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Research Article
Effectiveness of Natural Antioxidants on Physiological, Anatomical Changes and Controlling Downy, Powdery Mildew and Rust Diseases in Pea Plants

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Abstract

Background and Objective: Pea (*Pisum sativum* L.) was attacked by many foliar diseases. Downy mildew, powdery mildew and rust are the most common diseases that affect the quantity and quality of this crop. To control these diseases, certain natural organic acids, e.g., citric, benzoic, salicylic, boric, sorbic acids and also coumarin were foliar sprayed to examine the possibility of avoiding the adverse effect of biotic stress. **Materials and Methods:** The experiment was conducted at Kafr-El-Dwar district, Beheira Governorate, Egypt during 2 successive summer seasons of 2019 and 2020 to evaluate pea plants foliar spray with some organic acids and coumarin at the rate of 0.25 and 0.50% against some foliar diseases. **Results:** The results proved that citric and benzoic acids at 0.50% induced the highest reduction of downy, powdery mildew and rust, while coumarin recorded the lowest reduction at the same concentration and other tested materials came moderately. Moreover, all tested substances gave clear increases in crop parameters and alleviated the harmful effects of biotic (diseases) stress. Acceleration in the recovery in terms of enhancing photosynthetic pigments, indole acetic acid, phenolic, some compatible solutes and antioxidant content was recorded. Also, a positive variation in anatomical structure, e.g., the leaflet blade thickness, upper and lower epidermal layers, vascular bundles, midrib zone, palisade and spongy tissues was noticed. **Conclusion:** Conclusively, foliar spraying with tested materials alleviated the harmful effects of biotic stress and accelerated the recovery and improved pea plants productivity.

Key words: Pea, downy, powdery mildew, rust, physiology and anatomy

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Pea (*Pisum sativum* L.) is a widely grown vegetable crop for human use. Peas are regarded a matter because of their great nutritional value, which includes vitamins, protein and carbohydrates[1]. Numerous diseases harm pea output quantity and quality. The most important of these diseases are downy mildew, powdery mildew and rust. *Peronospora viciae*, which is found in many parts of the world, causes downy mildew, one of the deadliest diseases afflicting pea plants. As a systemic disease of this pathogenic organism destroys seedlings, while local infections affect leaves and pods. Mycelia and oospores of the organism *P. viciae* can be found in seed coatings. On pea plants, different types of downy mildew disease symptoms can be noticed. Throughout the crop life cycle, systemic, local and pod infections can be identified as three different symptom types[2].

Pea powdery mildew disease result of *Erysiphe pisi* is an air-borne disease of around the world conveyance. It is one critical disease that infection the pea plant. The disease can reduce the yield by 25-50%, at the same time it reduces total yield biomass, number of seeds per pod, number of pods per plant, plant height and number of nodes. The malady to influences green pea quality[3], *Uromyces fabae* is responsible for pea rut disease, which causes partial defoliation for all the green parts of the plant including pods of susceptible varieties in warm humid regions, responsible for huge economic losses.

Pea plants under biotic and abiotic stressful conditions, signalling intermediates (Reactive Oxygen Species (ROS)) overproduced and cause oxidative stress[4]. These particles are known to be mindful of damage of cellular membranes throughout lipid peroxidation and capacity to destruct chlorophyll, proteins and DNA[5]. To cope with osmotic stress, plants accumulate proline, Glycine Betaine (GB), soluble proteins and soluble carbohydrates[4]. The molecular basis of non-host resistance presumably inducible responses of a large array of proteins and other organic molecules produced which represents constitutive barriers before infection or during pathogen attack[6,7]. Constitutive defences tools are morphological and cell walls, structural barriers, e.g., trichomes, thorns epidermis layer, etc., chemical compounds, e.g., phenolics, metabolites, nitrogen compounds, etc., enzymes and proteins[8,9].

Not only by defending the plant against invasion but also by strengthening plant strength and rigidity, which confers tolerance or resistance to biotic stress. Chemical resistance inducers are commonly utilised as bioactive agents to combat soil-borne and foliar plant diseases[10]. In this concern, phenolic compounds as a chemical inducer (vanillic, garlic, salicylic, cinnamic, P-coumaric, benzoic acids and coumarin) are considered protecting particles facing biotic and abiotic stress[11,12]. Many plant phenolic compounds have antibacterial properties, act as signal molecules or function as precursors to lignin as structural polymers[13]. The degree of plant resistance to diseases was proportional to the rise in phenolic content[14]. Chemical inducers encourage specific enzymes catalyzing biosynthetic responses to form resistance compounds such as polyphenols, pathogenesis-related proteins that enhance the resistance of the plant to pathogen[15,16].

Salicylic acid could be a phenolic endogenous plant development bio controller that works as an antioxidant compound that contributes to the administration of physiological processes which is considered one of the defence mechanisms against biotic stress[17]. Also, foliar spray with salicylic acid swelled thickness of both midvein and lamina of flyers of Egyptian lupine ‘Giza 2’ due to extend initiated in thickness of palisade and light tissues to extend initiated measure in midvein bundle[18]. The valuable effects of salicylic acid on plant growth and productivity of cowpea plants are well-recorded[19].

Benzoic acid is known to provide plants with abiotic stress tolerance[20]. Abdallah *et al.*[21] reported that benzoic acid (carboxyl) is a natural antioxidant synthesized by plants and diffuses in the rhizosphere area to enable the assimilation of mineral nutrients. The increments in nutrient uptake helped plants in sandy soil to resist the nutrients shortage adverse effects and consequently improved yield quantity and quality.

Citric acid is a regular component in many vase solution preparations that regulate the pH that reduce bacterial creation and improve the water conductance in cut flowers xylem[22]. Citrate and malate are intermediate organic acids in the Krebs cycle which is participating in oxidative phosphorylation that produces cellular energy[23].

Boric acid is a feebly acidic hydrate of boric oxide with gentle clean, antifungal and antiviral properties. Boron is a fundamental plant micronutrient taken up through the roots generally within the frame of boric acid[24].

Sorbic acid may be a common natural compound (unsaturated greasy acid) that has antimicrobial movement against moulds, yeasts and organisms[25].

The objective of this work was to evaluate the protective role of natural organic compound to biotic stress through increment in biochemical constituents and improvement anatomy measurements and consequently the productivity of pea plants.

MATERIALS AND METHODS

Field trials: Throughout cultivation season (2019/2020) field trials were carried out at Kafr-El-Dwar district, Beheira Governorate, Egypt to evaluate foliar spray with some organic acids and coumarin against some foliar diseases on pea plants, i.e., downy mildew, powdery mildew and rust. The forecasting weather throughout the experimental periods from November, 2019 until March, 2020 (C.F. https://www.timeanddate.com/weather/Egypt/Cairo/historic?month=12&year=2019) was recorded.

Field-tested materials: Pea seeds cv. Master B were get from Vegetables Crop Research Department, Horticultural Research Institute, Agricultural Research Center, Egypt. Organic acids, i.e., salicylic, sorbic, citric, boric and benzoic acids in addition to coumarin were obtained from Al-Gomhoria Company Ltd., for chemicals and medicinal instruments, Cairo, Egypt.

Field experiments: Evaluation of the suppressing effectiveness for foliar spraying pea with organic acids, i.e., citric, benzoic, salicylic, sorbic and boric acids in addition to coumarin at 0.25 and 0.50% concentrations against diseases powdery mildew, downy mildew and rust were performed under natural infection conditions in the field. At the growing season both diseases, powdery mildew and downy mildew started to appear in December and were intensively recorded in January, while, rust disease started to appear and was recorded at the end of February.

The field was divided into plots 3×3 m^2 and each plot consisted of five rows with 15 hills/row on the eastern side. Pea Master B cultivar seeds were sown as two seed/hill, two edges of each hill 20 cm between holes. The seeds were sown on 15 November, 2019. The organic acids and coumarin mentioned before were applied twice at concentrations of 0.25 and 0.50%, where the first one was applied at 30 days after sowing and the second one was at 30 days after the first application. The experimental design was a completely randomized block design with 3 replicates for each treatment. The disease severity was calculated 70, 80 and 90 days after sowing for above mentioned studied diseases and it was determined under natural field conditions based on a score chart of 0-5 (0-no infection, 1-1-10, 2-10.1-15, 3-15.1-25, 4-25.1-50 and 5->50% according to Elgamal and Khalil[26] using the following equation:

$$P\ (\%) = \frac{\sum(n\times y)}{SN}\times 100$$

Where:

P = Disease severity
n = Number of infected leaves in each category
y = Numerical values of each category
S = The highest rating value
N = Total number of the infected leaves

On the other hand, the efficacy of each treatment was calculated as follows:

$$\text{Efficacy (\%)} = \left(\frac{\text{Control-Treatment}}{\text{Control}}\right)\times 100$$

Growth measurements: The averages of the morphological measurements in terms of plant height (cm), number of branches/plant, number of pods/plant, the weight of pod (g), hundred seed weight (g), length of the pod (cm), seed yield weight/plot (kg) and seed yield (ton/feddan) were recorded at harvest (90 days after sowing).

Physiological studies

Chemical analysis: Some biochemical constituents, in fresh leaves, including photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids and total pigments) were measured using the method of Lichtenthaler and Buschmann[27]. Total Soluble Sugars (TSS) were extracted according to Gomez *et al.*[28] and determined according to Albalasmeh *et al.*[29]. The antioxidant activity (DPPH radical scavenging) was determined using the method of Liyana-Pathirana and Shahidi[30]. Indole acetic acid content was extracted and analyzed by the method of Gusmiaty *et al.*[31]. Phenolic content was measured as described by Maurya and Singh[32]. Total Soluble Protein (TSP) was determined according to the methods of Bonjoch and Tamayo[33]. Proline content was extracted and calculated according to the methodology of Tamayo and Bonjoch[34]. Free Amino Acids (Free AA) were extracted as outlined by Kalsoom *et al.*[35] and determined with the ninhydrin reagent method reported by Verslues[36].

Anatomical studies: For the anatomical study, samples were taken from the middle part of the leaflet blade. Samples were fixed and killed in FAA (formalin:glacial acetic acid:alcohol 5:5:90) for 48 hrs. Samples were washed in 50% ethyl alcohol, dehydrated and cleared in tertiary butyl alcohol series, embedded in paraffin wax of 54-56°C m.p. Vertical sections were cut at 15 µm by a rotary microtome, adhered by Haupt's adhesive and stained with the crystal violet-erythrosin combination, cleared in carbol xylene and mounted in Canada balsam, sections performed by the freehand made technique[37]. The observation and documented using an upright light microscope [Carl Zeiss then photoed by eyepiece digital camera (HIROCAM 5)]. Measurements were done, using a micrometre eyepiece and an average of 5 readings were calculated.

Statistical analysis: The obtained results were statistically analyzed[38]. Least Significant Difference (LSD) at 0.05 level of probability was applied for means comparison.

RESULTS

Efficacy of foliar spraying with different organic acids and coumarin against downy mildew, powdery mildew and rust on pea under field condition: Table 1 revealed that, all tested treatments significantly reduced the incidence of previously mentioned foliar diseases. Among the used all the tested treatments, citric and benzoic acids recorded the highest reduction in diseases severity of all tested diseases followed by salicylic and sorbic acids, respectively while coumarin caused recorded the lowest effect.

The highest reduction of downy mildew was obtained with citric and benzoic acids at 0.5% concentration by 72.63 and 71.40%, respectively, while coumarin recorded the lowest reduction by (46.43%) at the same concentration. Meanwhile, other treatments showed a moderate effect. The same results were obtained for the two other diseases with some differences in the reading values. The applied treatments have demonstrated their effect against fungal activities and were convenient in overcoming these diseases.

Changes in pea crop parameters under field conditions: Five organic acids i.e., citric, benzoic, salicylic, sorbic and boric acids in addition to coumarin at the concentrations of 0.25 and 0.50% were tested to study their effect on yield components of pea plants.

Table 2 demonstrate that all tried inducers gave a clear increase of crop parameters. This effect was increased with increasing inducers concentrations. Among the used inducers, citric acid caused the highest yield parameters followed by benzoic, salicylic and sorbic acids, respectively while coumarin caused the lowest. Where significant differences were found in some measures as a result of treatments such as plant height (cm), number of branches/plant, number of pods/plant, seed yield weight/plot (kg) and seed yield ton/feddan, while there were no significant differences between them, in the other treatments such as weight of pod (g), hundred seed weight, length of the pod (cm).

Changes in photosynthetic pigments of pea plants: Table 3 represents the influence of chlorophyll a, chlorophyll b, carotenoids and total pigment contents of a pea when treated with organic acids (salicylic, sorbic, citric, boric and benzoic) and coumarin.

All spraying treatments of the previous materials, generally, significantly ($p<0.05$) increased photosynthetic pigments compared to the control treatment. The highest ($p<0.05$) values were recorded when plants were sprayed with citric acid followed by benzoic and salicylic acids over that recorded for the other treatments. The maximum increases were obtained with citric acid foliar spraying at 0.50 mg L^{-1}.

Table 1: Efficacy evaluation of foliar spray with different organic acids against some foliar diseases of pea

		Disease severity (%)					
Treatments	Concentration (%)	Downy mildew	Reduction (%)	Powdery mildew	Reduction (%)	Rust	Reduction (%)
Control	0.00	55.83±1.36	0.00	39.23±5.28	0.00	28.20±1.31	0.00
Salicylic acid	0.25	24.70±0.75	55.75	15.43±0.63	60.67	8.37±0.49	70.32
	0.50	18.08±1.46	66.33	9.63±0.92	75.45	5.43±0.58	80.74
Sorbic acid	0.25	19.20±0.46	65.61	11.67±0.54	70.25	6.20±1.21	78.01
	0.50	15.97±0.62	71.40	8.87±0.81	77.39	4.00±0.44	85.86
Citric acid	0.25	20.83±1.23	62.69	11.90±0.83	69.67	6.16±0.56	78.16
	0.50	15.28±0.64	72.63	6.81±0.76	82.64	4.40±0.51	84.40
Boric acid	0.25	27.86±2.26	50.09	19.30±0.53	50.80	12.23±0.59	56.63
	0.50	21.82±0.95	60.92	15.97±1.24	59.29	8.33±0.41	70.46
Benzoic acid	0.25	19.44±1.40	65.18	17.80±0.25	54.63	11.67±0.90	58.62
	0.50	17.69±1.22	68.31	14.67±0.49	62.61	6.33±0.32	77.55
Coumarin	0.25	32.80±1.48	41.25	23.03±1.11	41.29	14.03±0.52	50.25
	0.50	29.91±0.73	46.43	18.80±0.81	52.08	12.60±1.21	55.32
LSD 5%		4.83	-	NS	-	3.65	-

SE: Standard error, LSD: Least significant difference at $p<0.05$ and NS: Not significant

Table 2: Effect of spraying pea plants with different organic acids and coumarin on crop parameters under field conditions (Mean±SE)

		Average of crop parameters							
Treatments	Concentration (%)	Plant height	Number of branches/plant	Number of pods/plant	Weight of pod (g)	100-seed weight (g)	Length of pod (cm)	Seed yield weight/plot (kg)	Seed yield (t/fed)
Control	0	64.00±1.57	8.50±1.21	10.33±0.88	7.20±0.81	46.00±1.15	9.30±0.42	8.80±0.49	3.91±0.15
Salicylic acid	0.25	73.40±0.83	15.50±1.37	11.33±0.88	9.80±0.62	48.80±1.37	11.20±0.47	11.00±0.60	4.22±0.24
	0.5	77.50±2.25	21.50±1.27	18.00±1.17	11.50±0.93	69.20±1.42	12.30±0.47	12.30±0.44	4.58±0.31
Sorbic acid	0.25	95.00±1.66	27.00±0.64	16.00±0.58	9.80±0.64	51.00±1.00	9.70±0.61	9.50±0.36	4.89±0.13
	0.5	102.0±1.25	35.33±1.30	25.67±1.54	7.50±0.66	53.60±1.59	10.10±0.49	10.30±0.69	5.10±0.34
Citric acid	0.25	95.30±1.24	11.33±1.45	12.33±0.88	10.30±0.93	54.80±0.62	11.00±0.35	11.50±0.75	5.11±0.23
	0.5	97.20±1.22	25.00±1.57	20.00±1.15	11.50±1.14	60.80±1.17	12.50±0.76	12.00±0.87	5.47±0.15
Boric acid	0.25	65.33±1.68	17.33±0.99	13.33±0.88	8.10±0.44	43.50±1.32	9.50±0.32	9.70±0.59	4.31±0.23
	0.5	75.67±1.59	32.00±1.49	22.67±0.88	10.20±0.66	48.00±1.01	11.33±0.96	10.80±0.38	4.80±0.20
Benzoic acid	0.25	66.67±1.30	9.67±0.96	8.50±0.67	8.20±0.99	45.00±0.61	10.20±0.61	9.20±0.32	4.09±0.20
	0.5	75.67±7.78	19.00±0.85	13.00±1.00	9.00±0.64	51.60±1.17	11.33±0.38	10.00±0.51	4.44±0.30
Coumarin	0.25	80.00±2.11	21.50±1.32	19.50±1.72	9.70±0.79	52.80±1.61	12.00±0.74	10.90±0.64	4.85±0.16
	0.5	83.33±7.57	17.33±1.09	13.00±0.58	10.00±0.95	54.40±1.53	13.20±0.54	10.70±0.47	4.76±0.14
LSD 5%		11.37	4.69	3.63	NS	NS	NS	2.11	0.79

SE: Standard error, LSD 5%: Least significant difference at p<0.05 and NS: Not significant

Table 3: Effect of foliar spraying with different organic acids and coumarin on photosynthetic pigments of pea plants (Mean±SE)

Treatments	Concentration (%)	Chlorophyll a	Chlorophyll b	Carotenoids	Total pigments
Control		8.67±0.23	1.71±0.07	2.57±0.21	12.95±0.52
Salicylic acid	0.25	10.87±0.16	2.35±0.04	2.31±0.04	15.53±0.16
	0.50	12.30±0.07	4.63±0.20	2.20±0.02	19.13±0.28
Sorbic acid	0.25	10.61±0.45	1.70±0.11	2.78±0.27	15.09±0.83
	0.50	12.04±0.42	2.96±0.51	2.92±0.10	17.92±1.04
Citric acid	0.25	16.02±0.34	4.61±0.17	3.88±0.24	24.51±0.74
	0.50	17.60±0.57	4.49±0.22	4.01±0.01	27.01±0.36
Boric acid	0.25	9.70±0.11	3.96±0.04	2.80±0.07	16.46±0.08
	0.50	11.39±0.19	3.95±0.13	3.05±0.01	18.39±0.05
Benzoic acid	0.25	13.88±0.40	3.94±0.02	3.19±0.01	21.01±0.43
	0.50	14.63±0.20	4.14±0.09	4.20±0.01	22.97±0.30
Coumarin	0.25	9.47±0.29	2.31±0.11	3.48 ±0.11	15.26±0.29
	0.50	10.25±0.15	3.46±0.01	3.92±0.05	17.63±0.10
LSD 5%		1.12	0.62	0.60	1.93

SE: Standard error, LSD 5%: Least significant difference at p<0.05 and NS: Not significant

Changes in biochemical constituents: The changes in Total Soluble Sugar (TSS), Total Soluble Protein (TSP), proline, Free Amino Acid (FAA), total phenols, Indol Acetic Acid (IAA) and total antioxidants (DPPH %) were explored in Table 4.

It is clear that, all tested materials (chemical resistance inducers) (salicylic, sorbic, citric, boric, benzoic acids and coumarin) at both concentrations (0.25 and 0.50%) induced a significant (p<0.05) increase in all tested parameters compared with untreated plants. The highest value was observed in response to the highest concentration in general. It was observed that the maximum increases in the most biochemical constituents of pea plants were observed when the plants were sprayed with citric acid.

Effect of foliar spraying organic acids and coumarin on the anatomical characteristics of pea (*Pisum sativum* L.) leaves: Microscopically counts and measurements of specific histological characters in transverse sections through the blade of mature foliage leaf developed a the median portion of the leaflet of pea plant sprayed with 0.50% of citric acid and those of untreated plant is assumed in Table 5 and Fig. 1a-e.

As well, microphotographs explain these treatments are shown in the Fig. 1a-e. It is clear from Table 5 and Fig. 1b that, foliar application with citric acid at 0.50% recorded the highest increase in the thickness of upper and lower epidermal layers of pea leaflet by +92.08 and +70.38%, respectively, but foliar application with coumarin at the at 0.50% led to decrease in the thickness of the upper epidermal layer of pea leaflet by -6.59 and -15.31%, respectively less than the untreated plants. Also, the highest increase is recorded in the length and width of the vascular bundle by +50.82 and +52.83%, respectively more than the control (Fig. 1a) when a foliar application with citric acid at 0.50% (Fig. 1b), in this regard the length and width of vascular bundle of are slightly increased by +3.23 and 3.42%, respectively over the control when a foliar application with boric acid at 0.50% (Fig. 1c). Although the foliar application with coumarin at the same concentration led

Table 4: Effect of foliar spraying with different concentrations of organic acids and coumarin on some compatible solutes, total phenols and indoles of pea plants (Mean±SE)

Treatments	Concentration (%)	Compatible solutes (mg g^{-1} dry weight) TSS	TSP	Proline	FAA	Phenol (mg g^{-1} dry weight)	IAA (mg g^{-1} dry weight)	DPPH (%)
Control		291.92±4.88	189.20±11.22	76.79±0.50	965.76±17.60	123.21±3.99	28.72±0.58	29.85±1.01
Salicylic acid	0.25	345.74±2.50	209.84±14.77	98.45±0.11	1164.53±73.71	130.58±1.08	31.08±0.93	35.45±1.25
	0.50	423.48±3.13	224.89±1.31	116.33±0.95	1576.59±32.39	142.60±0.46	51.21±2.44	45.42±0.51
Sorbic acid	0.25	426.04±4.62	174.91±3.54	113.12±0.44	1760.87±0.94	159.07±4.28	36.16±0.67	38.62±2.43
	0.50	409.39±4.67	236.97±9.11	111.00±0.35	2124.71±84.92	155.02±0.17	43.46±0.35	35.75±1.81
Citric acid	0.25	299.73±3.60	169.06±1.58	86.26±0.02	1528.02±19.81	153.03±0.21	30.56±0.87	26.82±0.47
	0.50	373.86±5.60	244.35±1.86	127.31±0.29	2186.82±62.51	162.85±1.81	42.75±2.02	48.04±1.45
Boric acid	0.25	233.91±8.66	162.71±1.18	96.98±0.58	1134.82±16.05	146.62±1.96	35.89±1.10	27.50±0.28
	0.50	368.03±3.18	206.91±9.19	96.71±0.48	1729.81±31.05	156.28±0.71	41.94±4.76	39.88±1.54
Benzoic acid	0.25	314.81±1.76	156.80±0.70	88.97±0.01	1884.09±12.38	160.41±6.57	28.14±0.58	27.37±0.55
	0.50	440.99±8.46	187.07±8.24	105.39±0.25	2100.28±46.53	180.12±3.93	46.67±0.56	30.58±1.07
Coumarin	0.25	257.54±5.94	174.47±0.06	81.47±0.85	1287.90±60.59	153.45±2.45	35.21±0.57	25.13±0.08
	0.50	330.16±4.88	251.31±2.63	94.75±0.53	1404.73±6.15	157.87±4.47	39.47±0.99	46.44±1.35
LSD 5%		89.98	20.10	1.91	210.20	13.06	5.74	4.65

SE: Standard error, LSD 5%: Least significant difference at p<0.05, TSS: Total soluble sugar, TSP: Total soluble protein, FAA: Free amino acid, IAA: Indol acetic acid and DPPH: Total antioxidants (DPPH)

Table 5: Effect of foliar spraying of organic acids and coumarin on the anatomical characteristics in pea plant leaves

	Characters							
	Epidermis thickness (µm)				Vascular bundle thickness (µm)			
	Upper epidermal layer (µm)		Lower epidermal layer (µm)		Length (µm)		Width (µm)	
Treatments	Absolute value	%±of control	Absolute value	%±of control	Absolute value	%±of control	Absolute value	%±of control
Control	14.27	0.00	13.71	0.00	244.61	0.00	192.340	
Salicylic acid	18.36	+28.66	16.9	+23.26	258.23	+5.56	194.97	+1.36
Sorbic acid	22.76	+59.49	21.2	+54.63	247.87	+1.33	195.51	+1.64
Citric acid	27.41	+92.08	23.36	+70.38	368.95	+50.83	293.98	+52.84
Boric acid	17.46	+22.35	19.66	+43.39	252.52	+3.23	198.93	+3.42
Benzoic acid	20.76	+45.48	17.41	+26.98	260.77	+6.6	199.18	+3.55
Coumarin	13.33	-6.59	11.61	-15.31	130.63	-46.59	188.48	-2.06

	Characters					
	Mesophyllic tissue (µm)					
	Palisade tissue		Spongy tissue		Midrib zone thickness (µm)	
Treatments	Absolute value	%±of control	Absolute value	%±of control	Absolute value (µm)	%±of control
Control	57.35	0.00	136.59	0.00	410.03	0.00
Salicylic acid	98.1	+71.05	222.67	+63.02	419.87	+2.39
Sorbic acid	105.6	+84.13	235.04	+72.07	549.76	+34.07
Citric acid	128.65	+124.32	289.73	+112.11	632.09	+54.15
Boric acid	88.08	+53.58	219.4	+60.62	477.68	+16.49
Benzoic acid	95.41	+66.36	227.29	+66.4	546.66	+33.32
Coumarin	51.64	-9.95	128.61	-5.84	402.15	-1.92

to a sharp decrement in the length of the vascular bundle by-46.59%, the led to a slight reduction in the width of the vascular bundle by -2.06% (Fig. 1d) less than the untreated plants (Fig. 1a). The alteration occurred on the thickness of leaflet blade either increasing or reduction corresponding to changes in the thickness of both of upper and lower epidermal layers, palisade and spongy tissues. In Table 5 and Fig.1b, it was clear that foliar application with citric acid at 0.50% recorded the highest increase in the thickness of mesophyllic tissue either palisade or spongy tissue by +124.32 and +112.11%, respectively more than the untreated plants (Fig. 1a). But the foliar application with coumarin at 0.50% recorded the decrement in the thickness of palisade and spongy tissues by -9.95 and -5.84%, respectively less than the untreated plants.

Although the foliar application with sorbic acid at 0.50% (Fig. 1e) led to an increase in the thickness of the midrib zone by +34.07% more than the control (Fig. 1a).

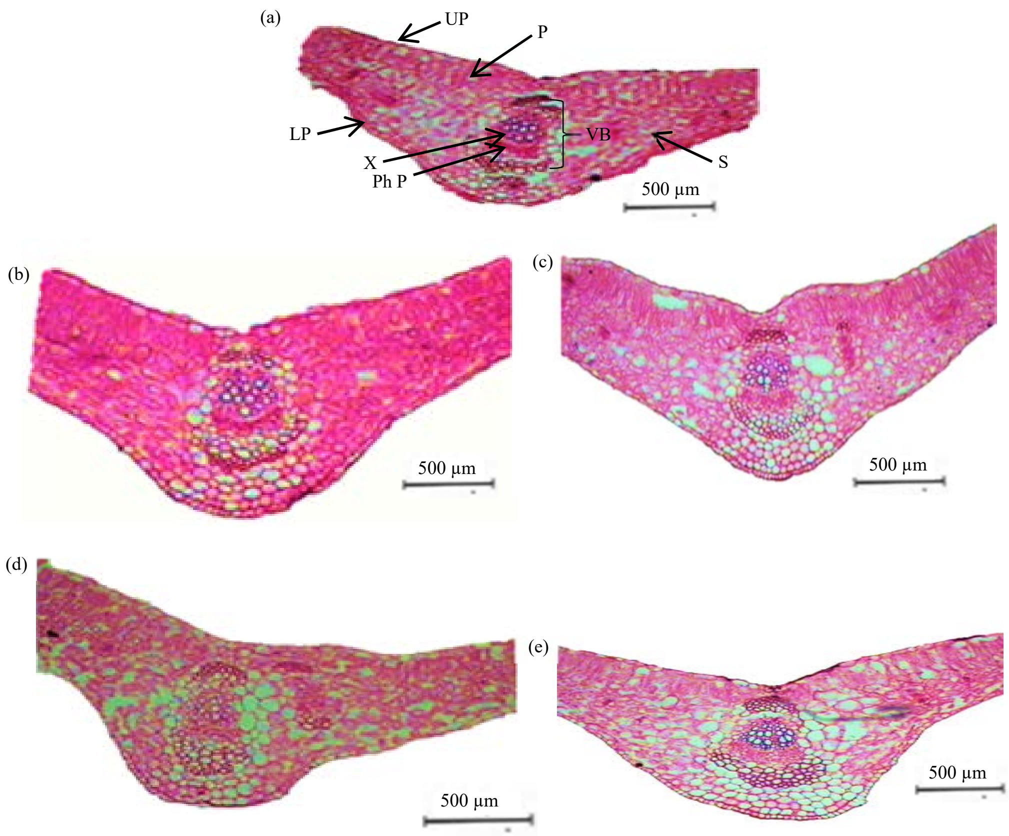

Fig. 1(a-e): Changes in transverse sections of the terminal leaflet blade of the fourth upper compound leaf on the mainstem of pea (*Pisum sativum* L.) plants grown under downy mildew, powdery mildew and rust diseases and application of the studied organic acids and coumarin at 0.50%, (a) Untreated plant leaves, (b) Plant leaves treated with citric acid, (c) Plant leaves treated with boric acid, (d) Plant leaves treated with coumarin and (e) Plant leaves treated with sorbic acid

L: Lower epidermis, P: Palisade tissue, Ph: Phloem, S: Spongy tissue, U: Upper epidermis, Vb: Vascular bundle, X: Xylem vessels

DISCUSSION

In Egypt, it is necessary to note that the conditions of the climate in these periods were characterized by high humidity and low temperatures which prevailed causing the emergence of fungal diseases on pea plants. During the experimentation season temperature and humidity were in ranges of 13-16°C and 69-74%, respectively and rainfall recorded 23.1-59.2 mm in the same season. Ash *et al.*[39] showed that in early fall and late summer, under favourable weather conditions, humid nights, suitably warm, this leads to the emergence of fungal diseases.

Pea (*Pisum sativum* L.) was attacked by many foliar diseases. Downy mildew, powdery mildew and rust are the most common of these diseases. To control these diseases, certain chemical inducers have been utilized. Among the all tested treatments, citric and benzoic acids (Table 1) recorded the highest reduction in diseases severity for all tested diseases followed by salicylic and sorbic acids, respectively while coumarin recorded the lowest effect. The highest reduction of downy mildew was obtained with citric and benzoic acids at 0.5% concentration, while coumarin recorded the lowest reduction at the same concentration. Meanwhile, other treatments showed a moderate effect. The same results were obtained for the two other diseases with some differences in the reading values. In the present study, the efficiency of used chemical inducers could be attributed to their dynamic action in enhancing resistance and eliciting natural immunity of the host plants that may promote their defence mechanisms to fight the pathogens[40-42]. In this regard, various studies reported that salicylic acid proved to have stimulation activity in resisting disease. It has been reported that foliar application of cotton plants with potassium citrate and salicylic acid induced a significant rise in plant development and yield advantages as well as augmentation of the leaf chemical composition[43]. Moreover, Dalie *et al.*[44] found that organic acids affected the plasmic membrane throughout changing its electrochemical properties thus increasing its permeability. With low pH values, a maximal concentration of protons is resulted and cause the augmentation in acid spreading across the plasmic membrane and cytoplasm. The results obtained herein are in agreement with Khalil *et al.*[45], who explained that salicylic acid, potassium sorbate and ascorbic acid spraying treatments on pea plants suppressed the incidence of disease by about 12.4 and 17.1%. Also, Elgamal and Khalil[26] studied that the effect of the foliar spray against powdery mildew on luffa *in vitro* and under field conditions with organic acids and essential oils, the results showed that all treatments significantly reduced powdery mildew of luffa caused by *P. xanthii* compared to untreated plants. In this regard, Ismail and Afifiorcid[46] indicated that use some organic acids for control of rust disease on a bean plant. They added that salicylic, citric and fulvic acids were the most effective treatments in the reduction of rust under field conditions. It also led to an increase in growth parameters, total carbohydrates, chlorophyll and protein in bean plants over control. Fulvic, citric and salicylic acids were the most effective treatments in this regard. On the other hand, the highest activities of defence-related enzymes i.e., peroxidase, polyphenol oxidase and catalase were recorded in treated bean plants with organic acids.

All tested inducers gave a clear increase of crop parameters (Table 2). These results are in agreement with Ismail and Afifiorcid[46], who indicated that, use some organic acids for the control of rust disease on bean plants. Results showed that salicylic, citric and fulvic acids were the most effective treatments in the reduction of rust under field conditions. It also led to an increase in growth parameters, total carbohydrates, chlorophyll and protein in bean plants over control. On the other hand, the highest activities of defence-related enzymes i.e., peroxidase, polyphenol oxidase and catalase were recorded in treated bean plants with organic acids. Similar results were recorded by Ramadan *et al.*[47] on flax plants when treated with benzoic acid and coumarin and Abdallah *et al.*[48] on wheat plants using salicylic acid.

All photosynthetic pigments (Table 3) showed generally a significant increase in pea plants when treated with organic acids (salicylic, sorbic, citric, boric and benzoic) and coumarin. Abd El-Gawad and Bondok[17] found that total chlorophyll significantly decreased in leaves of infected tomato plants. They attributed the reduction in total chlorophyll of tomato leaves due to tomato mosaic virus infection to be a significance of the released transported toxins that induce the liberation of ROS[49]. Also, a high level of lipid peroxidation mediating cell damage in tomato tissues decreased its biomass and chlorophyll content[15]. The enhancement in these parameters of pigments induced by citric acid spraying is in line with Darandeh and Hadavi[50] since the foliar application of citric acid at 0.075 and 0.15% w/v increased the chlorophyll contents of Lilium plants. Also, Hu *et al.*[51] found that, foliar spraying of citric acid (0.2, 2 and 20 mM) ameliorate the adverse effect on chlorophyll content of heat-stressed tall fescue (*Lolium arundinaceum*) plants. Concerning the effect of salicylic acid on photosynthetic pigments, the maximum increase was observed in response to the highest concentration (0.50 mg L^{-1}). Vicente and Plasencia[52] stated that SA is an important regulator of photosynthesis due to its effects on leaf and chloroplast structure, stomata closure, chlorophyll and carotenoid contents. Khodary[53] attributed the increase in photosynthetic capacity effect, with SA treatments on *Zea maize* plants under salinity stress, to its stimulatory effects on Rubisco activity and pigment contents. Moreover, SA is an antioxidant compound intense in the chloroplast and reported to protect the photosynthetic apparatus in drought stress conditions throughout scavenging the excessive ROS[54]. The primitive influence photosynthetic pigments induced by benzoic acid treatments are in harmony with Anjum *et al.*[55] on soybean and Ramadan *et al.*[47] on flax plants. They explained that the improvement effect of benzoic acid on photosynthetic pigments resulted from the increase in gas exchange, stomatal conductance, transpiration and photosynthetic rates. Coumarin also affected photosynthetic pigments, Saleh *et al.*[56] on *Vicia faba* plants and Ramadan *et al.*[47] on flax plants found that application of coumarin significantly increased its content. Khairy and Roh[57] recorded a similar trend of improvement in chlorophyll contents of salinity stressed tobacco plants when treated with benzoic acid and p-coumaric acid. It is well established that boron has a direct role in photosynthesis as reviewed[58] and its deficiency reduces chlorophyll and soluble protein contents of leaves, which results in Hill reaction's inhibition and net photosynthetic rate[59].

It is clear that all tested materials (salicylic, sorbic, citric, boric, benzoic acids and coumarin) at both concentrations (0.25 and 0.50%) induced a significant increase in all determined parameters (Table 4) compared with untreated plants. Concerning the effect of salicylic acid on the tested biochemical constituents, similar results were shown by Abdallah *et al.*[48] on wheat plants who reported that plant resistance to stress improved by the significant increase in TSS, proline, FAA and phenolic contents. In this concern, Tawfik *et al*.[60] decided that increasing TSS, proline and free amino acids improved plant cells tolerance to salinity stress throughout increasing osmotic pressure in the cytoplasm as well as relative water contents essential for plant growth. In addition, Ashraf and Foolad[61] stated that proline induces vital roles in preventing damaging effects of osmotic stresses expressed in forms of osmotic adjustment, stabilization and protection of enzymes, proteins and membranes. Also, Ahmadi *et al*.[62] stated that proline content increased in grape buds under cold stress when treated with SA. In this concern, Jagesh *et al*.[63] demonstrated that the osmotic adjustment in stressed plants resulted from the accumulation of high concentrations of osmotic compatible solutes, e.g., proline, glycinebetaine, soluble sugars and free AA. They also noticed that these substances express their important role in the adaptation of cells to various adverse environmental conditions via raising cytoplasm's osmotic pressure, stabilizing proteins and membranes and maintaining the relatively high-water content obligatory for plant growth and cellular functions. Amino acids are primary metabolites that play essential roles in plant immunity against a wide range of pathogens. The variation in plant tissues amino acid quantity may determine the chance of environment for the pathogenic attackers like fungi, bacteria and viruses. This results finally either in strengthening plant defence to resist pathogenic attack effectively or surrender before vigorous infection[64]. The defences to biotic stress include morphological and structural barriers, chemical compounds and proteins and enzymes. These confer tolerance or resistance to biotic stresses by protecting products and by giving them strength and rigidity. The improvement effect of the used tested inducers on pea growth parameters (Table 2) may be attributed to the enhancement in the biosynthesis of photosynthetic pigments (Table 3) and/or its antioxidant effect (Table 4). These results are in the line with those recorded on maize using SA[65] and Anjum *et al*.[55] on soybean using benzoic acid. Ramadan *et al*.[47] found an increase in total soluble sugar, total soluble protein, proline, free amino acids, total phenols, IAA contents and DPPH% of flax plants when sprayed with benzoic acid or coumarin. Also, they added that the increase in endogenous IAA content due to foliar spraying of the same materials resulted in enhancement of growth rate which stimulate cell division and/or enlargement[21]. In addition, amino acids play a vital role in enhancing secondary metabolites which alleviated the harmful effects of stress on plants[66]. Khairy *et al*.[57] found that osmoprotectants (total soluble and insoluble sugar, totally soluble and insoluble protein) and antioxidant (total phenols) of *Vicia faba* plant' leaves significantly increased with coumarin application.

The results in Table 5 and Fig. 1a-e indicated that foliar application with 0.50% citric acid on pea plants led to the highest increase in the thickness of upper, lower epidermal layers, length and width of the vascular bundle and mesophyllic tissue either palisade or spongy tissue. Also, foliar application with boric acid at 0.50% produced a slight increase in the length and width of the vascular bundle more than the control. Although the foliar application with coumarin at the same concentration led to a decrease in the thickness of the upper epidermal layer and a sharp decrement in the length of the vascular bundle, a slight reduction in the width of the vascular bundle was recorded compared to the untreated plants. In this regard, Ali *et al*.[67] on maize (*Zea mays* L.) reported that treatment with citric acid led to increasing upper and lower epidermal layers, length of the vascular bundle and mesophyll tissue, but the width of the vascular bundle was similar to the control. Concerning foliar application with salicylic acid, the results showed an increase in thickness in both palisade and spongy tissues. Moreover, the foliar application with sorbic acid at the same concentration led to an increase in the thickness of the midrib zone more than the control. These results are in agreement with Gomaa *et al*.[18] who reported that foliar spraying with salicylic acid increased the thickness of all studied layers containing both palisade and spongy tissues. Some investigators confirmed the present findings using salicylic acid on other field crop plants, for instance, Farouk and Osman[68] on *Phaseolus vulgaris* L.; Cárcamo *et al*.[69] on *Zea mays* L., Nour *et al*.[70] on bean and Gomaa *et al*.[18] on *Lupinus termis* L. They found that salicylic acid application increased the thickness of the midvein and lamina of leaves.

CONCLUSION

The tested organic acids and coumarin at 0.25 and 0.50% are highly recommended to be applied twice for promoting vegetative growth, increasing productivity from pod and seeds, based on induced favourable changes in anatomical structures and endogenous secondary metabolites against powdery mildew, downy mildew and rust diseases that occurrence on pea plants under natural field conditions. The highest concentration (0.5%) of all tested materials were the most effective.

SIGNIFICANCE STATEMENT

From environmental and human health points of view, natural chemical inducers (natural organic antioxidants and phenolic compounds) proved to be of a great impact compared to pesticides which threaten globalization. The plant researchers should focus on the use of biological resources such as salicylic, sorbic, citric, boric, benzoic acids and coumarin to avoid the adverse effects of using other chemicals on the quantity and quality of the produced plants. This study represents guidance for researchers to uncover the critical areas of the positive impact for using similar substances which not only protect the plants from air-born diseases but also improve the morphological, physiological and anatomical structure. Thus, a natural material application for plant nutrition produce more healthy plants (resistant to biotic stresses and metabolic disorders).

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