them in the two seasons. Meanwhile, FA did not reveal any significant changes in this respect. Also, the interaction treatments clarify that the combination of KC at 2000 ppm plus FA at 100 $\mu g.L^{-1}$ gave the highest results in both seasons. In this respect, Stakhova *et al.* (2000) found that the increased content of FA in the leaves stimulates the synthesis of the dependent amino acids and so increases the quality of pea and barley. Also, Taha *et al.* (2014) found that spraying mango with KC improved fruit quality. In addition, Emam *et al.* (2011) showed that foliar application of FA improved the quality of flax. Moreover, application of FA, resulting an increase of quality yield of white button mushroom (Dahmardeh *et al.,* 2015).

In the present study, two possibilities can be suggested to explain the positive effect of KC on the tuber quality indicators. The first, potassium citrate is potassium salt of citric acid which considered one of the most important organic acids in the respiratory pathways into plant cell. The mitochondrial citric acid cycle provides the energy for ATP synthesis which is essential for different biochemical and physiological processes (Taiz and Zeiger, 2002). The second possibility, potassium is considered one of the most important factors affecting the growth and yield of potato. Many literatures have proved that potassium plays a key role in increasing crop yield and improving the quality of potato plants (Ibrahim, 2011; Abd El-Latif *et al*., 2011). These Effects could be attributed to the importance of potassium in improving the translocation of carbohydrates from sources to the tubers. Additionally, potassium ion transport across chloroplast and mitochondrial membranes is related closely to the energy status of plants, aid in protein synthesis in the ribosomes (Barker and Pilbeam, 2007) and has favorable effect on CO_2 assimilation (Pier and Berkowitz, 1987). In addition, the enzyme responsible for synthesis of starch, starch synthetase, is also activated by potassium (Malvi, 2011). For that, the synergistic effect of the combination of both examined substances (KC and FA) on the tuber quality indicators may be due to increase the synthesis and activity of some essential enzymes for different physiological processes.

Table 6: Effect of foliar application of potassium citrate (KC) and folic acid (FA) on some tuber quality indicators of potato plants in the two seasons (2013 and 2014).

Treatments		Mean weight/ tuber (g)	Volum (cm^3)	Specific gravity	Starch (%)	Dry matter (%)
2013 season						
Potassium citrate (ppm)						
0		121.9 c	112.8 c	1.0811 c	13.98 c	20.40 c
1000		149.2 b	136.2 b	1.0956 b	16.81 b	23.40 b
2000		166.4 a	150.9 a	1.1022 a	18.33 a	25.02 a
Folic acid (μg/l)						
0		131.8 b	120.4 b	1.0944 a	16.57 a	23.15 a
50		149.5 ab	136.7 ab	1.0922 a	16.23 a	22.79 a
100		156.2 a	142.8 a	1.0926 a	16.32 a	22.88 a
KC	FA	Interaction treatments between potassium citrate and folic acid				
0	0	120.4 e	111.3 e	1.083 c	14.02 d	20.45 d
	50	119.7 e	111.0 e	1.080 c	13.57 d	19.97 d
	100	125.6 de	116.0 de	1.080 c	14.36 d	20.80 d
1000	0	133.0 cd	121.3 cd	1.096 b	17.03 bc	23.64 bc
	50	153.1 b	139.7 b	1.096 b	17.03 bc	23.64 bc
	100	161.4 b	147.7 b	1.093 b	16.36 c	22.92 c
2000	0	142.1 c	128.7 c	1.103 a	18.67 a	25.37 a
	50	175.5 a	159.3 a	1.100 a	18.10 ab	24.77 ab
	100	181.5 a	164.7 a	1.103 a	18.23 ab	24.91 ab
2014 season						
Potassium citrate (ppm)						
0		120.4 c	111.4 c	1.0800 b	13.97 c	20.39 c
1000		151.1 b	138.3 b	1.0933 a	16.20 b	22.75 b
2000		166.2 a	151.1 a	1.1000 a	17.78 a	24.4 a
Folic acid (μg/l)						
0		129.8 b	118.9 b	1.0913 a	16.06 a	22.61 a
50		152.0 a	139.2 a	1.0908 a	15.95 a	22.49 a
100		155. 9 a	142.8 a	1.0907 a	15.94 a	24.47 a
KC	FA	Interaction treatments between potassium citrate and and folic acid				
0	0	104.9 e	96.7 f	1.083 bcd	14.77 cd	21.24 cd
	50	126.8 d	117.7 e	1.077 d	13.35 d	19.74 d
	100	129.6 d	120.0 e	1.080 cd	13.77 d	20.18 d
1000	0	137.0 cd	125.3 de	1.093 a-c	16.41 a-c	22.98 a-c
	50	157.4 b	144.0 bc	1.097 ab	16.42 a-c	22.98 a-c
	100	158.8 b	145.7 bc	1.090 a-d	15.78 bc	22.30 bc
2000	0	147.6 bc	134.7 cd	1.097ab	17.00 ab	23.60 ab
	50	171.8 a	156.0 ab	1.100 a	18.09 a	24.76 a
	100	179.3 a	162.7 a	1.103 a	18.26 a	24.94 a

Mean within a column and their interaction following with the same latter are not significantly different according to Duncan multiple range test at the probability of 0.05 levels.

Symptoms:

Symptoms developed on potato plants expected to virus infection were recorded after 45 and 65 day post planting and virus disease percentages depending on developed symptoms were recorded after 65 days post planting (Table 7). Generally potato plants treated with KC combined with FA showed mild symptoms and less virus disease percentage than the other treatments. Non-treated plants showed severe symptoms and it had highly disease percentage compared with all treated plants. After 45 days after planting, most the combination treatments of KC with FA did not show any external symptoms. Foliar application of KC at 2000 ppm plus 50 or 100 μg.L^{-1} FA showed mild mosaic. Additionally, KC at 1000 ppm showed leaf roll, KC at 2000 ppm showed mild mosaic and leaf roll, non-treated plants showed mosaic and leaf roll. After 65 days post planting, symptoms were mosaic on potato plants treated with 2000 ppm KC plus FA at 100 μg.L^{-1} followed by 1000 ppm KC plus FA at 100 μg.L^{-1} and 1000 ppm citrate plus FA at 100 μg.L^{-1}, yellowing, mosaic and leaf roll on plants treated with 2000 ppm KC plus FA at 50 μg.L^{-1}, mosaic and leaf roll on both single concentrate KC at 1000 or 2000 ppm, also on plants treated with FA at 50 μg.L^{-1}, yellowing and mosaic on plants treated with folic at 100 μg.L^{-1}, severe yellowing, severe mosaic and leaf roll on non-treated plants. Virus disease percentage depending on the expressed symptoms on potato plants were estimated, generally plants treated with combination of KC at 2000 ppm plus FA at 100 μg.L^{-1} was the lowest in percentage of disease infection compared with the other treatments, it was 2.5% in the first season and 5.1% at the second one, followed by KC at 1000 ppm plus FA at 50 μg.L^{-1} followed by KC at 1000 ppm plus FA at 100 μg.L^{-1} and folic KC at 1000 ppm plus FA at 50 μg.L^{-1} was 5.1% in the first season, in the second season it was 7.6% for KC at 2000 ppm plus FA at 50 μg.L^{-1}, followed by KC at 1000 or 2000 ppm plus FA at 50 μg.L^{-1} with 10.2%. Plants treated with KC at concentration 1000 or 2000 ppm was 7.6% in the first season, while, KC at 1000 ppm was 12.8% in the second season, followed by FA at 100 μg.L^{-1} was 12.8% at first season and 17.9% in the second season followed by FA at 100 μg.L^{-1} increased 15.3% in the first season but 20.5% in the second season. The highest percentage of disease was the non- treated plants it was 20.5% in the first season and 25.6% in the second season.

Table 7: Effect of interaction between foliar application of potassium citrate and folic acid on symptoms and virus disease percentage (%) of potato plants in the two seasons (2013 and 2014).

Treatments		Symptoms* after 45 days			Symptoms after 65 days					Disease (%)
		M.M	M	L.R	Y	S.Y	M	S.M	L.R	
Potassium citrate (ppm)	Folic acid (μg/l)	2013 season								
0	0	-**	+**	+	-	+	-	+	+	20.5
	50	+	-	-	-	-	+	-	+	15.3
	100	+	-	-	+	-	+	-	-	12.8
1000	0	-	-	+	-	-	+	-	+	7.6
	50	-	-	-	-	-	+	-	-	5.1
	100	-	-	-	-	-	+	-	-	5.1
2000	0	+	-	+	-	-	+	-	+	7.6
	50	+	-	-	+	-	+	-	+	5.1
	100	-	-	-	-	-	+	-	-	2.5
2014 season										
0	0	-	+	+	-	+	-	+	+	25.6
	50	+	-	-	-	-	+	-	+	20.5
	100	+	-	-	+	-	+	-	-	17.9
1000	0	-	-	+	-	-	+	-	+	12.8
	50	-	-	-	-	-	+	-	-	10.2
	100	-	-	-	-	-	+	-	-	7.6
2000	0	+	-	+	-	-	+	-	+	10.2
	50	+	-	+	+	-	+	-	+	7.6
	100	-	-	-	-	-	+	-	-	5.1

**: M.M= Mild mosaic, M= mosaic, S.M= sever mosaic, LR= leaf roll, Y= yellowing, S.Y= sever yellowing, **:+= symptoms, - = no symptoms.*

DAS-ELISA values:

Serological detection with DAS-ELISA values and relative virus concentration were illustrated in Table (8). All examined treatments of potato plants with DAS-ELISA showed negative reaction with PVX, on the other hand all of them showed positive reaction with PVY and the lowest relative concentration of PVY was noticed on potato plants foliar application with KC at 2000 ppm plus FA at 100 μg.L^{-1}. On the contrast, the highest relative concentration of PVY was on non-treated plants. Reaction with PLRV was positive with all treatments except combination between foliar application of KC and FA, the highest relative concentration of PLRV was showed in non-treated plants. In this connection, KC at 5 mM was reported as strong inhibitors of *Tobacco mosaic virus* multiplication in protoplast (Kassanis *et al.*, 1975). The protective effect of KC and FA against the viral diseases might be related indirectly with increasing the phenolic compounds (Table 3) and the activity of their related enzymes (Table 4) including PAL which is considered the key enzyme for phenolic acid synthesis and most another secondary metabolites and PPO which responsible for protecting the plants from pests and diseases. This role may be attributed to that phenolic compounds have antioxidant activity against reactive oxygen species. In this respect they were reported to be higher than that of vitamins C and E (Stewart *et al.,* 2000). The substantial progress in the synthesis of these secondary metabolites by the treatment of KC or FA either as individual or in combination could be due to the importance of them in enhancing the different physiological processes especially photosynthesis which is considered the main pathway for phenolic acid synthesis via shikimic acid (Taiz and Zeiger, 2002). These results were confirmed by enhancing the growth parameters (Table 2) and turned directly on the performance of plants through increase the yield of tubers and their quality indicators.

Table 8: Effect of interaction between foliar application of potassium citrate and folic acid on DAS-ELISA values for three viruses (PVY, PVX and PLRV) of potato plants in the two seasons (2013 and 2014).

Treatments		PVY	*±	PVX	±	PLRV	±	PVY	±	PVX	±	PLRV	±
Potassium citrate (ppm)	Folic acid (µg/l)	2013 season						2014 season					
0	0	0.363 a	+	0. 149 c	-	0. 353 a	+	0.358 a	+	0. 185 a	-	0. 373a	+
	50	0.289 c	+	0.172 ab	-	0.330 b	+	0.308 b	+	0.178 a	-	0.293 c	+
	100	0.291 c	+	0.141c	-	0.284c	+	0.285 c	+	0.103 e	-	0.258 d	+
1000	0	0.328 b	+	0.156 bc	-	0.352 a	+	0.300 bc	+	0.168 b	-	0.301 bc	+
	50	0.352 ab	+	0.148 c	-	0.179 d	-	0.311 b	+	0.135 d	-	0.156 e	-
	100	0.331 ab	+	0.157 bc	-	0.143 e	-	0.292 bc	+	0.101 e	-	0.150 e	-
2000	0	0.290 c	+	0.172 ab	-	0.336 b	+	0.356 a	+	0.152 c	-	0.293 c	+
	50	0.352 ab	+	0. 183 a	-	0.292 c	+	0.336 a	+	0. 133 d	-	0.311 b	+
	100	0.276 c	+	0.144 c	-	0.178 d	-	0.283 c	+	0.156 c	-	0.121 f	-

*ELISA value of negative control: 0.105, *+= infected, -= non infected*

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