USING SALICYLIC ACID, FOLIC ACID AND/OR MANCOZEB IN CONTROLLING TOMATO EARLY BLIGHT BIOTIC STRESS AND THEIR EFFECTS ON GROWTH, YIELD, FRUIT QUALITY, AND STRESS-RELATED ENZYMES

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ABSTRACT

Two field experiments were conducted to study the effect of foliar applications of salicylic acid (200 ppm), folic acid (100 ppm) and/or Mancozeb (50% and 100% of recommended dose) on the growth, yield, quality, enzymes activities related to stress, and disease severity% of tomato plants cv. "Fayrouz" grown under biotic stress conditions of early blight (*A. solani*) disease. The results showed significant effects of the applied treatments on all the studied characters with a noticeable superiority of the treatments of SA+FolA+50%$_{Rec}$ followed by 100%$_{Rec}$ without significant difference between them, which reflects on high mean values of growth, chlorophyll, yield, fruit quality, total phenol, and enzymes activities, in both seasons. Also, the best-applied treatments were related with the highest significant increases in leaves' total phenol content in addition to enhancing the activity levels of POD, PPO, and CAT enzymes, which were found to significantly decrease the disease severity%, in both seasons. It could be suggested to reduce the recommended dose of mancozeb fungicide up to 50% by using 200 ppm of salicylic acid with 100 ppm of folic acid for ameliorating the deleterious effects of early blight and producing tomato safer for human consumption and eco-friendly.

Keywords*:* leveas, growth, enzymes, mancozeb, early blight, diseases, chlorophyll.

حامض الساليسيليك وحمض الفوليك و/أو المانكوزيب في مكافحة الإجهاد الحيوي لمرض اللفحة المبكرة للطماطم وتأثيراتها على المحصول وجودة الثمار والإجهاد

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المستخلص

أجريت تجربتان حقايتان لدراسة تأثير التطبيقات الورقية لحمض الساليسيليك (200 جزء في المليون) وحمض الفوليك (100 جزء في المليون) و/أو المانكوزيب (50٪ و100٪ من الجرعة الموصي بها) على النمو والمحصول والجودة وأنشطة الإنزيمات المرتبطة بالإجهاد، كذا شدة المرض٪ لنباتات الطماطم صنف "فيروز" تحت ظروف الإجهاد الحيوي لمرض اللفحة المبكرة (*A. solani*). أظهرت النتائج تأثيراً معنوياً للمعاملات المطبقة على جميع الصفات المدروسة مع تفوق ملحوظ في معاملات SA + FolA + 50٪$_{Rec}$ يليها 100٪$_{Rec}$ دون اختلاف معنوي بينهما، والذي انعكس على قيم المتوسطات العالية للنمو، الكلوروفيل والمحصول وجودة الثمار والفينول الكلي وأنشطة الأنزيمات في الموسمين. أيضًا، ارتبطت أفضل المعاملات المطبقة بأعلى زيادة معنوية في المحتوى الكلي للفينول في الأوراق بالإضافة إلى تعزيز مستويات نشاط إنزيمات POD وPPO وCAT، والتي وجدت أنها تقلل بشكل كبير من شدة المرض في كلا الموسمين. مما سبق، يمكن اقتراح تقليل الجرعة الموصي بها من مبيد المانكوزيب حتى 50٪ باستخدام 200 جزء في المليون من حمض الساليسيليك مع 100 جزء في المليون من حمض الفوليك لتخفيف الآثار الضارة للفحة المبكرة وإنتاج طماطم أكثر أمانًا للاستهلاك البشري وصديق للبيئة.

الكلمات المفتاحية: الطماطم، حامض السليسيليك، حامض الفوليك، مناكوزيب، اللفحة المبكرة

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INTRODUCTION

The tomato crop (*Solanum lycopersicum* L.) is one of the main solanaceous vegetable crops in Egypt and all over the world. Egypt in 2019, occupied the fifth rank in tomato production, which contributes about 3.74% of the total world production of tomatoes (FAOSTAT). Therefore, researchers are always interested in studying various means to improve the different performances of tomato plants grown under normal or under biotic, and abiotic stresses conditions, which enhance tomato growth and yield. Among the various types of biotic stresses (diseases) that confront the tomato crop, the early blight (*Alternaria solani*) is considered one of the most common harmful tomato diseases, which attacks tomato plants wherever are grown and almost every season, which causes high leaf deterioration and damage to the fruits (11). The conventional method for controlling early blight is using synthetic fungicides, which were found to be an effective approach for this disease control, as reported by Farooq et al. (17), Abdou et al. (2), and Sharma et al. (37), especially those based on copper and mancozeb where inhibiting mycelium growth and the sporulation (30). However, there are hazardous consequences of using such synthetic fungicides on the environment, humans, and treated plants (12; 42). Therefore, the modern plant protection systems search for decreasing the used doses of fungicides by involving new eco-friendly means, which mostly do not act directly on the pathogen but promote defense mechanisms of the treated plants that are called induced systemic resistance, as found in salicylic acid (16) and folic acid (8). Each salicylic acid (SA) and folic acid (FolA) was found to be effective treatments that have a positive and significant influence on many physiological processes of the treated plants that reflect on growth, yield, and quality when plants are grown under normal or stressed conditions (25; 27). Concerning tomato plants, the foliar application of such bio-stimulants were found to improve many morphological and physiological aspects of tomato plants as found with SA (1) and with FolA (45), which revealed that the foliar application of FolA at 100 ppm resulted in the highest mean values of vegetative growth, chlorophyll, nutrient content, yield and its components, and quality attributes of the final product. Moreover, many earlier studies found a significant potential for each of SA and FolA as ameliorative agents for biotic or a biotic stress condition. For instance, SA was stated as an important agent for overcoming the biotic stress conditions as found with rarely blight in tomatoes (5; 21). Also, Abdou et al. (2) showed that the high efficacy of SA in reducing the growth of *A. solani* caused early blight on tomato plants even as linear growth *in vitro* or as disease severity *in vivo*. Ibrahim et al. (19) found significant enhancement of foliar treatment of FolA application on all studied characters of potato plants grown under the biotic stress of viral diseases. In addition, Wittek et al. (43) found a synergistic relationship between FolA and SA where the FolA application was found to activate the gene expression of the SA in leaves of Arabidopsis plants grown under biotic stress of *A. brassicicola*. *Vice versa*, Puthusseri et al. (32) found that the foliar application of SA resulted in an accumulation increase of internal levels of folates in Arabidopsis. The aims of this investigation are (a) to study the influence of foliar spray of salicylic acid and/or folic acid on the performance of tomato plants grown under the biotic stress of early blight disease, and (b) to examine the possibility of reducing the recommended dose of mancozeb by mixing with salicylic acid (SA) and/or Folic acid (FolA) for controlling the early blight in tomato.

MATERIALS AND METHODS

Two field experiments were conducted on a private farm in Itay Al-barud, El-Buhaira Governorate (Latitude 30° 67' N, Longitude 30° 89' E), which its soil type was characterized as clay. The experimental area was cultivated with tomato F_1 hybrid namely Fayrouz on 16 of May 2020 and 19 of May 2021.

Experiment layout and treatments

The Followed experimental layout was Randomized Complete Block Design (RCBD) with four replicates. Each experimental plot area for each treatment consisted of four rows with 13 m length and 1 m width for each with planting distance of 0.5 m between seedlings, Accordingly, the area of each plot was 52 m^2 with 104 plants. A guard row was left among the experimental plots to protect against interferences of treatments. Each replication of the experiment was consisted of nine randomly distributed treatments that were:

1- Control (tomato plants treated with tap water)
2- Salicylic acid at 200 ppm (SA)
3- Folic acid at 100 ppm (FolA)
4- Salicylic acid at 200 ppm (SA) with folic acid at 100 ppm (SA+FolA)
5- 50% of the recommended dose of mancozeb fungicide as 1.25 gL^{-1} ($50\%_{Rec}$)
6- Full recommended dose of mancozeb fungicide as 2.5 gL^{-1} ($100\%_{Rec}$)
7- Salicylic acid at 200 ppm with 50% of the recommended dose of mancozeb fungicide as 1.25 gL^{-1} ($SA+50\%_{Rec}$)
8- Folic acid at 100 ppm with 50% of the recommended dose of mancozeb fungicide as 1.25 gL^{-1} ($FolA+50\%_{Rec}$)
9- Salicylic acid at 200 ppm (SA) with Folic acid at 100 ppm and 50% of the recommended dose of mancozeb fungicide as 1.25 gL^{-1} ($SA+FolA+50\%_{Rec}$).

All the above-mentioned treatments were applied on tomato plants as a Foliar application that was repeated three times, which started at 30 days from transplanting with 10 days intervals. All other agricultural practices (irrigation, fertilization, weed control… etc.) were done following the instructions of commercial tomato production suggested by Ministry of Agriculture and Land Reclamation of Egypt.

Recorded data

After 70 days from transplanting, three randomly selected tomato plants from each treatment were labeled for recording the mean values of vegetative growth, chlorophyll, and leaves chemical composition characters as follow:

Vegetative growth characters:

Leaves number $plant^{-1}$ was counted and the mean values of were calculated. In the same way, shoot fresh weight was recorded in grams then the tomato shoots dried in a forced hot air oven under 70°C until weight was constant, and the shoots dry weight was recorded for each treatment.

Leaves chlorophyll and chemical composition:

The tomato leaves' chlorophyll was determined by a nondestructive method using a SPAD-502 chlorophyll meter. Concerning tomato leaves' chemical composition; nitrogen, phosphorus, and potassium as % in tomato leaves were each determined according to the methods illustrated by Temminghoff and Houba (40).

Yield and its components:

During the harvesting season, the tomato fruits number were counted from three randomly selected tomato plants for each treatment and fruits number pl^{-1} means were calculated. average tomato fruits weight was measured in grams. The weight of tomato fruits for each gather for each plot were taken and the yield of each plot were recorded. Total yield fad^{-1} was estimated by multiplying the mean values of fruits yield $plot^{-1}$ by the factor of 76.92, which derived from dividing the net area of faddan (4000 m^2) on the area of the experimental plot (52 m^2).

Fruits quality characters:

According to the methods described by **Ranganna (34)**, the tomato fruit samples were taken from the second harvest to determine total soluble solids (TSS, as Brix°) by using a hand refractometer; ascorbic acid (mg 100 g^{-1} FW) by using the direct colorimetric method using 2,6-dichlorophenol-indophenol dye; and lycopene (mg 100 g^{-1} FW) by spectrophotometer at 503 nm.

Enzymes activities

Crude enzyme extract:

The sample of one of leaves (after 70 days from sowing) was homogenized in 2 ml of 0.1 M sodium phosphate buffer (SPB) pH 6.5 at 4 °C. The filtrate was centrifuged at 20.000 rpm at 4 C min. The supernatant served as an enzyme extract for enzyme assay of peroxidase (POD), polyphenol oxidase (PPO), and catalase (CAT).

Polyphenol oxidase activity (PPO):

Polyphenol oxidase activity was estimated as described by **Mayer and Harel (23)** with some modifications. The polyphenol oxidase activity was expressed as change in absorbance at 495 nm per min/g fresh leaves.

Peroxidase activity (POD):

Peroxidase activity was assayed calorimetrically according to the method described by **Amako et al. (4)**. Peroxidase enzyme activity was expressed as change absorbance at 430 nm per min/g fresh leaves.

Catalase activity (CAT):

Catalase activity was assayed by measuring the rate of disappearance of H_2O_2 at 240 nm according to the methods of (Cakmak and Horst, 1991). The decrease in absorbance at 240 nm was recorded for 1 min by spectrophotometer one unit was definer as amount of enzyme necessary to decompose in mol H_2O_2/min/g fresh leaves under the condition of the assay.

Total phenols:

According to the method of **Singleton and Rossi (39)** the total phenols of the tomato leaves were determined using spectrophotometer at 765 nm using folinn-ciocalteu reagent. The total phenols content was expressed as mg of gallic acid equivalents per gram of fresh weight

Disease assessments

For assessing disease severity, ten plants were selected randomly in each replication (plot) due to recording disease severity individually for each one using 0-5 rating scale described by **Pandey et al. (29)** where, 0 = No symptoms, 1 ≤ 10% of surface area of leaf, stem and fruit infected by early blight, 2 = 11-25% of Foliage of plant covered with a few isolated spot, 3 = Many spot coalesced on the leaves, covering 26-50% of surface area of plant, 4 = 51-75% of surface area of the plants infected, fruits also infected at peduncle end, defoliation and blightening started, sunken lesions with prominent concentric ring on stem, petioles and fruits and 5 ≤ 75% surface area of the plants part blighted, severe lesion on stem and fruit rotting on peduncle end. Early blight disease severity% (DS%) was assessed according to the Following formula:

$$\text{Disease severity\%} = \sum \left(\frac{n \times v}{5N}\right) \times 100$$

Where (n) = Number of plants in each category; (v) = Numerical values of symptoms category; (N) = total number of plants; (5) = Maximum numerical value of symptom category.

Efficacy (Ef) percentage of different treatments as previously mentioned was calculated based on mean of DS%e during the two seasons 2020 and 2021. Efficacy-I % (Ef-I %) calculated for comparison all tested treatments with untreated control (Mahmoud et al., 2013) as Follows:

$$\text{Ef} - \text{I\%} = \frac{\text{DS\% in control} - \text{DS\% in treatments}}{\text{DS\% in control}} \times 100$$

Statistical analyses

Data were statistically analyzed using CoStat program. A Least Significant Difference test (LSD) at 0.05 probability level was Followed to verify the significance between the examined treatments by using the same program.

RESULTS AND DISCUSSION

Vegetative growth characters:

The effect of foliar application with SA, FolA acids and mancozeb on vegetative growth parameters (fresh and dry weights of shoot and leaves number) of tomato under biotic stress of early blight disease were presented in **Table 1**. All treatments under investigation significantly increased vegetative growth parameters and alleviating the biotic stress of early blight on tomato plants comparing with untreated plants. Combination between SA+FolA increased mean values of all traits comparing with FolA application, and without any significant with SA alone. Also, foliar application of SA or FolA with mancozeb at 50 $\%_{Rec.}$ significantly increased the traits comparing with the untreated plant. While, the foliar application with SA+FolA+50$\%_{Rec}$ recorded the highest mean values of all vegetative growth parameters followed by 100$\%_{Rec}$ with no significant effect except with dry shoot weight in the 1st season.

With regard to the stimulatory effect of SA on different estimated characteristics of tomato growth, it is found that the exogenous application of SA improves the physiological status of tomato plants that exhibited in plant growth aspects through keeping suitable levels of endogenous phytohormones i.e., Auxin, gibberellin and cytokinin **(1; 24)**. Moreover, SA was stated as an important agent for overcoming the biotic stress conditions as found with *A. solani* infection **(9)**. In addition, **Abdou et al. (2)** stated that SA has high efficacy in reducing the growth of *A. solani* caused early blight on tomato plants even as linear growth *in vitro* or as disease severity *in vivo*.

The present study proved that foliar application of FolA has the ability to improve tomato growth characters where may be due to the role of FolA in many cellular reactions such as, metabolism of amino acids, synthesis of methionine and the formation of lignin, and chlorophyll and also in the photo-respirations cycle, which led to increase in growth **(7; 27)**. Also, **Zamanipour (45)** on tomato stated that the foliar application of FolA at 100 ppm resulted in the highest mean values of vegetative growth characters.

Also, the superiority of mixing SA+FolA comparing with their solo treatments could be due to the existence of some kind of synergistic effect between them on the physiological status of the treated plants as shown by **Wittek et al. (43)** and **Puthusseri et al. (32)**.

Moreover, the results of this study may reveal a potential for using SA (200 ppm) and FolA (100 ppm) for reducing the recommended dose of the tested fungicide (mancozeb) by 50% for confronting the biotic stress of early blight that positively consequences on the agro-environment if compared with using the full recommended dose of mancozeb.

Table 1. Effects of different salicylic acid, Folic acid and mancozeb treatments on tomato shoot fresh and dry weights, and leaves number in 2020 and 2021 seasons.

Treatments	Shoot FW (g)		Shoot DW (g)		Leaves No. pl^{-1}	
	2020	2021	2020	2021	2020	2021
Control	506.37g	515.40f	68.64i	70.14g	54.33g	55.33h
SA	527.53d	536.57cd	70.83f	72.22d	70.67d	68.67e
FolA	513.27f	521.75ef	69.44h	70.85f	60.67f	58.33g
SA+FolA	529.46d	538.88cd	71.06e	72.31d	72.33cd	70.67d
$50\%_{Rec.}$	521.32e	530.40de	70.15g	71.70e	64.67e	60.67f
$100\%_{Rec.}$	551.29a	561.98a	72.97b	74.59a	85.00a	82.67a
SA+$50\%_{Rec.}$	543.44b	553.63ab	72.26c	73.87b	80.33b	78.67b
FolA+$50\%_{Rec.}$	536.67c	547.94bc	71.65d	73.36c	75.33c	74.00c
SA+FolA+$50\%_{Rec.}$	553.55a	565.89a	73.23a	74.65a	86.00a	83.33a
Mean	531.43	541.38	71.14	72.63	72.15	70.26

*** The means with the same letter(s) do not differ significantly under 0.05 confidence level**

Leaves chlorophyll and chemical composition:

It is shown from the data presented in Table 2, the effect of foliar application with salicylic, folic acids, and mancozeb on tomato leaves chlorophyll, N, P, and K% grown under biotic stress of early blight. All treatments under investigation were increased significantly the chlorophyll, N, P, and K% mean values in both seasons compared with control. Also, the combined treatment of SA+FolA has significantly increased the mean values of chlorophyll and N% compared with the solo treatments of SA or FolA, in both seasons. whereas, the SA+FolA treatment did not show any significant difference in P and K% compared to SA treatment, in both seasons. The effect of SA and FolA were have noticeable effects on the nutrients and chlorophyll composition of leaves of tomato plants (1; 45) especially under stressed conditions (25; 27). Also, these findings were pointed to some kind of synergistic relationship between AS and FolA that increased their ameliorative effect in the combined treatment compared with the individual treatment (32; 43). Moreover, the results of this study revealed that the treatment of SA+FolA+$50\%_{Rec.}$ was found to be the most ameliorative treatment for tomato plants confronting the biotic stress of early blight followed by $100\%_{Rec.}$ treatment comparing with other treatments. Thus, it could to declare the ability of reducing the recommended dose of mancozeb by 50% if combined with 200ppm of SA with 100 ppm of FolA.

Table 2. Effects of different salicylic acid, folic acid and mancozeb treatments on tomato leaves chlorophyll (SPAD), N, P, and K in 2020 and 2021 seasons.

Treatments	Characters							
	chlorophyll (SPAD)		N%		P%		K%	
	2020	2021	2020	2021	2020	2021	2020	2021
Control	45.23i	46.19i	1.99g	2.06f	0.498g	0.512f	2.09g	2.17f
SA	52.17f	53.00f	2.20d	2.28d	0.541d	0.548d	2.31d	2.39d
FolA	47.64h	48.61h	2.07f	2.16e	0.517f	0.527e	2.18f	2.27e
SA+FolA	53.41e	54.54e	2.28c	2.37c	0.543d	0.553d	2.34d	2.44d
50%Rec.	49.85g	51.05g	2.14e	2.23d	0.529e	0.540d	2.25e	2.32e
100%Rec.	60.32b	61.41b	2.43a	2.52a	0.576a	0.588ab	2.55a	2.63b
SA+50%Rec.	58.04c	59.04c	2.36b	2.44b	0.565b	0.576bc	2.47b	2.58b
FolA+50%Rec.	55.83d	56.71d	2.31bc	2.39bc	0.555c	0.572c	2.40c	2.50c
SA+FolA+50%Rec.	61.33a	62.41a	2.46a	2.54a	0.581a	0.593a	2.58a	2.68a
Mean	53.76	54.77	2.25	2.33	0.545	0.557	2.35	2.44

*** The means with the same letter(s) do not differ significantly under 0.05 confidence level**

Yield and its components:

The listed mean values in Table 3 revealed that the yield components (average fruit weight, fruit number plant^{-1}, and kg plot^{-1}) and total yield of tomato plants suffering from early blight biotic stress were significantly alleviated due to the foliar application of the examined treatments comparing with control, in both seasons of study. Also, it is clearly noticed that the SA+FolA treatment was significantly ameliorate the yield and its components mean values of biotic stressed tomato plants comparing with the individual treatments of SA or FolA, in both seasons. These findings could be due to the significant role of each of SA and FolA in maintaining the adequate levels of different physiological processes of stressed plants with early blight disease that resulted in enhancing the nutrients uptake, chlorophyll content, and growth, which in sum increased the mean values of yield and its components as found with SA (1) and FolA (27). Zamanipour (45) stated that the foliar application of FolA at 100 ppm resulted in tomato yield performances. Moreover, SA was found to have another side of ameliorative effect under biotic stress of early blight that related to the ability of SA to control the *A. solani* fungi caused early blight (2; 9). Along with the role of FolA in inducing systemic resistance (8), it also was found to induce the SA-dependent immunity against *Alternaria* biotic stress (43). Moreover, the results found the SA+FolA+50%$_{Rec.}$ gave the most significant ameliorative effect against the biotic stress of early blight compared with other examined treatments followed by 100%$_{Rec.}$, which did not differ significantly with SA+FolA+50%$_{Rec.}$ in fruits No. plant^{-1}, fruits weight plot-^{1}, and total yield, in the second season only. Although the researchers were found that the mancozeb fungicide was a very effective treatment for controlling the early blight biotic stress in tomato that reflects on increasing the mean values of yield and its components traits (Palaiah P. et al., 2020; 37), our findings showed the ability for using SA (200ppm) with FolA (100 ppm) to reduce the recommended dose of mancozeb up to 50%, which could give better or the same ameliorative effect.

Table 3. Effects of different salicylic acid, Folic acid and mancozeb treatments on tomato average fruit weight, fruits number plant^{-1}, fruits weight plant^{-1}, total yield fad.$^{-1}$ in 2020 and 2021 seasons.

Treatments	Characters							
	Average fruit weight (g)		Fruits Number plant^{-1}		Fruits weight (kg plot^{-1})		Total yield fad.$^{-1}$ (Ton)	
	2020	2021	2020	2021	2020	2021	2020	2021
Control	99.63h	103.64i	18.00i	18.67e	185.59	199.13	14.28i	15.32e
SA	104.69f	110.20g	24.00g	25.00d	259.20	285.47	19.94g	21.96d
FolA	102.46g	106.73	21.67h	22.67d	230.02	249.53	17.69h	19.19d
SA+FolA	108.55e	113.84e	29.33e	32.67bc	327.23	385.67	25.17e	29.67c
50%$_{Rec.}$	107.46e	111.70f	27.67f	31.67c	307.34	365.76	23.64f	28.13c
100%$_{Rec.}$	118.45	123.69	36.00b	38.67a	442.28	494.49ab	34.02b	38.04a
SA+50%$_{Rec.}$	115.48c	119.74c	33.33c	36.33a	398.40	451.38	33.64c	34.72b
FolA+50%$_{Rec.}$	111.70	116.22	31.00d	33.33bc	359.09	400.98	27.62d	30.84c
SA+FolA+50	120.58a	124.91a	37.67a	39.33a	469.42	509.05	36.11a	39.16a
Mean	109.89	114.52	28.74	30.93	330.95	371.27	25.46	28.56

*** The means with the same letter(s) do not differ significantly under 0.05 confidence level**

Fruits quality characters: Data presented in Table 4, revealed the effect of salicylic acid, Folic acid and mancozeb treatments on quality of tomato fruits as TSS, ascorbic acid, lycopene, and leaves total phenols under biotic stress of early blight disease in 2020 and 2021 seasons. All treatments significantly affected in tomato fruit quality and reduce the biotic stress of early blight on tomato plants comparing with untreated plants, in both seasons. The foliar application with SA+FolA increased the fruit quality over the solo application of SA or FolA. Also, the data in Table 4 proved that foliar application of SA+FolA+50%$_{Rec.}$, gave the highest mean values of TSS, ascorbic acid, lycopene, and leaves total phenols followed significantly by the application of 100%$_{Rec.}$ except with ascorbic acid in the 1st season and lycopene in the 2nd season. The results of this study are harmonious with the vital role of each of SA and FolA in tomato plants, which published by other researchers (1; 45). Also, it is clear from the results of this experiment the possibility of a synergistic relationship between each of SA and FolA, as shown by SA+FolA treatment that gave higher mean values for the quality traits of the tomato fruits that faced early blight stress compared to the single treatments of SA or FolA, this synergistic relationship between SA and FolA and *vice versa* has been demonstrated by Wittek et al. (43) and Puthusseri et al. (32). Moreover, the results of this study revealed the possibility of reducing the hazardous effects of using the chemical fungicides by 50% by using the mixture of AS+FolA with 50% of the recommended dose of mancozeb fungicide for achieving the best tomato fruit quality characters under the biotic stress of early blight disease. This may be due to the potential of SA in ameliorating the biotic stress of *A. solani* fungi caused early blight (2; 9), in addition to the role of FolA in inducing systemic resistance (8) and stimulation of SA synthesis signals (43).

Table 4. Effects of different salicylic acid, Folic acid and mancozeb treatments on tomato fruits TSS, ascorbic acid, and lycopene, and leaves total phenols in 2020 and 2021 seasons.

Treatments	Characters							
	TSS (°Brix)		Ascorbic acid (%)		Lycopene (mg/100 g f.w.)		Total phenols (mg/g/f.w.) in leaves	
	2020	2021	2020	2021	2020	2021	2020	2021
Control	5.73i	6.84i	25.29e	27.80i	3.36i	3.42h	3.88g	3.95i
SA	6.73g	8.41g	26.29d	28.74g	4.67g	4.83f	4.54e	4.66f
FolA	6.16h	7.81h	25.76de	28.32h	3.96h	4.06g	3.99g	4.07h
SA+FolA	7.55e	9.65e	27.45c	29.59e	5.61e	5.73d	4.86d	4.95d
50%$_{Rec.}$	7.26f	9.36f	27.90bc	29.33f	5.37f	5.44e	4.28f	4.36g
100%$_{Rec.}$	9.27b	11.38b	29.39a	31.21b	7.29b	7.45a	5.43b	5.54b
SA+50%$_{Rec.}$	8.76c	10.68c	28.81ab	30.76c	6.73c	6.93b	5.17c	5.27c
FolA+50%$_{Rec.}$	8.13d	10.07d	28.18bc	30.14d	6.29d	6.41c	4.75d	4.82e
SA+FolA+50%$_{Rec.}$	9.47a	12.60a	29.60a	31.74a	7.47a	7.57a	5.65a	5.83a
Mean	7.68	9.65	27.63	29.74	5.64	5.76	4.73	4.83

*** The means with the same letter(s) do not differ significantly under 0.05 confidence level**

Enzymes activities:

Data concerning the effect of SA, FolA and mancozeb treatments on total protein, peroxidase (POD), polyphenol oxidase (PPO), and catalase (CAT) of tomato leaves during 2020 and 2021 seasons under biotic stress of early blight disease were presented in Table 5. Generally, the showed results indicated a significant effect of the examined treatments in increasing the mean values of total protein and the assessed antioxidant enzymes activities comparing with control, in both seasons of study. However, the treatment of SA+FolA was found to be significantly effective in ameliorating the biotic stress of tomato early blight when compared with the solo treatment of SA or FolA, in both seasons of study. This could be because of the synergistic effect that found between SA and FolA that found by Wittek et al. (43) and Puthusseri et al. (32). Also, It is known now that biotic stress leads to emergence of oxidative stress with its reactive oxygen species (ROS), which leads to a significant deterioration in the physiological state of plants (38). Hence the importance of antioxidant enzymes in these confronting of these stressful conditions was rise. There is many of researches that has shown the effective role of each of SA and FolA in depressing oxidative stress by significantly increasing levels of antioxidant enzymes (1; 20; 35; 36). Moreover, concerning leaves total protein, the highest significant mean values of the investigated parameters were scored with foliar application of SA+FolA+50%$_{Rec.}$ followed significantly by treatment of 100%$_{Rec.}$; while the POD, PPO, and CAT enzymes activities did not differ significantly between these two treatments, in both seasons of study. This indicates the possibility of substitute 50% of the used doses of mancozeb fungicide by 200 ppm of SA with 100 ppm of FolA.

Table 5. Effects of different salicylic acid, Folic acid and mancozeb treatments on tomato leaves total protein, peroxidase (POD), polyphenol oxidase (PPO), and catalase (CAT) in 2020 and 2021 seasons.

Treatments	Characters							
	Total protein (%)		POD (U min^{-1} mg^{-1} protein)		PPO (U min^{-1} mg^{-1} protein)		CAT (U min^{-1} mg^{-1} protein)	
	2020	2021	2020	2021	2020	2021	2020	2021
Control	7.97i	8.26i	0.66g	0.64g	0.39g	0.43g	0.30g	0.25g
SA	9.26f	9.62f	0.75de	0.81e	0.46e	0.55de	0.41e	0.43de
FolA	8.65h	9.02h	0.68fg	0.71f	0.40fg	0.48f	0.32fg	0.32f
SA+FolA	10.27e	10.66e	0.82c	0.86de	0.55cd	0.59cd	0.51cd	0.48cd
50%$_{Rec.}$	10.02g	10.38g	0.72ef	0.75f	0.44ef	0.51ef	0.37ef	0.37ef
100%$_{Rec.}$	12.24b	12.71b	0.93a	0.99ab	0.63ab	0.71ab	0.56ab	0.64ab
SA+50%$_{Rec.}$	11.57c	11.98c	0.88b	0.94bc	0.58bc	0.68b	0.53bc	0.58b
FolA+50%$_{Rec.}$	10.84d	11.22d	0.79cd	0.90cd	0.52d	0.62c	0.47d	0.52c
SA+FolA+50%$_{Rec.}$	12.54a	12.96a	0.97a	1.04a	0.66a	0.74a	0.59a	0.69a
Mean	10.37	10.76	0.80	0.85	0.51	0.59	0.45	0.48

*** The means with the same letter(s) do not differ significantly under 0.05 confidence level**

Disease severity:

Data illustrated in Table 6 reveal that all tested SA, Fol and fungicide singly or in combination had a great significant effect in decreasing the early blight disease severity percentage caused by A. *solani* on tomato plants during the two seasons 2020 and 2021 in comparing with control treatment under field conditions. Also, SA+FolA+50%$_{Rec}$. treatment followed by fungicide 100%Rec were scored highest significant decrease in disease severity% (8.77and 9.20, respectively). These results are in harmony with the findings of El-Shennawy and Abd El-All (14) who found that SA 30 mg/l had the greatest inhibitory effect on early blight disease of tomato plants. Al-Ani and Shaker (3) and EL-Tanany et al. (15) concluded that SA induced the production of proteins associated with reducing early blight disease intensity in tomato plants. The same trend was observed concerning efficacy (Ef-I%) where SA+50%$_{Rec.}$ treatment followed by

SA+FolA scored highest treatment efficacy in comparing with control in the first season. Also, the same trend cleared in the second season where the SA+Fol+50%$_{Rec.}$ and fungicide 100%$_{Rec.}$ was more effective in reducing disease severity with an average of 8.65 and 7.20, respectively followed by SA+50%$_{Rec.}$, SA+FolA and FolA+50%$_{Rec.}$with averages of 10.87, 13.60 and 17.73, respectively. These results are in agreement with results obtained by Vallad and Goodman (41) who found that SA can induce pathogenesis accumulation related proteins (PRP), which reduced several diseases incidence on many crops. Application of SA elicit production of tomatine (phytoalexin) in leaves and stems of tomato plants which is toxic to A. *solani* (6). Thus, these proteins induced in tomato plants are likely responsible for inducing systemic acquired resistance (SAR) in tomato plants and stimulated resistance R-genes to produce pathogenesis-related proteins (PRP) responsible for fungal resistance (6; 35). Ibrahim et al. (19) indicated that FolA at 50 and 100 µgL^{-1} enhanced the levels of biochemical constituents, enzyme activities, tolerance to viral diseases, tuber yield and quality of potato plants. Folic acid is a central cofactor for 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 (26; 31; 44).

Table 6. Effects of different salicylic acid, Folic acid and mancozeb treatments on disease severity% and efficiency in 2020 and 2021 seasons

Treatments	Percentage of disease severity (%)			
	2020	Efficiency	2021	Efficiency
Control	48.2a	-----	45.81	-----
SA	23.44c	51.37f	20.44	55.38f
Fol	34.08b	29,30g	30.04	34.42g
SA+Fol	16.8f	65.15c	13.6	70.31d
50%Rec.	20.73d	56.99e	19.81	56.76f
100%Rec.	9.2h	80.91a	8.65	81.12b
SA+50%Rec.	12.04fg	36.16b	10.87	76.27c
Fol+50%Rec.	19.13e	29.07d	17.73	61.3e
SA+Fol+50%Rec.	8.77h	81.8a	7.2	90.83a

*** The means with the same letter(s) do not differ significantly under 0.05 confidence level**

Liner relations with disease severity:

The liner relation between disease severity and each of total phenol content, POD, PPO, and CAT enzymes activities in tomato leaves in addition to total yield fad^{-1} were illustrated in figure 1, as an average of both seasons of study.

The results showed negative relationship between all the selected parameters and disease severity%. Also, Regression analysis shows that total phenol contents contribute to about 80.93% (R^2= 0.8093, *P*> 0.05), POD and PPO activities compounds contribute to about 82.18 and 81.02% (R^2 = 8218 and 8102, *P*> 0.05), CAT contribute to about 84.51% (R^2= 0.8451, *P*> 0.05), and total yield contribute to about 86.21% (R^2= 0.86.21, *P*> 0.05). these results emphasized on the importance of researching how to increase the levels of antioxidant enzymes activities that were found to have important role in decreasing disease severity%, which reflects on increasing the mean value of total yield as shown in aforementioned results. These results are in line with those of Ramamoorthy et al. (33). they mentioned that, the induction of defense enzymes involved in phenylpropanoid pathway accumulation of phenolics and PR-Proteins (Phenylalanine Ammonia-lyase (PAL), Peroxidase (POD) and Polyphenoloxidase (PPO) might have contributed to restriction of invasion of *Fusarium oxyporum* f.sp. *lycopersici* tomato roots. Hassan et al. (18) showed a positive relation between reduction of chocolate spot disease severity on faba bean plants and the increase in peroxidase activity and peroxidase isozymes as the results of application with chemical inducers. Also, El-Khallal (13) reported that both PPO and POD are important in defense mechanism against pathogens, through their role in the oxidation of phenolic compounds to quinines, causing increase in antimicrobial activity. Therefore, they may be directly involved in stopping pathogen development accelerating the cellular death of cells close to the infection site, preventing the advance of infection and/or by generating a toxic environment which will inhibit pathogen growth inside the cells. Our results suggested that, beside the ability of the previous agents to induce resistance against pathogens they could be used as protect treatments.

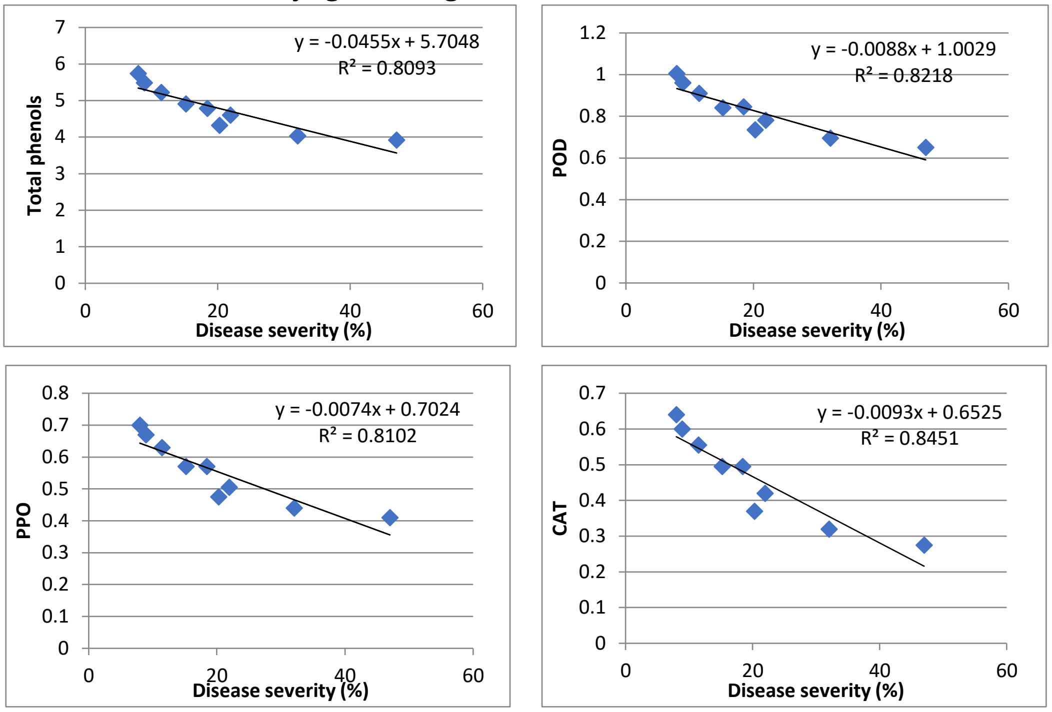

Figure1 . Liner relationship between disease severity and each of leaves total phenols content and POD, PPO, and CAT enzymes activities as a two seasons average

Conclusion

According to the results of this investigation, it could suggest the using of salicylic acid with folic acid for gaining better performance of growth, yield, and fruit quality of tomato cv." Fayrouz" under normal or stressed conditions. Also, the results are pointed out the ability of reduce the recommended dose of mancozeb fungicide in controlling tomato early blight disease up to 50% by adding 200 ppm of salicylic acid and 100 ppm of folic acid, which will have a positive impact on the production of tomato in a safer manner for human consumption, as well as reducing the risks of environmental pollution resulting from the use of synthetic pesticides.

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