https://dx.doi.org/10.4314/ejppri.v7i4.1

Egypt. J. Plant Prot. Res. Inst. (2024), 7 (4): 436 –447

وزارة الزراعة واستصلاح الأراضي
Ministry of Agriculture and Land Reclamation
مركز البحوث الزراعية
Agricultural Research Center

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

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Efficacy of foliar spraying of squash (*Cucurbita pepo*) with some elicitors for certain biochemical traits for *Bemisia tabaci* (Hemiptera: Aleyrodidae) protection

Yomna, N. M. Abd Allah; Lobna, R. Amin; Entesar, N. Haron and Tarek, R. Amin

Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

ARTICLE INFO

Article History

Received:1 /10/2024

Accepted:28 /11/2024

Keywords

Cucurbita pepo, *Bemisia tabaci*, elicitors, foliar spraying, biochemical traits and enzymes activity.

Abstract

Secondary metabolites in plants are bioactive scaffolds that are crucial for plant growth and survival in the environment. Also, to maintain a defence mechanism from pests. Four basic elicitors, (Benzoic acid, salicylic acid and acetyl salicylic (Aspirin) acid) at the rate of approximately 0.01 mol/l of each (i.e. 1.25, 1.4 and 1.8 g/l, respectively) and sulfonic acid was used at the rate of 1 % of product (Potassium hydroxide 0.6 g/l was added as a dissolver to sulfonic acid) were applied on squash plants *Cucurbita pepo* L. as a foliar treatments during vegetative growth stage at 15 days after planting in the open field to reduce the whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) infestation. The foliar application treatments of elicitors or inducers (Were applied at 15th after cultivation as one spray weekly for 3 weeks) over two successive growing seasons; 2022 and 2023 in autumn plantation. The effects of the four elicitors were close to each other in reducing *B. tabaci* numbers over the tested seasons compared to control treatments. The change in certain biochemical traits of the sprayed leaves particularly in different plant stages was successfully investigated. In the seedling or early stage (Represented by the period from seedling to 30 days after cultivation but leaves were taken for analysis after spraying at 21 days after cultivation), it was found in sprayed leaves that sulfonic, salicylic and benzoic acids treatments increased phosphorous versus control. Moreover, benzoic acid and sulfonic acid increased levels of total carbohydrates compared to those found in the control. However, salicylic, acetylsalicylic and sulfonic acids increased total phenols versus control. Sulfonic, acetylsalicylic, benzoic and salicylic acids increased titratable acidity. Sulfonic acid and benzoic acid increased the activity of alpha esterases. Also, sulfonic acid increased beta esterases activity versus control. Salicylic, acetylsalicylic and sulfonic acids increased peroxidase activity. In the late stage of plant (44 days after cultivation), data revealed that acetylsalicylic acid increased phosphorous content compared to control. Moreover, sulfonic, acetylsalicylic, salicylic and benzoic acids increased titratable acidity. While salicylic acid and sulfonic acid increased the activity of the peroxidase enzymes compared to control. This approach shed light on the efficiency of benzoic acid, salicylic acid, acetylsalicylic acid and sulfonic acid in reducing *B. tabaci* infestation via enhancement secondary metabolites and increasing defensive mechanisms of squash plants. In conclusion, these elicitors could be recommended to be used in combating the whitefly, *B. tabaci* in integrated pest management (IPM).

Introduction

The whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) has been considered one of the world's top 100 invasive species (International Union for the Conversation of Nature and Natural Resources (IUCN) list (http://www.issg.org). It is one of the most devastating, tropical and sub-tropical pests attacking agriculture crops and it affects the yield of most crops that it attacks (Wang *et al*., 2016).

Vegetable plants are exploited in a high range worldwide, but they attract a variety of pests and diseases and therefore they are found on the threatened list. *B. tabaci* has striking features such as inducing many different physiological changes to its host plants one of those is silverleaf in summer squash (Masuda *et al*., 2016).

Because of its unique ability to induce silverleaf disorders in squash, it is called a silverleaf whitefly. This disorder (Squash silverleaf) is economically one of the most important diseases to squash; *Cucurbita pepo* L. transmitted by *B. tabaci* around the world (Razze *et al.,* 2016).

Many pesticides have been used so far for pest control, but their indiscriminate use leads to resistance phenomenon, an increase in environmental pollution and disturbance of the natural ecological balance (El-Sherbeni *et al*., 2019). Plant defense may play an important role in the interaction of whiteflies with plants and viruses (Shi *et al*., 2016). Alternative eco-friendly approaches to pesticides which do not harm non-target organisms and increase plant resistance are demanded. Phenolic compounds are known as strong antioxidants that can scavenge free radicals. Salicylic acid has enhanced plant resistance against whitefly and thus reduced pest damage (Haider *et al*. 2023). Benzoic acid has protective roles against different environmental stresses such as heat, drought and chilling stress (Senaratna *et al.*, 2003 and Williams *et al.,* 2003). Tak *et al.* (2006) tested benzoic acid against adults of *Tyrophagus putrescentiae* (Schrank) (Sarcoptiformes: Acaridae) as acaricidal agents using direct contact toxicity bioassay. Exogenous application of phenolic compounds may have a significant practical application in agriculture and horticulture (Senaratna *et al*., 2000).

The present work hypothesized that benzoic, salicylic, acetyl salicylic and sulfonic acids as inducers for plant resistance, can be used in integrated pest management (IPM).

Materials and methods

The efficiency of four basic phenolic elicitors (i.e. Acetylsalicylic acid (Aspirin), benzoic acid, and salicylic acid) was obtained locally from El Nasr Company for Pharma-Ceutical Company, Cairo, Egypt, with a minimum of 99% purity and sulfonic acid was obtained locally and it was evaluated for reducing *B. tabaci* population on squash plants. Acetylsalicylic acid, benzoic acid, and salicylic acid were used at the rate of 1.8, 1.25, and 1.4 g/l, respectively to present approximately 0.01 mol/l of each compound, however, sulfonic acid was used at the rate of 1 % of product then chemical analysis of dry squash leaves was successfully conducted. These rates were selected to provide the same molecular amount per treatment. Potassium hydroxide at the rate of 0.6 g/l was added as a dissolver. These phenolic acids require an alkaline medium to be soluble in water. Tested phenolic acids and potassium hydroxide were mixed with water just before application. Squash seed eskandarani variety was sown on Oct.20th in both seasons. Field experiments were conducted in the Experimental Farm of Plant Protection Research Institute at Qaha region, southwest of Qalyubiya in Egypt over two successive growing seasons, 2022 and 2023 during autumn season. An area of about 525 m^2. Rows designed as 7 plants per row spaced

0.30 m apart. Social and agronomical practices of squash cultivation were performed consistently.

1. Application and sampling technique procedure:

The application was conducted by foliar spraying the plants weekly three times during the vegetative growth stage only. The application with the four tested elicitors started 15, 21 and 30 days after cultivation compared to control plots. The application was conducted using a knapsack sprayer with one nozzle at the rate of 200 L/4200 m^2. Thirty leaves were randomly picked up from each plot (Each one represented by 4 replicates) 15 days after the sowing date till the end of the season. Leaf samples were examined by a binocular stereomicroscope.

2. Chemical analysis of fresh squash leaves:

Leaf samples were collected from the two stages, the first was seedling stage or early stage (Represented by the period from seedling to 30 days after cultivation), but sampling of young, sprayed leaves was successfully taken for analysis 21 days after cultivation after one week from spraying in the vegetative growth stage. The second or later stage (The interfering between flowering and fruiting stage) were represented 44 days after cultivation. The late-stage leaves of squash were taken after 44 days from cultivation for analysis. Phytochemical analysis was conducted during the 2023 season.

2.1. Plant sample preparation:

The plant samples (Weighing 0.1-0.4 g) were weighed and stored at -20 ˚C and then processed as described in Ni *et al.* (2001). Briefly, the enzymes from the frozen plant samples were extracted using cold potassium phosphate buffer (0.1M, pH 7.0) contain 1 % (w/v) polyvinylpyrrolidone and 1 % (v/v) Triton X-100. The samples were macerated with 1 ml of the extracting buffer. Samples were further ground with another 1 ml of the extracting buffer. In total,2 ml of the extracting buffer was used for each sample. An aliquot (1.5 ml) of the extract was centrifuged at 10 000 g for 10 min at 4˚C. The supernatant was immediately frozen for future enzyme activity assays.

2.2. Determination of total proteins:

The **Bradford (1976)** technique was used to calculate the total amount of proteins. The absorbance at 595 nm was measured after two minutes and before one hr. later, compared to a blank made with five milliliters of protein reagent and one milliliter of phosphate buffer. The reference protein was bovine serum albumin.

2.3. Determination of total carbohydrates:

Total carbohydrates were estimated by the phenol sulphuric acid reaction of Dubois *et al*. (1956). Fill a boiling tube with one hundred milligrams of the plant specimen by weight. After adding 10 ml of 2.5 N HCl, hydrolyze by holding it in a boiling water bath for three hours, then cool at ambient temperature. Use sodium carbonate to neutralize it and bring it to effervescence. To prepare the supernatant for analysis, centrifuge and gather it (Sadasivam and Manickam, 1992). 0.5 ml of 20% w/v phenol was added to 100 microliters of the extract in a colorimetric tube. Next, quickly while shaking, 5 ml of concentrated sulfuric acid was added. At 490 nm, the distinctive orange-yellow hue absorbance is quantified in relation to blank (Distilled water).

2.4. Determination of phenols:

Extraction was performed as described by Kähkönen *et al*. (1999). Using an electric homogenizer, two batches of ten milliliters of 80% aqueous methanol were extracted from five grams of grounded plant seedlings. After centrifuging the samples for 10 mins at 3000 rpm, the mixed extracts were transferred into tiny conical flasks that had been previously weighed. There was less pressure used to extract the methanol. Weighing the solid residue (crude extract), 5 ml of ∆ H2O was added to dissolve it. The amount of total phenolics in extracts was determined by Folin – Ciocateu method as modified by Singleton and Rossi (1965). Two hundred microliters of plant extracts were introduced into test tubes; 1 ml of Folin- Ciocalteu reagent and 0.8 ml of sodium carbonate (7.5%) were added. Absorption at 760 nm was measured against a blank containing everything except the sample. Gallic acid standard (5 gm%) was used.

2.5. Determination of nitrogen (N):

The protein nitrogen is transformed during digestion by H_2SO_4 into ammonium sulphate. After being steam-distilled, this salt releases ammonia, which is gathered in a solution of boric acid and titrated against standard acid. The nitrogen content of the sample is determined by computation, as 1 milliliter of 0.1 N acid is equal to 1.4 milligrams of N. (Sadasivam and Manickam, 1991).

2.6. Inorganic Phosphorus (P) determination:

With the aid of an industrial kit from Spain's Quimica Clinica applicada S.A, the phosphate ion was found. P and molybdate combine to form phosphor-molybdate, which is then reduced to a molybdenum blue that may be detected by photometry at 650 nm. Results were achieved by comparing the acquired zero adjustment against the reagent blank to a reference standard (Conc. 4 mg %).

2.7. Potassium (K) determination:

On a radiometer FLM3 flame photometer, measurements of ions were performed. Potassium chloride (5±0.5 mmol/L) and 14±1.4 mmol/L of sodium chloride were kept at ambient temperature (25 °C) in the standard solution. 500 ml of distilled water were mixed with 5 ml of concentrated lithium chloride (300 ±5 mmol/L) to create a blank for zero adjustment (Chapman and Pratt, 1961).

2.8. Determination of Titratable acidity:

Estimation of acidity (%) was conducted by using standard AOAC method (Association of Official Agricultural Chemists). Where 50 ml of distilled water was added to 10 ml fruit juice sample. Then 3-4 drops of phenolphthalein indicator were added, and it was titrated against 0.1N sodium hydroxide. The following formula was used to calculate the percentage acidity (Ranganna, 2001). %acidity= Titre value x Equivalent weight of acid x 100 / volume of sample taken x1000.

2.9. Estimation of certain enzyme activities:

Nonspecific esterases: Alpha esterases (α-esterases) and beta esterases (β-esterases) were determined according to Van Asperen (1962) using α-naphthyl acetate or β-naphthyl acetate as substrates, respectively. Quantitative Determination of Peroxidase: Peroxidase activity was determined according to the procedure given by Hammerschmidt *et al.* (1982).

3. Statistical analysis of data:

The effects of tested elicitors compared with the control were elucidated by one-way analysis of variance (ANOVA) by using two ANOVA in SAS 9.1 (Anonymous, 2003). All tests were considered significant at $p < 0.05$ and all the means were represented along with their standard errors (±SE). The collected values were pooled from three replicates. Duncan's multiple range tests confirmed the relevance of variable treatments.

Results and discussion

1. Effect of applying different elicitors against *Bemisia tabaci* infestation:

1.1. Effect on *Bemisia tabaci* oviposition preference and nymphal infestation in 2022 season:

In general, the control plots showed oviposition preference to squash plants versus plots sprayed with elicitors. The data graphically illustrated in Figure (1) revealing that, *B. tabaci* eggs/squash leaf were significantly affected by the tested elicitors compared to control in 2022 season. The observed pattern in plots treated with benzoic acid and acetylsalicylic acid harbored moderate infestation, and the mean number reduced to (37±16.9) and (39.1± 20.1), respectively. However, salicylic acid reduced egg numbers to (25.2± 6.8) and sulfonic acid to (24.6 ± 7.4) versus control (72.9 ±33.3) (Mean±SD) (*P*=0.0001) (F=40.39) (LSD=9.3). The nymphal infestation in the same year showed a different pattern, there were no significant differences among the tested elicitors in reducing *B. tabaci* nymphal infestation (*P*< 0.05), however, there was a significant difference between treated plots and control. The highest infestation rate of *B. tabaci* nymphs / squash leaf was estimated in the control plots (12.59± 4.04) (*P*=0.001) (F=7.57). In the treated plots the tested elicitors reduced infestation to (7± 2.03), 6.3± 1.5), (5.2± 1.1) and (4.6± 1.3) with acetylsalicylic acid, salicylic acid, benzoic acid and sulfonic acid, respectively, versus control (LSD=3.6).

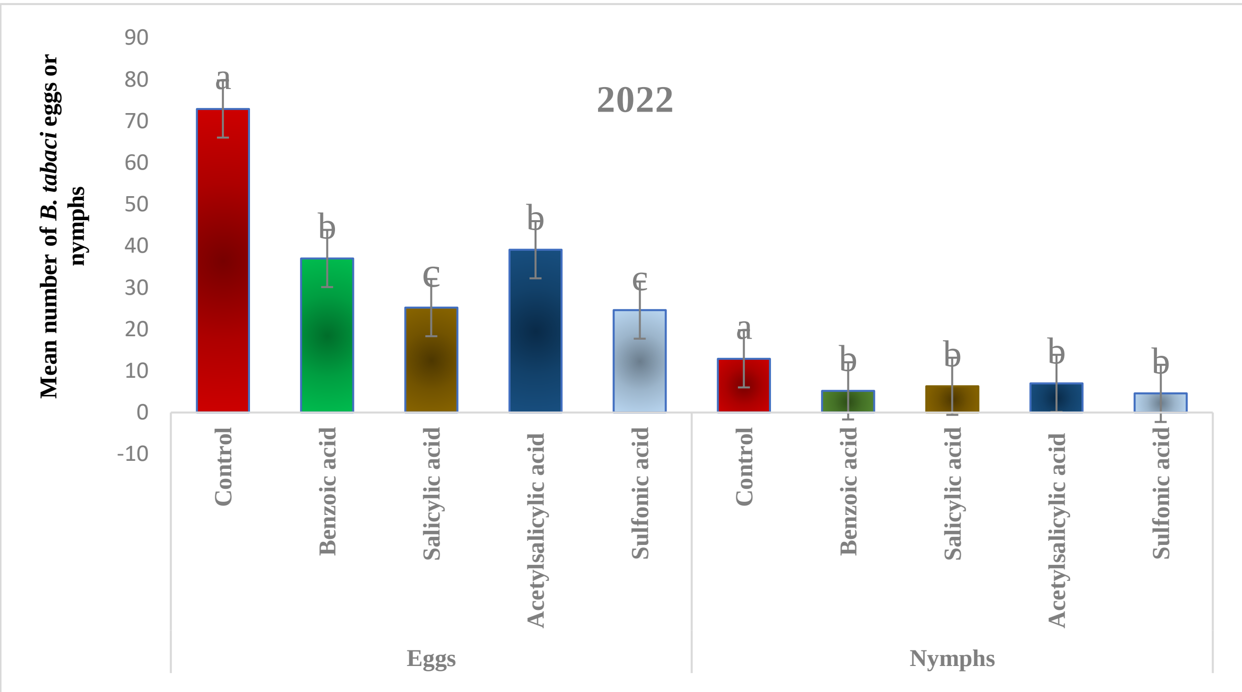

Figure (1): Mean seasonal counts (±SE) of *Bemisia tabaci* eggs and nymphs per leaf of squash treated with four elicitors versus control squash leaves over 2022 season. Bars topped with different letters are significantly different by Duncan's multiple range test (*P*< 0.05).

1.2. Effect on *Bemisia tabaci* oviposition preference and nymphal infestation in 2023 season:

The illustrated data in Figure (2) revealed that the infestation rate with *B. tabaci* increased in the second season than the first one. The highest means of depositing *B. tabaci* eggs /squash leaf was recorded in check or control (195.6± 42.8) (*P*=0.0001). However, treated plots with tested elicitors were less preferred to depositing *B. tabaci* eggs, acetylsalicylic acid reduced egg numbers to 154.5± 34.3, followed by salicylic acid 126.9 ±32.6 ([LSD= 34.87). On the other hand, the egg numbers in treated plots with benzoic acid and sulfonic acid were in the lowest category. Benzoic acid recorded 92.6± 23.25 and sulfonic acid reduced the deposited egg numbers to the lowest rate in this season with 82.9± 13.8 (F=15.93). The nymph population in this season had similar patterns as the previous one and the whitefly nymphs reduced in plots treated with benzoic acid (20.7 ± 6.9) or acetylsalicylic acid (20.8 ± 5.22) or salicylic acid (15.5± 4.1) and the last one ranked in the last group which had the lowest nymphal population across the sampling period (LSD= 17.2) (F= 1.57).

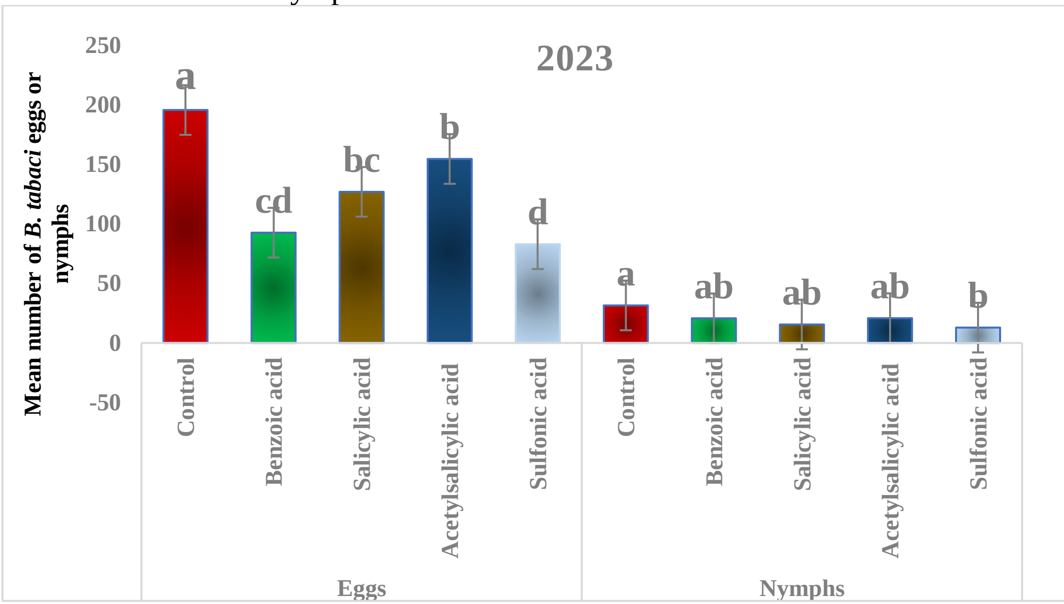

Figure (2): Mean seasonal counts (±SE) of *Bemisia tabaci* eggs and nymph of squash of squash treated with four elicitors versus control squash leaves over 2023 season. Bars topped with different letters are significantly different

2. Change in chemical constituents and enzymes activity over plant growth stages:

To clarify, if foliar spraying of different elicitors influenced chemical constituents in squash which can act as defense mechanism against *B. tabaci* population. Estimation of certain biochemical constituents and enzymes activity during vegetative squash growth stages was demanded.

2.1. Early stage:

The results obtained in Table (1) revealed the effect of tested elicitors on certain phytochemical constituents and enzymes activity in the early stage (Seedling stage). The total protein in the squash leaves is not affected by the tested elicitors except benzoic acid reduced total protein in the tested leaves (11.4 mg/g fresh weight) versus control (12.2 mg/g fresh wt.). Besides, the tested elicitors didn't change the nitrogen content in the squash leaves. However, a significant difference at $P<0.05$ in phosphorous content was found. The foliar spraying with benzoic acid, salicylic acid and sulfonic acid increased the phosphorous contents in sprayed leaves (0.58, 0.59 and 0.61 mg/g fresh wt., respectively) and the lowest content was estimated in leaves of acetylsalicylic acid (0.49 mg/g fresh weight) versus control (Table 1). There was no significant difference between potassium content in control leaves 2.5 mg/g fresh wt. and treated leaves with salicylic acid (2.5 mg/g fresh weight) or sulfonic acid (2.4 mg/g fresh weight) or benzoic acid (2.3 mg/g fresh wt.), these elicitors didn't change potassium content. However, acetylsalicylic acid significantly reduced potassium content (2.2 mg/g fresh weight) LSD=0.2. Benzoic acid and sulfonic acid increased total carbohydrates (21.9 and 21.6 mg/g fresh wt., respectively) versus control (17.7 mg/g fresh weight). On the other hand, the salicylic acid and acetylsalicylic acid didn't affect carbohydrate content in squash versus control (Table 1). Concerning the antioxidant, phenol, it was found that salicylic acid, acetylsalicylic acid and sulfonic acid increased to 1.8, 1.7 and 1.86 mg/g fresh wt., respectively versus control (1.3mg/g fresh weight). Benzoic acid didn't change phenol content in this stage. Concerning titratable acidity, acetylsalicylic acid and sulfonic acid increased it as 67.2 and 68.5 ug NaOH /gm fresh weight, respectively, followed by benzoic acid and salicylic acid 57.6 and 53.1 ug NaOH /gm fresh weight, respectively, versus control 47.1 ug NaOH /gm fresh weight. The activity of alpha esterases was affected with spraying of sulfonic acid which increased these enzymes to high rate (99.4 ug α- naphthol/min /gm fresh weight) followed by benzoic acid (82.4 ug α- naphthol/min /gm fresh weight) versus 68.1 ug α- naphthol/min /gm fresh weight in control. On the other hand, salicylic acid and acetylsalicylic acid didn't influence the activity of alpha esterases versus control. Besides, sulfonic acid increased beta esterases in squash leaves 32.5 ug β-naphthol/min /gm fresh weight versus 26.9 ug β-naphthol/min /gm fresh wt. in control. However, acetylsalicylic acid decreased the activity of beta esterases (22.5 ug β-naphthol/min /gm fresh weight) versus control. However, acetylsalicylic acid decreased the activity of beta esterases (22.5 ug β-naphthol/min /gm fresh weight) as compared with the control. Salicylic acid and acetylsalicylic acid highly affected the activity of peroxidase enzymes 387 and 381 ∆ O.D./min./ gm fresh weight, respectively, followed by sulfonic acid 342 ∆ O.D./min./gm fresh weight versus 296∆ O.D./min./ gm fresh weight in control. However, benzoic acid didn't affect the peroxidase activity versus control (279 ∆ O.D./min./ gm fresh weight).

2.2. Late stage:

The results presented in Table (2)indicated the effect of tested elicitors on certain chemical squash elements and enzymes activity in the late stage. In this stage, the tested elicitors didn't affect the total protein or nitrogen content. Acetylsalicylic acid increased phosphorous content (0.63 mg/g fresh wt.) versus control (0.55 mg/g fresh weight). However, salicylic acid reduced phosphorous to the lowest level in sprayed leaves (0.45 mg/g fresh wt.) as compared with control treatment (Table 2). Benzoic acid and sulfonic acid decreased potassium contents in sprayed leaves (1.71 and 1.66 mg/g fresh weight, respectively) compared with 1.98 mg/g fresh weight in control treatment. There wasn't significant difference between control and either salicylic acid or acetylsalicylic acid (Table 2). However, benzoic acid, salicylic acid and sulfonic acid reduced total carbohydrate in sprayed squash leaves as 10.61 and 7.6 and 9 mg/g fresh weight, respectively versus unsprayed leaves in control 12.55 mg/g fresh weight. As the leaves became older the elicitors didn't increase the total phenols versus control. However, titratable acidity was still affected with these elicitors in this stage as it increased in sprayed leaves with the tested elicitors; benzoic acid, salicylic acid, acetylsalicylic acid and sulfonic acid (70.4, 73.8, 82.3 and 86.3 ug NaOH /gm fresh wt., respectively) versus 60 ug NaOH /gm fresh weight. in the control leaves (Table 2). All the tested elicitors didn't affect the activity of alpha esterases. Concerning, beta esterases activity only benzoic acid decreased the activity of this enzyme (12.45 ug β-naphthol/min /gm fresh weight) compared to control (17 ug β- naphthol/min /gm fresh weight) and the remaining elicitors didn't alter the activity of this enzyme. In other word, salicylic acid and sulfonic acid increased the activity of the peroxidase in the older leaves 352 and 366 ∆ O.D./min/ gm fresh weight, respectively versus 304 ∆ O.D./min/ gm fresh wt. in unsprayed leaves in the control plots.

Table (1): Effect of the tested elicitors on certain squash biochemical constituents and enzymes activity versus control over the early stage (Mean ± SE) in 2023 season.

Elicitor	Total protein (mg/g fresh wt.)	Nitrogen (mg/g fresh wt.)	Phosphorous (mg/g fresh wt.)	Potassium (mg/g fresh wt.)	Total carbohydrates (mg/g fresh wt.)	Total phenols (mg/g fresh wt.)	Titratable acidity (ug NaOH /gm fresh wt.)	Alpha esterases (ug α-naphthol/min /gm fresh wt.)	Beta esterases (ug β-naphthol/min /gm fresh wt.)	Peroxidases (Δ O.D./min /gm fresh wt.)
Control	12.2±0.5^{a}	1.9±0.16 ^{a}	0.54±0.04^{b}	2.5±0.16 ^{ab}	17.7±0.8^{b}	1.3±0.12 ^{b}	47.1±2.6^{c}	68.1±5 ^{cd}	26.9±2.2^{b}	296±10.4 ^{c}
Benzoic acid	11.4±0.3^{b}	1.8±0.06^{a}	0.58±0.01 ^{a}	2.3±0.05 ^{bc}	21.9±2.8^{a}	1.32±0.1 ^{b}	57.6±3^{b}	82.4±7.9^{b}	23.1±2.1^{bc}	279±8.5 ^{c}
Salicylic acid	12.3±0.2^{ab}	2±0.05 ^{a}	0.59±0.02 ^{a}	2.5±0.1 ^{a}	15.6±0.7^{b}	1.8±0.15 ^{a}	53.1±2^{b}	75.6±6.1^{bc}	26.2±2.5 ^{bc}	387±11 ^{a}
Acetylsalicylic acid	12.1±1.5 ^{ab}	2.08±0.4^{a}	0.49±0.02 ^{c}	2.2±0.08 ^{c}	15.6±1.2^{b}	1.7±0.17 ^{a}	67.2±2.6^{a}	60.8±3.1^{d}	22.5±1.5^{c}	381±16 ^{a}
Sulfonic acid	13.1±0.6 ^{a}	2.06±0.09 ^{a}	0.61±0.016^{a}	2.4±0.05 ^{abc}	21.6±3.3^{a}	1.86±0.15 ^{a}	68.5±2.3^{a}	99.4±10.1^{a}	32.5±2.6^{a}	342±20.3 ^{b}
F value	1.94	1.1	15.03***	5.5*	6.8*	10.72**	39.98***	13.92***	9.64**	36.93***
LSD	1.4	0.3	0.4	0.2	3.7	0.25	4.6	12.5	4.1	25.3

Means within each column not followed by the same letter are significantly different (*P* < 0.05)

Table (2): Effect of the tested elicitors on certain squash biochemical constituents and enzymes activity versus control over the late stage (Mean ± SE) in 2023 season.

Elicitor	Total protein (mg/g fresh wt.)	Nitrogen (mg/g fresh wt.)	Phosphorous (mg/g fresh wt.)	Potassium (mg/g fresh wt.)	Total carbohydrates (mg/g fresh wt.)	Total phenols (mg/g fresh wt.)	Titratable acidity (ug NaOH /gm fresh wt.)	Alpha esterases (ug α-naphthol/min /gm fresh wt.)	Beta esterases (ug β-naphthol/min /gm fresh wt.)	Peroxidases (Δ O.D./min /gm fresh wt.)
Control	14.5±0.10 ^{a}	2.51±0.09 ^{a}	0.55±0.02^{bc}	1.98±0.18 ^{ab}	12.55±0.68^{a}	0.82±0.07 ^{a}	60±4.6 ^{c}	72.9±4.9 ^{ab}	17±2.1^{a}	304±10 ^{b}
Benzoic acid	13.96±0.50 ^{a}	2.32±0.08 ^{a}	0.48±0.02 ^{cd}	1.71±0.11^{c}	10.61±0.45^{b}	0.63± 0.04 ^{b}	70.4±2.1 ^{b}	63.2±8.7 ^{b}	12.45±2.2^{b}	293±30 ^{b}
Salicylic acid	13.86±1.15^{a}	2.31±0.19 ^{a}	0.45±0.02 ^{d}	2.18±0.08 ^{a}	7.6±0.66^{c}	0.62±0.04 ^{b}	73.8±1.0 ^{b}	67.3±7.9 ^{b}	16.1±2^{a}	352±27^{a}
Acetylsalicylic acid	14.2±1.59 ^{a}	2.36±0.26 ^{a}	0.63±0.08 ^{a}	1.77±0.10 ^{bc}	13.18±1.05 ^{a}	0.74±0.11 ^{ab}	82.3±4.9 ^{a}	65.9±4.5 ^{b}	16.4±2^{a}	332±12^{ab}
Sulfonic acid	14.27±0.95 ^{a}	2.38±0.16 ^{a}	0.60±0.04^{ab}	1.66±0.15 ^{c}	9±1.39^{bc}	0.85±0.13 ^{a}	86.3±3.5 ^{a}	82.8±10.8 ^{a}	18.1±0.6^{a}	366±26^{a}
F value	0.2	0.67	9.53**	8.9**	20.1***	4.7*	25.44***	3.02	3.95*	5.56*
LSD	1.8	0.3	0.1	0.2	1.7	0.2	6.5	14.1	3.4	41.3

Means within each column not followed by the same letter are significantly different (*P* < 0.05)

The elicitors, benzoic acid, salicylic acid, acetylsalicylic acid and sulfonic acid have been widely used as an abiotic stress mitigating agent in various crops. These elicitors induced physiological and biochemical changes in plants and could be used successfully in integrated pest management. The obtained results referred that, in general *B. tabaci* oviposition preference reduced in all treated plots by exogenous application with benzoic acid, salicylic acid, acetylsalicylic acid or sulfonic acid versus control over the two growing seasons. But the salicylic acid and sulfonic acid significantly reduced egg numbers in the first season than the other tested elicitors. Moreover, in the second season sulfonic acid was still superior in reducing *B. tabaci* oviposition preference versus control plots followed by benzoic acid. The promising results of efficacy of these elicitors were recorded with reducing nymphal infestation. All the tested elicitors significantly reduced the population of *B. tabaci* nymphs compared to check or control treatment in the first season, 2022. In the second season, 2023 sulfonic acid was superior in reducing *B. tabaci* population than the other tested elicitor. Exogenous application of salicylic acid to tomato plants increased the number and quantity of plant volatiles especially the quantity of methyl salicylate and δ-limonene and in Y-tube olfactometer assays, methyl salicylate and δ-limonene repelled the whiteflies (Shi *et al*., 2016). Salicylic acid and other plant inducers enhanced the systemic acquired resistance in sweet pepper causing high defense against whitefly population and it could be compatible with an integrated pest management program (Zayed *et al*., 2022). Senaratna *et al.* (2003) found that benzoic acid, sulfosalicylic acid and methyl salicylic acid were effective in inducing tolerance to heat, drought and chilling stress. Salicylic acid plays an anti-nutritive compound in plants (Conrath *et al*., 2015). The change in biochemical constituents and enzymes activity in this study over the seedling stage (Which included all the foliar spraying treatments application under investigation) and over the older squash stage was also studied. AS a result, the tested elicitors influenced certain biochemical constituents. In the early stage, it was detected in the lowest infested leaves with *B. tabaci* which were sprayed with sulfonic acid increased phosphorus, carbohydrates, phenols, and titratable acidity. and activities of alpha esterases, beta and peroxidase enzymes in squash leaves. Salicylic acid increased in the younger leaves the content of phosphorous, total phenols (1.8 mg/g fresh weight), titratable acidity and the activity of peroxidases. Acetylsalicylic increased total phenols, titratable acidity and peroxidases activity in the younger leaves. However, benzoic acid increased the phosphorous content, total carbohydrates, titratable acidity and alpha esterases activity. The younger leaves which harbored more eggs than the late one and oviposition preference were significantly affected by plant age (Abd Allah *et al*. 2022 b and c). From the current study we can conclude that the application of these antioxidants may alter oviposition preference in this stage by changing certain chemical constituents which are responsible largely in repelling *B. tabaci* adults. In the late-stage acetylsalicylic acid increased phosphorous content versus control. Titratable acidity percentage increase in sprayed leaves with the tested elicitors; benzoic acid, salicylic acid, acetylsalicylic acid and sulfonic acid versus control. Salicylic acid and sulfonic acid increased the activity of peroxidase in the older leaves versus control. All the tested elicitors reduced population under investigation and by estimating certain biochemical traits we found that these antioxidants in different plant ages increase plant immunity by inducing biochemical changes. In other words, phosphorous, carbohydrates, phenols, titratable acidity and activity of certain alpha and beta esterases and peroxidase increased with some of these elicitors and thus help in plant growth and its immunity against different diseases which led to good quality and quantity of the squash besides their promising role in reducing *B. tabaci* population. Abd Allah *et al*. (2022 a) clearly indicated that many organic and inorganic compounds affect plant health. Singh and Usha (2003) stated that salicylic acid plays significant role in regulating the drought response of plants and suggest that salicylic acid could be used as a potential growth regulator for improving plant growth under water stress. Alakhdar and Abou-Setta (2021) found that acetylsalicylic, benzoic and salicylic acids reduced *Tetranychus urticae* Koch (Acari: Tetranychidae) infestation level on *Phaseolus vulgari*s L. and the associated predators weren't significantly affected. They emphasized that these compounds enhanced %, phosphorous%, potassium% and total phenol in the sprayed leaves compared to control. Nehela *et al.* (2021) found that benzoic acid and its hydroxylated derivatives enhanced vegetative growth and yield traits. We suggested that benzoic acid, salicylic acid, acetylsalicylic acid and sulfonic acid can be involved in any IPM program as a promising elicitor sharing in reducing *B. tabaci* infestation on squash.

Besides, the role of the tested elicitors under investigations in plant growth and yield production as they were excellent source of nutrition for plants based on the many previous studies. In this work, we elaborated their superior role in reduction *B. tabaci* build up attacking squash plants and recommend them in any IPM program.

Acknowledgments

Authors express deep thanks to Prof. Dr. Mohamed M. Abou-Setta Emeritus, Chief Researcher, Plant Protection Research Institute, Agriculture Research Center for great support in carrying out this work.

References

Abd Allah, Y. N. M.; Metwally, S. A. G.; Refaei, B.M.; Ibrahim, S.A. and El Sawaf, B.M. (2022 a): *Bemisia tabaci* (Hemiptera: Aleyrodidae) infestation and yield cantaloupe production in relation to inorganic fertilization. Egyptian Journal of Plant Protection Research Institute, 5 (1): 19–28.

Abd Allah, Y. N. M.; Metwally, S. A. G.; Refaei, B.M. and El Sawaf, B.M. (2022 b): Leaf morphological characters can be a factor for intra-varietal preference of whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) among certain cantaloupe cultivars. Egyptian Journal of Plant Protection Research Institute, 5 (1): 78–93.

Abd Allah, Y. N. M.; Refaei, B.M.; Metwally, S. A. G.; Ibrahim, S.A. and El Sawaf, B.M. (2022 c): Interaction between certain weather factors and plant age of cantaloupe on *Bemisia tabaci* (Hemiptera: Aleyrodidae) infestation over different sowing dates. Egyptian Journal of Plant Protection Research Institute, 5 (1):1-18.

Alakhdar, H.H. and Abou-Setta, M.M. (2021): Efficacy of three elicitors on *Tetranychus urticae* Koch (Acari: Tetranychidae) infestation level and its associated natural enemies on *Phaseolus vulgaris* L. and their effects on plant parameters. Phytoparasitica, 49: 935–942.

Anonymous (2003): SAS Statistics and graphics guide, release 9.1. SAS Institute, Cary, North Carolina 27513, USA.

Bradford, M.M. (1976): A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72: 248-254. http://dx.doi.org/10.1016/0003-

2697(76)90527-3

Chapman, H.D. and Pratt, P.F. (1961): Methods of analysis for soils, plants and waters. University of California, Los Angeles, 60-61: 150-179.

Conrath, U.; Beckers, G.J.M.; Langenbach, C.J.G. and Jaskiewicz, M.R. (2015): Priming for enhanced defense. Annu. Rev. Phytopathol., 53: 97–119. https://doi.org/10.1146/ annurev-phyto-080614-120132

Dubois, M.; Gilles, K.A.; Hamilton, J.K.; Rebers, P.A. and Smith, F. (1956): Colorimetric Method for Determination of Sugars and Related Substances. Analytical Chemistry, 28: 350-356. http://dx.doi.org/10.1021/ac60111a017

El-Sherbeni, A. E.; Khaleid , M.S.; Abd Allah, S.A. and Ali, O.S.M. (2019): Effect of some insecticides alone and in combination with salicylic acid against aphid, *Aphis gossypii*, and whitefly *Bemisia tabaci* on the cotton field. Bulletin of the National Research Centre, 43, 57. https://doi.org/10.1186/s42269-019-0103-0

Haider, I.; Riaz, M.; Ali, S.; Ali, Q.; Noman, A.; Hussain, D.; Nadeem, I.; Akhtar, M.F.; Abbas, A.; Aslam, A.; Mustafa, H.S.B.; Hassan, E.U.; Zubair, M.; Saleem, M. and Malik, M.K. (2023): Efficacy of different insecticides alone and in combination with salicylic acid against cotton whitefly *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae). Pakistan Journal of Agricultural Research, 36 (1): 58-62.

Hammerschmidt, R.; Nuckles, E. and Kuc, J. (1982): Association of enhanced peroxidase activity with induced systemic resistance of cucumber to colletotrichum lagenarium. Physiologia Plantarum, 20: 73-80. http://dx.doi.org/10.1016/0048-4059(82)90025-x

Kähkönen, M. P.; Hopia, A. I.; Vuorela, H. J.; Rauha, J. P.; Pihlaja, K.; Kujala, T. S. and Heinonen, M. (1999): Antioxidant activity of plant extracts containing phenolic compounds. Journal of Agricultural and Food Chemistry. 47(10): 3954–3962. https://doi.org/10.1021/JF990146L

Masuda, K.; Kato, M. and Saito, T. (**2016**): Reduction in carotenoid and chlorophyll content induced by the sweet potato whitefly, *Bemisia tabaci*. Scientia Horticulturae, 200: 102-104. https://doi.org/10.1016/j.scienta.2016.01.010.

Nehela, Y.; Taha, N.A.; Elzaawely, A.A.; Xuan, T.D. A.; Amin, M.; Ahmed, M.E. and El-Nagar, A. (2021): Benzoic acid and its hydroxylated derivatives suppress early blight of tomato (*Alternaria solani*) via the induction of salicylic acid biosynthesis and enzymatic and nonenzymatic antioxidant defense machinery. Journal of Fungi, 7(8): 663. https://doi.org/10.3390/jof7080663

Ni, X.; Quisenberry, S.S.; Heng-Moss, T.; Markwell, J.; Sarath, G.; Klucas, R. and Baxendale, F. (2001): Oxidative responses of resistant and susceptible cereal leaves to symptomatic and nonsymptomatic cereal aphid (Hemiptera:Aphididae) feeding. Journal of Economic Entomology, 94:743-751

Ranganna, S. (2001): Handbook of analysis and quality control for fruit and vegetable products, Ranganna, S. (Ed.). Tata McGraw-Hill Publications, New Delhi, 719-724.

Razze**, J. M.;** Liburd**, O. E. and** McSorley**, R. (2016):** Preference of *Bemisia tabaci* biotype B on zucchini squash and buckwheat and the effect of *Delphastus catalinae* on whitefly populations. Pest Management Science, 72 (7): 1335-1339.

Sadasivam, S. and Manickam, A. (1991): Biochemical methods for agricultural sciences
- Carbohydrates. Publishing for one

world. Wiley Eastern Limited and Tamil Nadu Agricultural University. Coimbatore-641003, 8-9.

Sadasivam, S. and Manickam, A. (1992): Biochemical Methods for Agricultural Sciences. Wiley Eastern Ltd., New Delhi.

Senaratna, T.; Merritt, D.; Dixon, K.; Bunn, E.; Touchell, D. and Sivasithamparam, K. (2003): Benzoic acid may act as the functional group in salicylic acid and derivatives in the induction of multiple stress tolerance in plants. Plant Growth Regulation, 39(1): 77-81.

Senaratna, T.; Touchell, D.; Bunn, E. and Dixon, K. (2000): Acetyl salicylic acid (Aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. Plant Growth Regulation, 30 :157–161.

Shi, X.; Chen, G.; Tian, L.; Peng, Z.; Xie, W.; Wu, Q.; Wang, S.; Zhou, X. and Zhang, Y. (2016): The Salicylic acid-mediated release of plant volatiles affects the host choice of *Bemisia tabaci*. International Journal of Molecular Sciences, 17(7): 1048.

Singh, B. and Usha, K. (2003): Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. Plant Growth Regulation, 39(2): 137-141.

Singleton, V. and Rossi, J. (1965): Colorimetry of total phenolic compounds with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16: 144-158.

Tak, J. H.; Kim, H. K.; Lee,S. H. and Ahn, Y. J. (2006): Acaricidal activities of paeonol and benzoic acid from *Paeonia suffruticosa* root bark and monoterpenoids against *Tyrophagus putrescentiae* (Acari: Acaridae). Pest Manag. Sci., 62: 551-557.

Van Asperen, K. (1962): A study of house fly esterase by means of sensitive colourimetric method. Journal of Insect Physiology, 8: 401-416. Doi:10.1016/0022-1910(62)90074-4

Wang, Y.; Xiao, D.; Wang, R.; Li, F.; Zhang, F. and Wang, S. (2016): Deep sequencing-based transcriptome analysis reveals the regulatory mechanism of *Bemisia tabaci* (Hemiptera: Aleyrodidae) nymph parasitized by *Encarsia sophia* (Hymenoptera: Aphelinidae). PLoS ONE 11(6): e0157684. https://doi.org/10.1371/journal.pone.0157684

Williams, M.; Senaratna, T.; Dixon, K. and Sivasithamparam, K. (2003): Benzoic acid induces tolerance to biotic stress caused by *Phytophthora cinnamomi* in *Banksia attenuata*. Plant Growth Regulation, 41:89–91. Doi: 10.1023/A:1027355604096

Zayed, M. S.; Taha, E.-K.A.; Hassan, M.M. and Elnabawy, E.S.M. (2022): "Enhance Systemic Resistance Significantly Reduces the Silverleaf Whitefly Population and Increases the Yield of Sweet Pepper, *Capsicum annuum* L. var. annuum" Sustainability, 14 (11): 6583. https://doi.org/10.3390/su14116583