Journal of Horticultural Science & Ornamental Plants 8 (3): 161-172, 2016
ISSN 2079-2158

DOI: 10.5829/idosi.jhsop.2016.8.3.1183

Improving Growth, Yield and Resistance to Viral Diseases of Potato Plants Through Modifying Some Metabolites Using Zinc Sulphate and Jasmonic Acid

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Abstract: Two field experiments were conducted during the seasons of 2014 and 2015 to study the effect of foliar application of $ZnSO_4$ at 0, 25 and 50 ppm and JA at 0, 10 and 20 µM individually and in combination on potato plants grown under drip irrigation system. The results indicated that the growth parameters in term of shoot fresh and dry weights were increased significantly ($P \leq 0.05$) in $ZnSO_4$ applied plants; whereas, JA inhibited both traits compared to the untreated plants in both seasons. A similar trend was observed in respect to the concentration of photosynthetic pigments (chlorophyll and carotenoids). On the other hand, total soluble proteins and phenolic compounds were responded positively and significantly to both examined compounds. There were significant increments in the weight of tubers.plant^{-1} and the total yield of tuber (ton. fed^{-1}) by both investigated compounds. In general, the combination treatment of $ZnSO_4$ at 50 ppm + JA at 10 µM gave the highest results in this respect. Symptoms developed on expected plants to be viruses (PVY, PVX and PLRV) infected revealed that the treatment of $ZnSO_4$ at 50 ppm + JA at 10 or 20 µM decreased the percentage of disease incidence in comparison to the other treatments. These results were proved serologically by DAS-ELISA test. The concentrations of PVY and PLRV were decreased significantly in $ZnSO_4$ and/or JA treated plants compared to the controls in both seasons; whereas, PVX was not found in all treatments. The correlation coefficient between all studied biochemical constituents and the concentration of viruses in term of the values of DAS-ELISA test revealed that there was inverse and strong significant correlation between the concentration of total soluble proteins or phenolic compounds on one side and the concentration of PVY or PLRV on the other one. No significant relationships were detected for photosynthetic pigments in this respect.

Key words: Potato · Zinc sulphate · Jasmonic acid · Viral diseases · Biochemical constituents and ELISA

INTRODUCTION

Potato is among the most important food crops and considered the largest vegetatively propagated one in the world [1]. It is a rich source for carbohydrates, minerals, vitamins and antioxidants; furthermore, it is used as a raw material for multiple industries [2-3].

Many constraints were found to hinder the expansion of potato cultivation; for instance, in Egypt, the high cost of imported seed tubers, the spread of viral diseases and the scarcity of water resources are considered the most critical ones in this respect. Therefore, utilization of the recent irrigation systems especially drip irrigation instead of the traditional methods could help in solving a part of this problem. Moreover, increasing the growth, yield and resistance to viral diseases of potato plants may require some modifications on the physiological and biochemical levels. In this regard, several compounds were applied exogenously to induce these responses. Among these substances zinc sulphate ($ZnSO_4$) as a source for Zn and Jasmonic acid were selected to investigate their effects individually and in combination as a foliar application on potato plants in this study.

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Zinc (Zn) is involved in many cellular functions in all living organisms; in human; it plays an important role in improving the immune system, memory functions, keeping the hair, skin healthy and intact structure for body muscles [4]. In the higher plants; it is considered an essential micronutrient; it acts as an integral component for enzyme structures and proteins which involved in DNA replication and gene expression [5]. Furthermore, it is implicated in nitrogen, protein, carbohydrates metabolism and auxin synthesis [6]. Zinc sufficient supply leads to stabilize the cellular membranes, ribosomes and help in increase the growth and yield of plants [7-9].

Jasmonic acid (JA) is a natural growth regulator found in plant tissues; it is involved in several physiological processes including regulating the growth and development under biotic and abiotic stresses [10], promotion senescence, dormancy, tendril coiling and ethylene synthesis [11]. In potatoes; JA and its derivatives such as methyl jasmonate (MeJA) and tuberonic acid (TA) and tuberonic glucoside (TAG) were found to be closely related to the tuberization of potato plants [12]. It had been found that JA has a stronger positive effect on stolon tuberization rate, number of tuber and the final tuber weight of potato grown *in vitro* [13].

For all above mentioned reasons; this investigation aimed at improving the yield of potato crop and its resistance to viral diseases through modifying some physiological and biochemical aspects using zinc sulphate and jasmonic acid as foliar application on potato plants grown under drip irrigation system.

MATERIALS AND METHODS

Two field experiments using drip irrigation system were conducted during the seasons of 2014 and 2015 in Taba Farm, Sadat City, Menofia Governorate, Egypt, to investigate the effect of foliar application of zinc sulfate heptahydrate ($ZnSO_4.7H_2O$) at 0, 25 or 50 ppm, jasmonic acid (JA) at 0, 10 or 20 µM and all their possible combinations on the growth, biochemical constituents and the productivity of potato plants. The texture of the experimental soil was loamy sand and its physical and chemical properties are shown in Table (1).

The Experimental Layout: Imported certified potato seed tubers of cv. Draga were purchased from Daltex Company, El-Tawfikia, Behira Governorate. The seed tubers were planted on January 28th in both seasons. The experiment was arranged in split plot design with three replicates. The foliar applications of $ZnSO_4$ were carried out in the main plots and the foliar applications of JA treatments were distributed in the sub-plots. The plot area was 15 m (length) x 3 m (width) with 70 cm between rows and 30 cm plant spacing. Each sub-plot 5 m (length) x 3 m (width) contains 5 rows with 16 plant in a row and totally 80 plants for each one. The three inner rows (3 rowsX16 plant) = 48 plant were used to investigate the different characteristics in each sub –plot of this study.

Irrigation Procedure: Drip irrigation system was used to supply plants with 100% of irrigation requirements (IR) during the period of this study. The daily average amount of IR (Table, 2) of potato plants were determined according to Food and Agricultural Organization (FAO) Penman- Monteith (PM) procedure, FAO 56 [14] using the climatic data [15] in the site of the experiment. Each plant in the rows had a single plastic irrigation dripper (4 L h^{-1}). The efficiency of these drippers was multiple tested in many sites by collecting the drainage water in graduated cylinders. The average of drainage water per each dripper was about 3.25 L.h^{-1} for all experiment. Water flow-meter was used each time to measure IR during the irrigation.

Fertilization and Cultural Management: During the preparation of experimental soil, 30 m^3 compost, 30 kg N as ammonium sulfate (20.5% N) and 75 kg P as calcium superphosphate (15.5% P_2O_5) were dressed in the soil per fed. After that, 150 kg N as ammonium nitrate (33.5% N) and 96 Kg K as potassium sulfate (48% K_2O) were applied through 10 equal doses with irrigation water; this procedure was started after full emergence until 66 days after planting (DAP). All the other cultural processes, disease and pest control programs were followed according to the recommendations of the Egyptian Ministry of Agriculture.

Foliar Application and Sampling: The foliar application of $ZnSO_4$ at 0 (distilled water), 25 or 50 ppm was applied at 35 and 49 DAP; whereas, JA was applied at 42 and 56 DAP. This procedure could help plants to assimilate the both examined compounds in their tissues during the most critical period for potato plants from emergence to the initiation of tubers. Furthermore, to avoid the washing effect of both compounds to each other which may occur when applied the two compounds simultaneously; Tween 20 at 0.05 ml L^{-1} was used as a wetting agent. Two samples were collected randomly from the inner rows; the first one was taken at 75 DAP to estimate plant growth in term of shoot fresh and dry weights; in addition, to determine the biochemical constituents in the leaf tissues. The second sample was taken at 110 DAP to estimate the yield of tubers at the end of the experiment.

Table 1: Physical and chemical properties of the experimental soil

Physical properties					
Season	Clay (%)	Silt (%)		Sand (%)	Soil type
2014	2.2	13.6		84.2	Loamy sand
2015	3.1	14.2		82.7	Loamy sand
Chemical properties (mg/100 g soil)					
Season	pH	N	P	K	$CaCO_3$
2014	8.1	1.22	1.95	4.29	2.50
2015	7.95	1.29	2.00	4.32	2.50

Table 2: Average amounts of applied water ($m^3.fed^{-1}$) in the seasons of 2014 and 2015

	Irrigation treatments (m^3. fed^{-1})				
Date	100% of water requirements (Well- watered)	Average of daily requirements	Date	100% of water requirements (Well- watered)	Average of daily requirements
Jan 28 – Feb 8 (12 days)	20.1	1.7	Mar 24- April 4 (12 days)	166.7	14
Feb 9-19 (11 days)	29	2.6	April 5-15 (11 days)	193.2	17.6
Feb 20- Mar 2 (10 days)	33.6	3.4	April 16-26 (11 days)	363.3	36.4
Mar 3-12 (10 days)	75	7.5	April 27- May 7 (11 days)	360.2	32.8
Mar 7-12 (6 days)	45	7.5	May 8-17 (Stop watering)	0.0	0.0
Mar13-23 (11 days)	128.8	11.7	Total (m^3 .fed^{-1})	1370	

Studied Parameters

Vegetative Growth: Samples from the inner rows for each sub plot at 75 DAP were collected randomly; shoot fresh weight was determined immediately using digital balance; whereas, the dry weight was estimated by drying the samples in air-forced ventilated oven at 70°C until a constant weight [16].

Biochemical Constituents

Leaf Pigments: Leaf pigments were extracted according to the method of Lichtenthaler and Buschmann [17] with some modifications. 0.2 g of fresh leaves was homogenized with acetone 80% in dark at room temperature. Absorbance was measured in a UV/ VIS spectrophotometer. The concentrations were calculated using the following equations:

Chl a (µg/ml) = 12.25 A663.2 - 2.79 A646.8
Chl b (µg/ml) = 21.50 A646.8 – 5.10 A663.2
Total Chl = Chl a + Chl b
Carotenoids (µg/ml) = (1000 A470 – 1.82 Chl a – 85.02 Chl b) / 198
After that the calculations were done as mg. g^{-1} f.wt.

Total Soluble Protein and Phenolic Compounds: Total soluble protein was extracted using 0.1 M potassium phosphate buffer (pH 7.0) and determined according to the method of Bradford [18]; 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 [19].

Yield and its Components: The number and weight of tubers per plant were recorded after harvesting at 110 DAP, then the yield per fed^{-1} was calculated.

Symptoms and Ratio of Disease Incidence: Symptoms expected to virus infection were recorded at 45 and 75 DAP. Ratio of disease incidence depending on developed symptoms were estimated after 75 days from planting in all treatments, each treatment was about 16 plant X 3 rows X 3 replicates with total number 144 plant for each treatment.

Percentage of Virus Infection: Percentage of virus infection was expressed as the disease percentage (DP) of plants which developed symptoms, 75 days after planting of potato plants divided by total plants in each treated area which is 144 plants (48 plant X 3 replicates).

$$DP = \frac{\text{Number of plants developed symptoms}}{\text{Total number of plants in each treated area}} X100$$

Serological Detection

Sample Collection: During first and second seasons, field samples were conducted to estimate the incidence of viruses in potato cv. Draga that developed symptoms expected to virus infection. In each sub-plot leaf samples (top, middle and bottom) were collected from symptomatic plants for serological assay at 75 DAP.

Serological Detection: Viruses were detected by double antibody sandwich (DAS) enzyme-linked immunosorbent assay (ELISA) on polystyrene microtitre plats [20]. 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: All experimental data were subjected to analysis of variance (two-way ANOVA) procedures (SAS Institute Inc, [21]). Duncan's test ($P \leq 0.05$) was used to determine significant differences between means.

RESULTS AND DISCUSSION

Growth Characteristics: Data presented in Table (3) show that shoot fresh and dry weights were responded positively to the foliar application of $ZnSO_4$; the highest significant ($P \leq 0.05$) increases were obtained by the high examined concentration at 50 ppm compared to the controls in both seasons. . In this respect, many previous literatures reported that foliar applied $ZnSO_4$ improved the growth characteristics of several plants including maize [22], orange [23], lentil [24] and rice [25]. These results could be attributed to that zinc is considered an essential element for higher plants; it plays an important role as an integral component of enzyme structures, the proteins which involved in DNA replication and gene expression [5]. Moreover, it has a crucial role in the synthesis of tryptophan and indole acetic acid (IAA, auxin) metabolism [26] which is considered one of the most important phytohormones responsible for regulating plant growth and development.

Adversely, applied JA caused significant ($P \leq 0.05$) decreases in the previous traits compared to the controls in both seasons; the lowest results were obtained with the high studied concentration at 20 µM. Jasmonic acids (JA) can affect multiple features of plant growth and development. Among these responses, it can induce senescence, degradation of chlorophyll [27] and inhibit photosynthesis by affecting the process of translation for the large subunits of Rubisco enzyme [28]. It can also trigger the ethylene by inducing the activity of its biosynthesis enzyme [29].

Concerning the effects of all possible combinations of $ZnSO_4$ and JA treatments, it was obvious that the treatment of $ZnSO_4$ at 50 ppm + JA at 0 µM gave the highest significant ($P \leq 0.05$) increments in comparison to the other treatments in the two seasons. These findings indicated in general that applied JA as foliar application reduced and reverse the stimulant effect of $ZnSO_4$ on potato plants under the circumstances of this study. This influence was more pronounced with elevating the concentration of applied JA.

Biochemical Constituents

Photosynthetic Pigments: Data in Table (4) show that the foliar application of $ZnSO_4$ at both examined concentrations increased significantly ($P \leq 0.05$) the leaf concentration of total chlorophyll compared to the untreated plants in both seasons. In this respect, no significant differences were detected between the two studied concentrations of $ZnSO_4$. In contrast, total chlorophyll was decreased significantly ($P \leq 0.05$) in JA applied plants compared to the controls in both seasons. All possible combinations of different treatments revealed that the treatment of $ZnSO_4$ at 25 ppm under 0 µM of JA gave the highest significant increases in the total chlorophyll compared to the other treatments in both seasons.

As for carotenoids, with exception of the treatment of $ZnSO_4$ at 50 ppm in the first season, carotenoids were responded positively and significantly ($P \leq 0.05$) by the different treatments of $ZnSO_4$ compared to the untreated plants in both seasons. In this respect, the maximum increases were achieved by the treatment of $ZnSO_4$ at 25 ppm in the two seasons. Adversely, the treatments of JA gave a similar trend to that of total chlorophyll but these decreases reached the level of significance ($P \leq 0.05$) between all different examined concentrations of JA in both seasons. The combined treatments of $ZnSO_4$ at 25 or 50 ppm with JA at 0 µM gave the highest significant ($P \leq 0.05$) results in carotenoids in comparison to the other treatments.

The positive effect of Zn on leaf pigments was noticed earlier in some previous studies [30-31]. This response may be due to that, applied Zn at optimal concentrations leads to decrease the level of reactive oxygen species (ROS) in plant tissues through increasing the activity of SOD enzyme [32]. It is also considered an essential element in the structure of several chloroplast proteins and as co-factor for numerous of enzymes which involved in the leaf pigment synthesis and photosynthesis [8].

On the other hand; the reduction in leaf pigments (chlorophyll and carotenoids) by the treatments of JA could be attributed to stimulate the biosynthesis of ethylene in plant tissues or increasing the activity of plasma membrane NADPH oxidase and the accumulation of ROS. All of these events lead to the onset of senescence and leaf pigments degradation [29, 33-34].

Table 3: Effect of foliar application of $ZnSO_4$ (0, 25 or 50 ppm), Jasmonic acid (0, 10 or 20 µM) and their combinations on shoot fresh and dry weight of potato plants at 75 DAP during the seasons of 2014 and 2015.

	2014				2015			
	Jasmonic acid (µM)							
$ZnSO_4$ (ppm)	0	10	20	Mean	0	10	20	Mean
	Shoot fresh weight (g)							
0	453.33 c	414.00 e	389.19 f	418.84 C	459.00 cd	425.67 e	408.33 e	431.00 C
25	484.33 b	460.67 c	433.67 d	459.56 B	486.33 b	468.00 bcd	456.67 d	470.33 B
50	506.22 a	486.67 b	466.33 c	486.41 A	512.73 a	483.80 b	476.67 bc	491.06 A
Mean	481.30 A'	453.78 B'	429.73 C'		486.02 A'	459.15 B'	447.22 C'	
	Shoot dry weight (g)							
0	78.01 de	66.20 f	55.46 g	66.56 C	84.22 c	75.26 de	67.78 e	75.75 C
25	91.53 b	80.60 cd	72.48 ef	81.54 B	96.00 b	88.60 bc	80.02 cd	88.20 B
50	98.80 a	87.17 bc	82.00 cd	89.32 A	105.27 a	87.53 bc	86.17 c	92.99 A
Mean	89.45 A'	77.99 B'	69.98 C'		95.16 A'	83.80 B'	77.99 C'	

Means followed by different letters are significantly different at $P \leq 0.05$ according to Duncan's multiple range test

Table 4: Effect of foliar application of $ZnSO_4$ (0, 25 or 50 ppm), Jasmonic acid (0, 10 or 20 µM) and their combinations on the concentrations of photosynthetic pigments of potato leaves at 75 DAP during the seasons of 2014 and 2015

	2014				2015			
	Jasmonic acid (µM)							
$ZnSO_4$ (ppm)	0	10	20	Mean	0	10	20	Mean
	Total chlorophyll (mg .g^{-1} f.wt)							
0	1.43 cde	1.37de	1.32e	1.37B	1.55bc	1.44cd	1.39d	1.46B
25	1.65 a	1.50bc	1.47bcd	1.54A	1.74a	1.58bc	1.53bcd	1.62A
50	1.57 ab	1.50bc	1.41cde	1.49A	1.64ab	1.57bc	1.51bcd	1.57A
Mean	1.55A'	1.45B'	1.40B'		1.64A'	1.53B'	1.48B'	
	Carotenoids (mg .g^{-1} f.wt)							
0	0.501c	0.405e	0.338f	0.414 C	0.479b	0.363d	0.313e	0.385 C
25	0.574a	0.532b	0.492c	0.533 A	0.565a	0.508b	0.472b	0.515 A
50	0.579a	0.438d	0.319f	0.414 C	0.569a	0.429c	0.360d	0.453 B
Mean	0.551 A'	0.458 B'	0.383C'		0.537 A'	0.434 B'	0.382C'	

Means followed by different letters are significantly different at $P \leq 0.05$ according to Duncan's multiple range test

Table 5: Effect of foliar application of $ZnSO_4$ (0, 25 or 50 ppm), Jasmonic acid (0, 10 or 20 µM) and their combinations on total soluble protein and phenolic compounds of potato leaves at 75 DAP during the seasons of 2014 and 2015

	2014				2015			
	Jasmonic acid (µM)							
$ZnSO_4$ (ppm)	0	10	20	Mean	0	10	20	Mean
	Total soluble proteins (mg .g^{-1} f.wt)							
0	2.23f	2.39de	2.45cd	2.36B	2.31e	2.42d	2.62b	2.45C
25	2.26ef	2.48bcd	2.53bc	2.42B	2.43d	2.60bc	2.61b	2.54B
50	2.34def	2.68a	2.60ab	2.54A	2.49cd	2.75a	2.68ab	2.64A
Mean	2.28B'	2.52A'	2.53A'		2.41B'	2.59A'	2.64A'	
	Phenolic compounds (mg .g^{-1} d.wt)							
0	1.98e	2.25c	2.31bc	2.18B	2.06e	2.55b	2.47bc	2.36B
25	2.11d	2.41a	2.39ab	2.30A	2.20d	2.55b	2.54b	2.43A
50	2.16d	2.47a	2.28c	2.30A	2.27d	2.67a	2.43c	2.46A
Mean	2.08B'	2.38A'	2.33A'		2.18C'	2.59A'	2.48B'	

Means followed by different letters are significantly different at $P \leq 0.05$ according to Duncan's multiple range test

Total Soluble Protein and Phenolic Compounds: Concerning the total soluble protein (Table 5), it is obvious that the foliar application of $ZnSO_4$ enhanced the total soluble proteins in leaf tissues. The highest significant ($P \leq 0.05$) results were obtained by the treatment of $ZnSO_4$ at 50 ppm compared to the untreated plants in both seasons. It is known that the Zn sufficient supply is essential for the structure of the ribosomes and their structural integrity in plant cell [8]. These organelles are responsible for the biosynthesis of proteins.

Regarding the effect of JA, it is clear that foliar application of JA provokes accumulation of total soluble protein concentration by both its examined concentrations compared to the control plants in both seasons. These effects were more pronounced in the combined treatments of JA at 10 or 20 μM with $ZnSO_4$ at 50 ppm than other treatments. Increasing the protein by exogenous application of JA was confirmed previously in several studies. This response may be explained by that applied JA may lead to several quantitative modifications in polypeptide profiles and accumulation of new specific proteins [35-36]. Increasing the soluble protein in the combined treatments of $ZnSO_4$ and JA may be attributed to the synergistic influence of both treatments on the synthesis of protein.

Phenolic compounds (Table, 5) were also responded positively and significantly ($P \leq 0.05$) to the treatments of $ZnSO_4$ in comparison to the untreated plants in both seasons. It is well documented that Zn is related to enhance the photosynthetic performance by activation numerous of photosynthesis related enzymes [8]. Furthermore, photosynthesis is the major source for phenolic compounds through production and promoting the different compounds of shikimic acid pathway [37].

As for the effect of JA on phenolic compounds, it can be observed that the treatments of JA (10 or 20 μM) improved significantly ($P \leq 0.05$) the concentration of phenolic compounds compared to the controls in both seasons; these results were in agreement with those of Ghasemnezhad and Javaherdashti [38]. In another previous study, JA was found to induce genes and enzymes which involved in the phenolic compounds biosynthesis such as chalcone synthase and phenylalanine ammonia lyase [39].

All different combination treatments of $ZnSO_4$ and JA exhibited that the treatment of $ZnSO_4$ at 50 ppm + JA at 10 μM gave the highest significant increases in phenolic compounds in both seasons. This trend could be explained by the synergistic and the interaction effect between the both investigated compounds.

Tuber Yield and its Components: Data presented in Table (6) show that with exception of the treatment of JA at 10 μM in the second seasons, no significant ($P \leq 0.05$) differences were detected between the different treatments of $ZnSO_4$ and/or JA. On contrast, significant ($P \leq 0.05$) differences were observed between the different studied treatments in respect to the weight of tubers.plant^{-1} and consequently.fed^{-1}. The foliar application of $ZnSO_4$ at 25 or 50 ppm achieved significant ($P \leq 0.05$) increases compared to the control in both seasons. The maximum increments were obtained by the higher examined concentration of $ZnSO_4$ at 50 ppm in the two seasons. These findings could be attributed to enhance growth (Table 3) and altering the biochemical constituents (Table 2) which reflected positively on the performance and potential yield of the $ZnSO_4$ applied plants.

Respecting the effect of JA on the weight of tubers per plant and per fed., it was obvious that applied JA improved the weight of the produced tubers compared to the untreated plants in both seasons. The highest recorded increases were obtained by the treatment of JA at 10 μM in the two seasons.

Many of previous literatures reported that JA plays an important role in inducing the tuberization of potato plants; for instance Pelacho and Mingo-Castel [13] found that JA had stronger positive effect on stolon tuberization rate, number of tuber and the final tuber weight of potato grown *in vitro*. Also, its endogenous concentration was increased 20 times more than the kinetin in the tissues during the induction of tuberization. Furthermore, a lot of derivatives of JA were found in plant tissues such as tuberonic acid (TA) and its glucoside (TAG) [12] and were found to be closely related to the tuberization of potato plants. In this study, improving the yield of the JA applied plants although decreasing the shoot growth in comparison to the untreated plants could be explained by all above mentioned reasons in addition to increase the rate of carbohydrates translocation from leaves to the tubers.

The different possible combinations between the examined concentrations of $ZnSO_4$ and /or JA revealed that the treatment of $ZnSO_4$ at 50 ppm + JA at 10 μM or $ZnSO_4$ at 50 ppm + JA at 20 μM gave the highest significant ($P \leq 0.05$) increases in the yield of the produced tubers compared to the other treatments in both seasons. These results could be attributed to the synergistic effect of both examined compounds on the several of physiological and biochemical events which related to tuberization and yield of potato plants.

Table 6: Effect of foliar application of $ZnSO_4$ (0, 25 or 50 ppm), Jasmonic acid (0, 10 or 20 µM) and their combinations on the yield and its components of potato during the seasons of 2014 and 2015.

	2014				2015			
	Jasmonic acid (µM)							
$ZnSO_4$ (ppm)	0	10	20	Mean	0	10	20	Mean
	No. tubers.plant^{-1}							
0	7.23 ab	7.70 a	7.00 b	7.31 A	7.34 abc	7.62 ab	6.88 c	7.28 A
25	7.17 ab	7.63 ab	7.53 ab	7.44 A	7.05 bc	7.39 abc	7.55 ab	7.33 A
50	7.16 ab	7.09 ab	7.41 ab	7.22 A	7.07 bc	7.62 ab	7.89 a	7.53 A
Mean	7.19 A’	7.47 A’	7.31 A’		7.15 B’	7.54 A’	7.44 AB’	
	Weight of tubers.plant^{-1} (g)							
0	669 f	711 e	655 f	678 C	657 d	729 c	674 d	687 C
25	718 de	767 bc	730 de	738 B	727 c	782 b	766 b	758 B
50	751 cd	813 a	788 ab	784 A	772 b	828 a	816 a	805 A
Mean	712 B’	764 A’	724 B’		719C’	780A’	752B’	
	Yield (ton.fed^{-1})							
0	13.38 f	14.21 e	13.09 f	13.56 C	13.14 d	14.58c	13.47 d	13.73 C
25	14.35 de	15.34 bc	14.60 de	14.76 B	14.53 c	15.64b	15.32 b	15.16 B
50	15.01 cd	16.25 a	15.74 ab	15.67 A	15.44 b	16.55a	16.31 a	16.10 A
Mean	14.25 B’	15.27 A’	14.48 B’		14.37 C’	15.59 A’	15.03 B’	

Means followed by different letters are significantly different at $P \leq 0.05$ according to Duncan's multiple range test

Symptoms: Developed symptoms on potato plants which had been expected to be virus infected naturally in the open field were recorded twice at 45 and 75 DAP respectively (Table, 7). It was obvious that all investigated foliar applications led to decrease the symptoms and percentage of diseases incidence compared to the untreated plants in both seasons. The best results and minimum percentage of the symptoms of viral diseases were obtained by the combined treatments of $ZnSO_4$ at 50 ppm + JA at 10 µM followed by $ZnSO_4$ at 50 ppm + JA 20 µM in both seasons.

Concerning the symptoms at 45 DAP; it can be observed that the untreated plants showed leaf roll concomitant with mosaic or mild mosaic in the two seasons respectively. The foliar application of $ZnSO_4$ at 25 ppm individually exhibited mosaic in both seasons whereas; $ZnSO_4$ at 50 ppm individually showed mild mosaic and leaf roll. The treatments of JA individually revealed that JA at 10 µM gave leaf roll in the two seasons while; JA at 20 µM exhibited mild mosaic and mosaic in both seasons respectively.

As for the effect of the combination treatments; it was obvious that the treatment of $ZnSO_4$ at 25 ppm + JA at 10 µM gave leaf roll in both seasons whereas; the treatment of $ZnSO_4$ at 25 ppm + JA at 20 µM revealed mosaic and mild mosaic in the two seasons respectively. The treatment of $ZnSO_4$ at 50 ppm + JA at 10 µM followed by $ZnSO_4$ at 50 ppm + JA at 10 µM didn’t show at mostly any symptoms to the viral diseases in this period of plant growth.

Respecting the symptoms at 75 DAP; it can be observed that the untreated plants showed sever symptoms including sever yellowing, leaf roll and stunting compared to the other treatments in both seasons. Moreover, sever mosaic or mosaic was also manifested in the two seasons respectively. The foliar application of $ZnSO_4$ at 25 ppm individually didn’t show any distinct improving in the symptoms whereas; the treatment of $ZnSO_4$ at 50 ppm reduced clearly these symptoms. Applied JA at 10 µM individually showed sever mosaic, leaf roll and stunting in both seasons while; JA at 20 µM individually exhibited sever mosaic and leaf roll in both seasons.

Concerning the influence of the combination treatments, it was obvious that the treatments of $ZnSO_4$ at 50 ppm + JA at 10 µM followed by $ZnSO_4$ at 50 ppm + JA at 20 µM reduced markedly the symptoms which had been expected to be resulted in viral diseases under open field conditions compared to the other treatments in both seasons.

ELISA: Serological detection with DAS-ELISA values; relative concentration to three of the most spread potato viruses (PVY, PVX and PLRV) in the world and under Egyptian conditions were showed in Table (8).

Regarding PVY; it can be observed that all samples which had been expected to be virus infected under open field conditions showed positive reactions with PVY under all different examined foliar treatments in this study.

Table 7: Effect of foliar application of $ZnSO_4$ (0, 25 or 50 ppm), Jasmonic acid (0, 10 or 20 μM) and their combinations on symptoms and virus disease percentage (%) of potato plants at 45 and 75 DAP during the seasons of 2014 and 2015

Treatments		Symptoms* at 45 DAP			Symptoms at 75 DAP						
		M.M	M	L.R	Y	S.Y	M	S.M	L.R	S	Disease (%)
$ZnSO_4$ (ppm)	JA (μM)				Season 2014						
0	0	-	+	+	-	+	-	+	+	+	27.0
	10	-	-	+	-	+	-	+	+	+	24.3
	20	+	-	-	+	-	-	+	+	-	25.6
25	0	-	+	-	-	+	-	+	+	+	24.3
	10	-	-	+	+	-	-	+	+	-	21.5
	20	-	+	-	+	-	-	+	-	-	23.6
50	0	+	-	+	+	-	+	-	+	-	23.6
	10	-	-	-	-	-	+	-	-	-	15.9
	20	-	-	+	-	-	+	-	+	-	17.3
					Season 2015						
0	0	+	-	+	-	+	+	-	+	+	20.8
	10	-	-	+	+	-	-	+	+	+	21.5
	20	-	+	-	+	-	-	+	+	-	20.8
25	0	-	+	-	-	+	-	+	+	+	18.75
	10	-	-	+	+	-	+	-	+	-	15.2
	20	+	-	-	+	-	-	+	-	-	16.6
50	0	+	-	+	+	-	+	-	+	-	17.3
	10	-	-	-	-	-	+	-	-	-	10.4
	20	-	-	-	-	-	+	-	-	-	11.8

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

Table 8: Effect of foliar application of $ZnSO_4$ (0, 25 or 50 ppm), Jasmonic acid (0, 10 or 20 μM) and their combinations on DAS-ELISA values for three viruses (PVY, PVX and PLRV) of potato leaves at 75 DAP during the seasons of 2014 and 2015

	2014							2015						
							Jasmonic acid (μM)							
$ZnSO_4$ (ppm)	0	±	10	±	20	±	Mean	0	±	10	±	20	±	Mean
	PVY													
0	0.478 a	+	0.379 b	+	0.411 ab	+	0.423 A	0.470 a	+	0.438 abc	+	0.420 a-d	+	0.443 A
25	0.443 ab	+	0.370 b	+	0.392 b	+	0.402 A	0.445 ab	+	0.367 d	+	0.398 bcd	+	0.403 B
50	0.411 ab	+	0.378 b	+	0.393 b	+	0.394 A	0.412 bcd	+	0.384 cd	+	0.416 a-d	+	0.404 B
Mean	0.444 A’		0.376 B’		0.399 B’			0.442 A’		0.396 B’		0.411 B’		
							PVX							
0	0.192 a	-	0.189 a	-	0.198 a	-	0.193 B	0.216 a	-	0.190 b	-	0.192 b	-	0.199 A
25	0.204 a	-	0.200a	-	0.205 a	-	0.203 A	0.195 b	-	0.202 ab	-	0.204 ab	-	0.200 A
50	0.199 a	-	0.200 a	-	0.200 a	-	0.199 AB	0.202 ab	-	0.207 ab	-	0.209 ab	-	0.206 A
Mean	0.198 A’		0.196 A’		0.201 A’			0.204 A’		0.199 A’		0.201 A’		
							PLRV							
0	0.523 a	+	0.460 bc	+	0.489 ab	+	0.491 A	0.568 a	+	0.493 b	+	0.513 b	+	0.524 A
25	0.462 bc	+	0.416 cd	+	0.419 cd	+	0.432 B	0.501 b	+	0.433 cd	+	0.367 c	+	0.457 B
50	0.428 cd	+	0.353 e	+	0.392 de	+	0.391 C	0.475 bc	+	0.324 e	+	0.389 d	+	0.396 C
Mean	0.471 A’		0.410 B’		0.433 B’			0.515 A’		0.417 C’		0.446 B’		

Means followed by different letters are significantly different at P =0.05 according to Duncan's multiple range test

The foliar applications of $ZnSO_4$ at 25 ppm or 50 ppm revealed decreases in the concentration of PVY as indicated by DAS-ELISA values. These decreases reached the level of significance ($P \leq 0.05$) compared to the untreated plants in second season. On the other hand, the treatments of JA at both investigated concentrations caused significant ($P \leq 0.05$) decreases in the concentration of PVY compared to the control plants in both seasons. All possible combinations showed that the treatment of $ZnSO_4$ at 25 ppm + JA at 10 μM gave the lowest values of DAS-ELISA for PVY in both seasons.

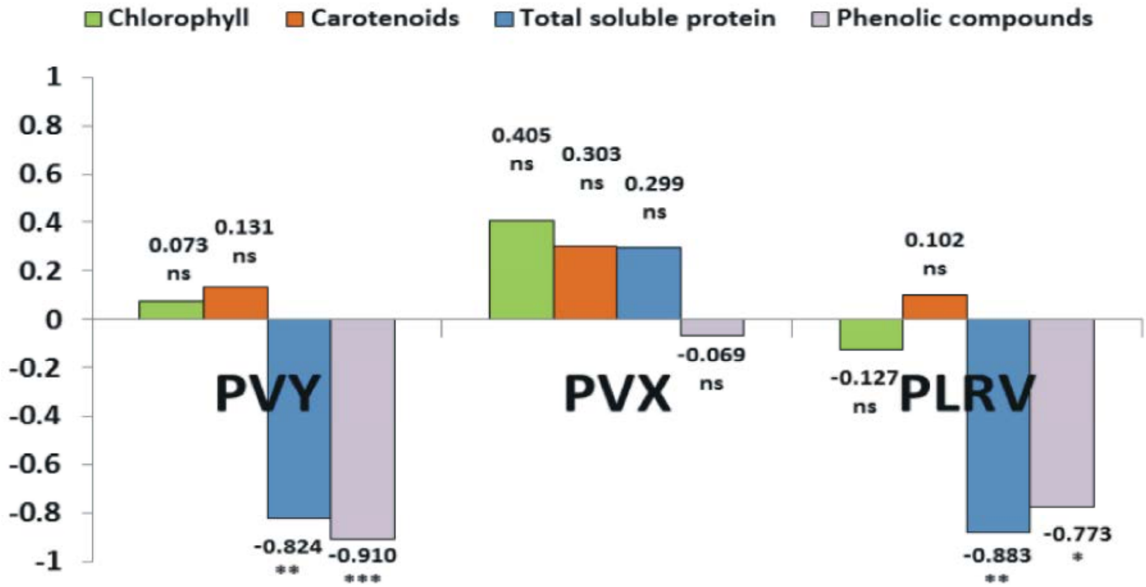

Fig. 1: Combined analysis for the correlation coefficient between the biochemical constituents (Chlorophyll, carotenoids, total soluble protein and phenolic compounds) and DAS-ELISA values for three viruses (PVY, PVX and PLRV) in potato leaves at 75 DAP as affected by the foliar application of $ZnSO_4$ (0, 25 or 50 ppm), Jasmonic acid (0, 10 or 20 µM) and their combinations during the seasons of 2014 and 2015.
Ns: non- significant *P=0.05 **P=0.01***P=0.001.

Respecting PVX; it can be seen that there were negative reactions with PVX with different foliar treatments under the circumstances of this study. These results could be attributed to that PVX is not transmitted by insects; it is transmitted mechanically. Moreover; the high quality of free virus imported seed tubers which used in this study may be the main reason for these results.

Concerning PLRV; it was obvious that PLRV showed a similar trend as PVY. All expected samples to have virus infection exhibited already positive reactions with PLRV. The foliar applications of $ZnSO_4$ revealed significant ($P\leq0.05$) decreases with DAS-ELISA values compared to the untreated plants. In this respect, the high examined concentration of $ZnSO_4$ at 50 ppm gave the lowest significant ($P\leq0.05$) decreases in the virus concentration in both seasons. On the other hand, the treatment of JA at 10 µM followed by 20 µM showed significant decreases in DAS-ELISA values in the two seasons. Furthermore; the combined treatment of $ZnSO_4$ at 50 ppm + JA at 10 µM was the highest efficient treatment in reducing significantly ($P\leq0.05$) the concentration of PLRV in leaf tissues under the conditions of this study.

In this study, reducing the symptoms and the concentration of viruses (PVY and PLRV) in leaf tissues of potato plants with applied $ZnSO_4$ as foliar application was in harmony with (Hamid [40]) who found that zinc deficit plants showed high susceptibility to virus diseases. In addition, enhancing the resistance to viral diseases of JA applied plants in comparison to the untreated ones could be attributed to its direct effect on the mechanisms of the systemic resistance [41-42]. Moreover, reducing the symptoms and the concentration of PVY and PLRV by the combined treatments of $ZnSO_4$ and JA could be attributed to the synergistic effect of both examined compounds on each other which caused significant ($P\leq0.05$) increases in the total soluble proteins and phenolic compounds (Table, 5) compared to the other treatments. These findings may reflect several increases in the activity of some resistance enzymes or increase the synthesis of specific proteins.

Correlation Coefficient: Correlation coefficient (Figure 1) between the biochemical constituents and the concentration of the examined viruses (PVX, PVY and PLRV) as indicated by the values of DAS-ELISA showed that there were significant inverse correlations between the concentrations of total soluble protein and phenolic compounds from one side and the concentrations of PVY and PLRV in the leaves on the other side. Meanwhile, no relationships were detected between the concentrations of chlorophyll and carotenoids with the concentration of the different investigate viruses (PVY, PVX and PLRV) under the circumstances of this study.

These results with more precisely indicated that the foliar applications of $ZnSO_4$ and/or JA may help potato plants to increase its resistance to viral diseases especially PVY and PLRV by increasing the concentrations of total soluble protein and phenolic compounds. The positive effect of $ZnSO_4$ and JA on the both chemical constituents was confirmed and discussed previously in this study (Table 5). Furthermore, it is well documented that phenolic compounds play an important role in the development of defense mechanisms against plant viruses [43]; whereas, the simultaneously increases of total soluble protein with resistance to the examined viruses may be due to increase specific resistance proteins or some of related enzymes in this respect. These results were confirmed in many previous studies [35-36, 43].

CONCLUSION

The results of this study revealed that the treatments of $ZnSO_4$ and/or JA individually or in combination had many significant effects on the growth, yield and some biochemical constituents of potato plants; in general, the treatment of $ZnSO_4$ at 50 ppm + JA at 10 μM gave the highest results in this respect. Also, it was obvious that there was an increase in the resistance to viral the diseases (PVY; PLRV) in the treated plants with both investigated compounds. An inverse significant correlation was found between the changes in the total soluble proteins or phenolic compounds with the changes in the concentration of PVY and PLRV in leaves of the $ZnSO_4$ and/or JA treated plants. These finding proved that the treatments of $ZnSO_4$ and/or JA individually or in combination had positive effect on the resistance of potato plants to viral diseases through modifying some biochemical metabolites.

ACKNOWLEDGMENT

The authors are grateful to Dr/ Ahmed El-Sawy; Climate Modification Department; Central Laboratory for Agriculture Climate; Agriculture Research Center; Ministry of Agriculture; Egypt for his great helpful efforts and provided us with climatic data and irrigation requirements of potato plants in the site of experiment.

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