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Research Article

Orobanche crenata control in three faba bean varieties by soaking seeds in acetylsalicylate solutions

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Abstract: The prevalence of *Orobanche crenata* on faba bean fields in the Mediterranean region is a serious problem. The chemical inducers, including salicylic acid and its analogues were reported to activate the systemic acquired resistance in plants to subsequent pathogen attacks. The effect of different concentrations of acetylsalicylate (AcSA) as presoaking treatments on *O. crenata* infected faba bean plants of three varieties was studied in a pot experiment under control conditions. At an early stage, before *Orobanche* establishment, soaking seeds in AcSA solutions at 0.4, 0.8, 1.2 and 1.6 mM varied in their effects on faba bean growth depending on their concentrations and tested varieties. Lower concentrations tended to reduce plant height and fresh and dry weight, but these growth parameters showed remarkable increases in concentration of 1.2 mM. At the budding stage, *O. crenata* infection significantly reduced plant biomass and pods yield for all tested varieties. Application of AcSA at o.8-1.6 mM showed a significant increase in fresh and dry biomass and pod yield of faba bean plants. AcSA at less than 1 mM resulted in a great enhancement effect on attached *Orobanche* by increasing the number and weights of attached tubercles and spikes. The highest AcSA concentration (1.6 mM) completely inhibited the growth and development *Orobanche* grown on all tested varieties and produced increases in host biomass and pod yield. Moreover, this treatment produced leaves with the highest levels of chlorophylls, phenolics, and antioxidant activity compared to other treatments. Further studies using relatively high rates of AcSA in field trials are necessary to validate their practical use.

Keywords: *Orobanche*, *Vicia faba*, holoparasite, acetylsalicylate, growth, yield

Introduction

In Egypt, faba *Vicia faba* L. is considered the main source of protein for the majority of the population, and it is consumed as a vegetable in many ways (El-Mergawi and Taie, 2014). Despite the fact that in the 1970's self-sufficiency from faba bean in Egypt was estimated to be 115%, this percentage has been dropping gradually since the 1980's, reaching about 35% in 2018 (Zeid and Hemeid, 2019). Such a drop in self-sufficiency is mainly due to the decline in the cultivated area from 152,000 ha in 1961 to about 32,500 in 2017 (FAOSTAT 2019). Among the major factors that decrease faba bean cultivation area is the spread *Orobanche* parasite due to heavy soil infestation with *Orobanche crenata* seeds. The obligate holoparasite *O. crenata* is considered the most devastating constraint for faba bean cultivation in

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the Mediterranean region (El-Dabaa *et al*., 2021). It produces thousands of seeds that can remain viable in the soil for more than 10 years and germinate in response to chemical signals from faba bean roots. After seed germination, the parasite attaches to the host root by a haustorium through which it absorbs water, minerals, and organic compounds, producing tubercles from which shoot arises and emerges from the soil to flower and set seeds (Brun *et al*., 2017). At this delicate stage, if pod filling has already been initiated on the faba bean plant, partitioning of assimilates between the two sinks (parasite and pods) occurs. However, if pod filling was not achieved by that time, the below-ground sink (parasite) suppresses the formation of the above-ground sink (pods) (Manschadi *et al*., 1997).

Several control methods have been implemented to control *Orobanche* parasite, but without complete success (Triki *et al*., 2018). The application of chemicals to induce systemic acquired resistance (SAR) to activate the plant's defense system is considered a new technology for plant pathogen control (Triki *et al*., 2018). The induction of SAR is associated with the activation of a wide range of resistance mechanisms and the production of a wide range of defense compounds (Sillero *et al*., 2012). Salicylic acid (SA) is an endogenous phenolic plant hormone that regulates growth and acts as a signaling molecule in activating the plant defense responses against many stress factors (Wani *et al*., 2017). The natural resistance of plants to pathogens and parasitic weeds is based on the signaling pathway and depends on the endogenous accumulation of SA (Yang *et al*., 2010). It involved in inducing SAR, which activates many defense compounds, including phenolic acids, coumarins, flavonoids, and lignin (Al-Wakeel *et al*., 2013). Exogenous SA and its analogues have been shown to induce resistance of host plants to control *Orobanche* parasite (Abbes *et al*., 2014; Perez-de- Luque *et al*., 2004). Madany *et al*. (2020) found that applying SA at 1 mM as seed priming mitigated the adverse effects of *O. ramosa* stress in tomato plants, especially at oxidative stress levels. Acetylsalicylate (AcSA) is a commercially manufactured form of SA, and in an aqueous solution, it hydrolyzed completely to SA (Matysiak *et al*., 2020). We found that the exogenous application of different AcSA rates caused higher effects on the growth of six plant species and accumulated more endogenous SA than those of corresponding SA rates (El-Mergawi and Abd El-Wahed, 2020). Recently, we studied the effect of AcSA and SA as seed soaking (0.5-1.5mM) or foliar spray (2-6 mM) on increasing faba bean tolerance to *O. crenata* infection. The results revealed that AcSA was more effective than SA in increasing faba bean tolerance to *O. crenata* infection, especially when used as seed soaking (El-Mergawi *et al*., 2022). Therefore, this study aimed to evaluate the potential of five AcSA concentrations used as seed soaking in increasing the tolerance of three faba bean varieties to *O. crenata* infection.

Materials and Methods

Pot experiment was conducted during the growing season (November-April) of 2020/2021 at the greenhouse of the National Research Centre, Giza, Egypt. Seeds of three faba bean (*Vicia faba*) varieties, Nubaria 5, Giza 716 and Nubaria 4 were obtained from Agricultural Research Centre, Giza, Egypt. Analytical standard of Acetylsalicylate (AcSA) was purchased from S D Fine-Chem Limited (India) and applied to seeds as seed soak treatments. The experiment was conducted as a completely randomized two -factor design with five replications. The first factor was the dose of AcSA, which was used for seed soaking and included 0, 0.4, 0.8, 1.2, and 1.6 mM. The second factor was the variety of faba beans, including Nubaria 5, Giza 716 and Nubaria 4. Seeds of each faba bean variety were subjected to five presoaking treatments by incubating seeds in AcSA solutions (200 ml/100 seeds) for 24 hrs. Five seeds were sown in each 30-cm diameter plastic pot containing about 5 kg sandy loam soil (48.4% sand, 41.1% silt, and 10.5% clay; pH 8.0). About 200 mg of *O. crenata* seeds were mixed with the soil of the infested pot. Commercial rhizobia and 8 g of superphosphate (15%, P2O5) were incorporated into the top 30 mm of the soil at sowing. Each variety has six treatments, five of *O. crenata*-infested groups for AcSA presoaking treatments and one non-infested group (control healthy). Pots of the three tested varieties were divided into fifteen groups (3 varieties × 5 AcSA treatments) with five replications and arranged in a randomized complete block design. The pots were placed in a greenhouse (25 ± 3 °C, 12 h photoperiod). After 20 days from sowing, seedlings were thinned to three uniform seedlings per pot. Plants were supplied with nitrogen (ammonium sulfate, 21% N, 6 g/pot) three times at 4, 6, and 8 weeks after sowing. Samples from all tested treatments were collected at the vegetative growth stage 40 days after planting (40 DAP). At the budding stage, 135 DAP, then the growth parameters of faba bean and *Orobanche* were determined. At 40-DAP, plant samples were collected, fast cleaned with distilled water to remove the AcSA- residues, and used to measure the vegetative growth parameters in terms of height (cm), number of leaves, and plant fresh and dry weight (g). Leaves of collected plants were subjected to determine chlorophyll content, total phenolics content and antioxidant activity. At 135-DAP, yield parameters were determined in terms of plant fresh weight (g), plant dry weight (g), number of pods plant^{-1} and weight of pods plant^{-1}. Attached *Orobanche* was collected from each pot, divided into tubercles (stages 2- 3) and spikes (stages 4-5). Their numbers and weights (g) per pot were determined.

***Chlorophyll content*:** Fifty milligrams of fresh leaf sample was extracted with 10 mL acetone 70%, and absorbance readings were taken with Shimadzu spectrophotometer (UV-240, Shimadzu, Japan) at 645 and 663 nm. Then, concentrations of chlorophyll a (Chl a), chlorophyll b (Chl b) and total chlorophylls (Total Chl) were calculated with the equations proposed by Lichtenthaler (1987).

***Total phenolics content*:** Phenolics in faba bean leaves were extracted with 70% acetone and determined according to the method previously mentioned by El-Mergawi and Taie (2014) using the Folin-Ciocalteu reagent. In brief, 0.1 ml of extract was added to 7.9 ml of distilled water, 0.5 ml of Folin-Ciocalteu reagent, 1.5 ml of sodium carbonate solution (200 g L^{-1}), and mixed vigorously. The mixture was allowed to stand for 1 h at room temperature, and then the absorbency was measured at a wavelength of 765 nm. Gallic acid was used as a standard, and the results were expressed as mg gallic acid equivalent g dry matter (mg GAE g^{-1} DW).

Antioxidant Activity: Antioxidant capacity or free radical scavenging activity was determined according to Brand-Williams *et al*. (1995) using 1,1-diphenyl-2- picryl-hydrazil (DPPH) reagent. In brief, 1.5 ml of freshly prepared methanolic DPPH solution (0.02 mg/ml) was added to 0.75 ml of previously 70% acetone extract and then stirred. The decolorizing was recorded after 5 min of reaction at a wavelength of 517 nm and compared with a blank control. The DPPH radical scavenging activity of the extracts was measured using the Trolox standard curve. Results were expressed as µmol Trolox g^{-1} dry sample (µmol Trolox g^{-1} DW).

Statistical analysis: We found that the obtained data were normally distributed for ANOVA, and the significance of treatments was determined by Duncan's multiple range test ($P \leq 0.05$) (Gomez and Gomez, 1984).

Results

Effects of AcSA and variety treatments on vegetative growth parameters of faba bean plants

Vegetative samples were collected at the early stage of faba bean growth (40 DAP) before *Orobanche* establishment.

Mean effects of AcSA concentrations

Data showed that the vegetative growth parameters of faba bean plants, such as plant height, number of leaves, and fresh and dry weights, varied in their response to different AcSA treatments (Table 1). However, the number of leaves did not show a significant effect with all AcSA treatments. Soaking seeds in AcSA at 0.4 mM possessed the lowest mean values of plant height, plant fresh and dry weights compared with control and the other AcSA treatments. By increasing AcSA concentration, an enhancement effect on plant height, fresh and dry weight was observed. These growth parameters reached their maximum values by 1.2 mM AcSA. This treatment enhanced the mean values of fresh and dry weight by 12.5% and 19.1%, respectively as compared with the control. Meanwhile, the higher AcSA concentration (1.6 mM) did not show a significant effect on various growth parameters when compared with the corresponding control.

Mean effects of tested varieties

In comparison between the three tested varieties, the number of fresh leaves and dry weight did not show significant differences (Table 1). Meanwhile, Nubaria 5 produced the tallest faba bean plants, followed by Giza 716.

Interaction effects between AcSA and varieties

Data showed that the growth parameters such as plant height, number of leaves, fresh and dry weights of three faba bean varieties varied in their response to AcSA treatments (Table 2). However, the number of leaves of three faba bean varieties did not show any significant response to all AcSA treatments. Soaking seeds in 0.4 or 0.8 mM AcSA decreased the plant height of the three tested varieties when compared with those of control plants. The lowest values of plant fresh and dry weights of Nubaria 5 and Giza 716 plants were observed for 0.4 mM AcSA, although this treatment produced the heaviest Nubaria 4 plants when compared with control. Meanwhile, the maximum value of plant biomass was obtained at 1.2 mM for Nubaria 5 and Giza 716.

Table 1 Mean effects of AcSA concentrations and tested varieties on vegetative growth parameters of faba bean, 40 days after planting.

Treatments	Plant height (cm)	Number of leaves $plant^{-1}$	Fresh weight (g $plant^{-1}$)	Dry weight (g $plant^{-1}$)
AcSA concentrations				
0.4 mM	42.1 b	9.50 a	10.0 c	1.09 b
0.8 mM	44.0 ab	9.17 a	11.5 b	1.20 b
1.2 mM	47.9 a	8.87 a	12.6 a	1.37 a
1.6 mM	47.8 a	8.77 a	11.1 b	1.09 b
Control	46.7 a	8.80 a	11.2 b	1.15 b
Varieties				
Nubaria 5	49.4 a	8.8 a	11.1 a	1.12 a
Giza 716	45.7 b	9.0 a	11.4 a	1.20 a
Nubaria 4	42.1 c	9.3 a	11.4 a	1.22 a

Means followed by the same letter (s) within the same column are insignificantly different at 0.05 level of probability.

Table 2 Interaction effects between AcSA and variety treatments on vegetative growth parameters of faba bean plants, 40 days after planting.

Variety	AcSA Treatments	Plant height (cm)	Number of leaves $plant^{-1}$	Fresh weight (g $plant^{-1}$)	Dry weight (g $plant^{-1}$)
Nubaria 5	0.4 mM	40.3	9.0	8.50	0.97
	0.8 mM	46.5	8.8	10.75	1.02
	1.2 mM	54.8	8.3	13.00	1.38
	1.6 mM	54.8	9.0	11.25	1.06
	Control	50.8	8.8	11.75	1.18
Giza 716	0.4 mM	45.5	9.5	8.75	0.89
	0.8 mM	44.0	8.8	11.75	1.30
	1.2 mM	44.5	9.3	12.75	1.40
	1.6 mM	47.0	8.8	12.25	1.26
	Control	47.0	8.8	11.50	1.16
Nubaria 4	0.4 mM	40.5	10.0	12.75	1.41
	0.8 mM	41.5	10.0	12.00	1.30
	1.2 mM	44.3	9.0	12.00	1.33
	1.6 mM	41.8	8.5	9.75	0.96
	Control	42.3	8.8	10.25	1.12
$LSD_{5\%}$		2.5	NS	1.63	0.26

Effects of AcSA and variety treatments on chlorophyll content

Mean effects of AcSA concentrations

Chlorophyll in the 4th leaves of three faba bean varieties was determined at vegetative growth, 40 DAP. The mean effects of soaking seeds in AcSA on chlorophyll a, b and total chlorophyll are illustrated in Fig. 1. All chlorophyll forms did not show any significant response to the application AcSA at 0.4-1.2 mM when compared with the control. A significant increase in chlorophyll a, b, and total chlorophyll was observed for 1.6 mM AcSA compared with control and other AcSA treatments.

Mean effects of tested varieties

As shown in Table 3, chlorophyll a, b and total chlorophyll did not show great variations between the three faba bean varieties. In comparison, except for the significant increase in chlorophyll b for Nubaria 5, levels of chlorophyll a, b and total chlorophyll did not show any significant variations between the three tested varieties.

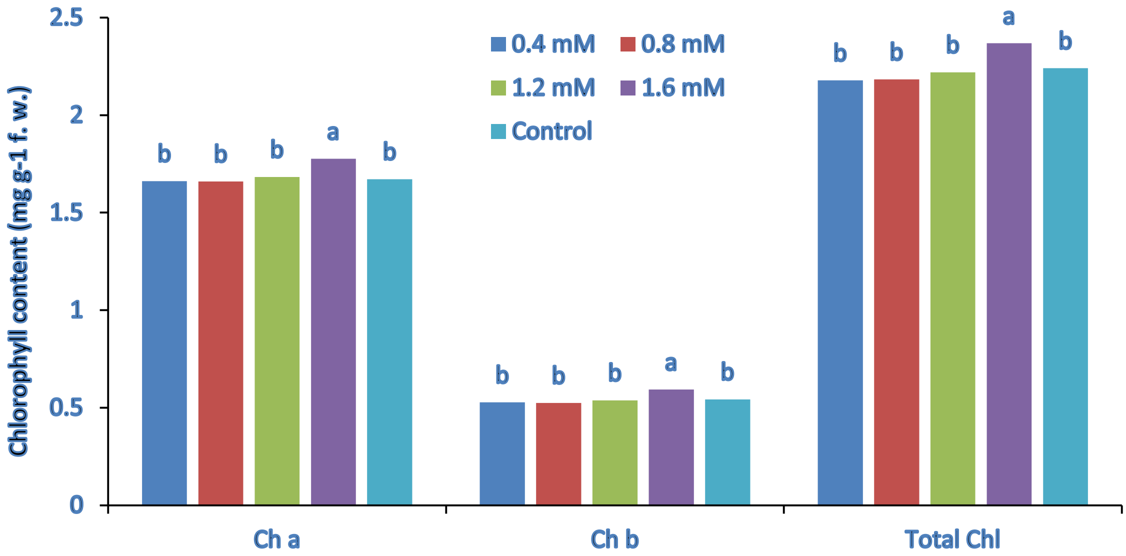

Figure 1 Mean effects of different AcSA concentrations on chlorophyll contents in leaves of vegetative faba bean plants, 40 days after planting. Means followed by the same letter (s) are insignificantly different at 0.05 level of probability.

Table 3 Mean values of chlorophylls, total phenolics and antioxidant activities in faba bean leaves of the three tested varieties, 40 days after planting.

Treatment	Chl a	Chl b (mg g^{-1} FW)	Total Chl	Total phenolics (mg GAE g^{-1} DW)	Antioxidant activity (µmol Trolox g^{-1} DW)
Nubaria 5	1.708 a	0.570 a	2.277 a	40.5 b	78.5 c
Giza 716	1.667 a	0.523 b	2.190 a	38.1 b	97.5 b
Nubaria 4	1.696 a	0.542 b	2.246 a	57.5 a	135.5 a

Chl: Chlorophyll. GAE: Gallic acid equivalent. Means followed by the same letter (s) within the same column are insignificantly different at 0.05 level of probability.

Interaction effects between AcSA and varieties

As shown in Table 4 chlorophylls contents in the tested varieties varied in their response to AcSA treatments. Chlorophyll a, b and total chlorophyll in leaves of Nubaria 4 plants did not show any significant response to various AcSA treatments. Meanwhile, levels of these chlorophyll compounds in Giza 716 and Nubaria 4 varied in their response between AcSA treatments. It can be observed that all tested varieties produced the highest values of chlorophyll a, b and total chlorophyll by AcSA at 1.6 mM.

Effect of AcSA and variety treatments on total phenolics and antioxidant activity

The effect of soaking seeds in different AcSA solutions on phenolics as antioxidant compounds and antioxidant activity in dry leaves of three faba bean varieties were determined at 40 DAP.

Mean effects of AcSA concentrations

All AcSA treatments showed a significant increase in total phenolic content and antioxidant activity as compared with control (Fig. 2). Levels of two antioxidants tended to increase as AcSA concentration increased. In comparison with the control, more than 20% increases in total phenolic contents and antioxidant activity were observed for different AcSA treatments. The highest increase in phenolics content (50%) and antioxidant activity (45.9%) was observed in a concentration of 1.6 mM.

Mean effects of tested varieties

As shown in Table 3, faba bean varieties varied significantly in their contents from phenolics and antioxidant activity. Among the three varieties, Nubaria 4 possessed the highest values of phenolics content and antioxidant activity. Plants of this variety increased phenolics content by 50.9% and 42.0% when compared with Giza 716 and Nubaria 5, which corresponded with 39.0% and 72.6% increases in antioxidant activity, respectively.

Interaction effects between AcSA and varieties

Among tested varieties, all AcSA treatments tended to increase total phenolic content and antioxidant activity as compared with the corresponding control in most cases (Table 4). The highest values of phenolic content and antioxidant activity in the three tested varieties were observed in a concentration of 1.6 mM. It increased phenolic contents in leaves of Nubaria 5, Giza 716 and Nubaria 4 varieties with 37.2%, 57.2% and 53.4%, respectively, relative to control. The corresponding increase in antioxidant activities of the three tested varieties constituted 37.6%, 78.4% and 32.2%, respectively.

Table 4 Interaction effects between AcSA and variety treatments on chlorophyll (Chl) and total phenolics contents and antioxidant activity of faba bean plants, 40 days after planting.

Variety	AcSA Treatment	Chlorophyll (mg g^{-1} FW)			Total phenolics	Antioxidant activity
		Chl a	Chl b	Total Chl	(mg GAE g^{-1} DW)	(µmol Trolox g^{-1} DW)
Nubaria 5	0.4 mM	1.608	0.506	2.114	42.9	82.2
	0.8 mM	1.768	0.601	2.369	38.7	75.5
	1.2 mM	1.661	0.561	2.222	39.9	79.6
	1.6 mM	1.781	0.612	2.393	46.8	90.0
	Control	1.721	0.576	2.287	34.1	65.4
Giza 716	0.4 mM	1.662	0.539	2.201	36.9	98.2
	0.8 mM	1.531	0.466	1.997	38.1	102.3
	1.2 mM	1.708	0.514	2.222	39.2	107.3
	1.6 mM	1.802	0.579	2.381	46.7	115.1
	Control	1.634	0.519	2.153	29.7	64.5
Nubaria 4	0.4 mM	1.715	0.537	2.220	49.4	124.7
	0.8 mM	1.681	0.505	2.185	60.7	142.1
	1.2 mM	1.680	0.538	2.218	65.8	145.0
	1.6 mM	1.745	0.585	2.330	67.5	151.6
	Control	1.659	0.544	2.278	44.0	114.7
$LSD_{5\%}$		0.118	0.055	0.170	3.6	10.3

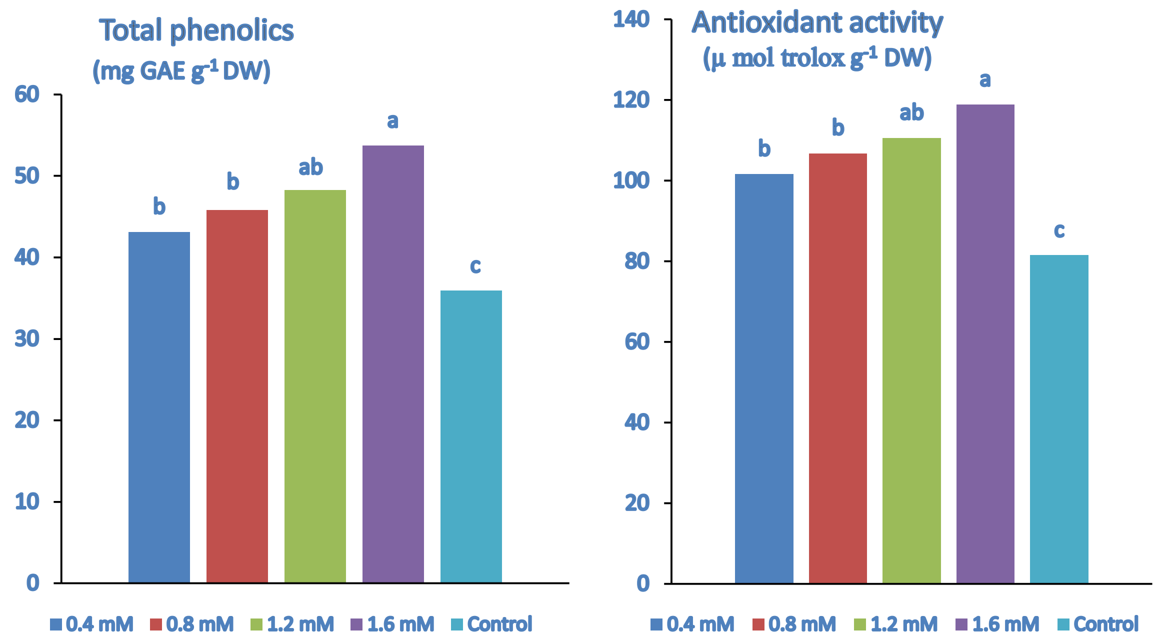

Figure 2 Mean effects of different AcSA concentrations on total phenolics and antioxidant activity in leaves of vegetative faba bean plants, 40 days after planting. Means followed by the same letter (s) are insignificantly different at 0.05 level of probability.

Effect of AcSA and variety treatments on biomass and pods yield

At the budding stage, we determined the effect of presoaking seeds in AcSA solutions on biomass and pod yield of infected faba bean plants for three faba bean varieties (Tables 5-6). By comparing with healthy plants, *O. crenata* infection significantly reduced the fresh and dry weight of plants, as well as the number and weight of pods per plant (Table 5). The mean values of these yield parameters showed more than 40% decreases as affected by Orobanche infestation compared to healthy, uninfected plants (Table 5).

Mean effects of AcSA concentrations

It can be observed that presoaking seeds in AcSA tended to improve biomass accumulation and pod yield of infected plants (Table 5). With few exceptions, fresh weight, dry weight, number of pods and weight of pods of infected plants responded positively to AcSA treatments. However, AcSA treatments failed to restore the infection-inhibiting effects on host biomass and pods to a full yield. Relative to the control, low AcSA concentration (0.4 mM) showed a significant increase in plant fresh and dry weights, accompanied by an insignificant decrease in the number and weight of pods per plant. Soaking seeds in AcSA o.8-1.6 mM tended to produce a significant increase in biomass and pod yield of infected plants. The highest values of tested yield parameters were observed mainly in concentrations of 1.6 mM followed by 0.8 mM. These treatments exhibited more than 20% increases in mean values of plant fresh and dry weights and more than 27% increases in the number and weight of pods relative to control (Table 5).

Mean effect of tested varieties

Regarding faba bean performance, plant biomass and pod yield varied among tested varieties (Table 5). The heaviest fresh and dry biomass and the highest number of pods were observed for Nubaria 4. On the contrary, among the three tested varieties, Giza 716 showed the lowest weight of fresh and dry weight accompanied by the highest weight of pods per plant.

Table 5 Mean effects of AcSA concentrations and tested varieties on plant biomass and pods yield of faba bean plants, 135 days after planting.

Treatments	Fresh weight (g plant^{-1})	Dry weight (g plant^{-1})	Number of pods plant^{-1}	Weight of pods plant^{-1} (g)
AcSA concentrations				
0.4 mM	75.2 c	13.82 bc	2.83 d	15.6 c
0.8 mM	84.4 b	15.51 b	3.92 c	24.9 b
1.2 mM	78.8 c	14.31 b	3.57 c	22.6 b
1.6 mM	88.5 b	15.45 b	4.60 b	25.0 b
Control	69.1 d	12.70 c	3.08 d	18.8 c
Non-infected	121.8 a	21.24 a	6.67 a	31.5 a
Varieties				
Nubaria 5	87.1 ab	16.44 a	3.66 b	23.4 ab
Giza 716	79.3 b	15.00 a	3.83 b	25.1 a
Nubaria 4	92.6 a	15.25 a	4.86 a	20.9 b

Means followed by the same letter (s) within the same column are insignificantly different at 0.05 level of probability.

Table 6 Interaction effects between AcSA and variety treatments on biomass and pods yield of faba bean plants, 135 days after planting.

Variety	AcSATreatments	Fresh weight (g plant^{-1})	Dry weight (g plant^{-1})	Number of pods plant^{-1}	Weight of pods plant^{-1} (g)
Nubaria 5	0.4 mM	72.4	14.12	3.0	19.3
	0.8 mM	82.0	15.99	3.5	22.7
	1.2 mM	76.5	14.92	3.0	23.6
	1.6 mM	106.8	19.22	4.5	26.9
	Control	76.9	15.00	2.7	21.2
	Uninfected	107.7	19.39	5.3	26.4
Giza 716	0.4 mM	76.1	14.54	2.0	14.1
	0.8 mM	84.4	16.12	4.0	30.0
	1.2 mM	73.7	13.72	3.9	23.1
	1.6 mM	77.9	14.71	5.3	25.8
	Control	58.0	11.08	2.8	23.2
	Uninfected	105.4	19.82	5.0	34.1
Nubaria 4	0.4 mM	77.1	12.80	3.5	13.4
	0.8 mM	86.8	14.41	4.3	22.1
	1.2 mM	86.1	14.29	3.8	21.2
	1.6 mM	80.9	13.43	4.0	22.4
	Control	72.4	12.02	3.8	12.2
	Uninfected	152.3	24.52	9.8	33.9
$LSD_{5\%}$		4.4	0.80	1.1	2.7

Interaction effects between AcSA and varieties

Great variation between tested varieties in their response to different AcSA concentrations was noticed (Table 6). Presoaking seeds in AcSA at 0.4 mM varied in their effects on fresh and dry weights between tested varieties. This treatment significantly reduced the biomass of infected Nubaria 5 plants and caused a significant increase in the biomass of Giza 716 plants when compared with the corresponding control, infected plants. However, such low AcSA concentration tended to reduce the number and weight of pods of the three tested varieties. With few exceptions, application of AcSA at 0.8-1.2 mM as seed soaking tended to produce significant increases in plant fresh and dry biomass as well as in the number and weight of pods of the three tested varieties. The highest pods yield per plant was observed at 1.6 mM AcSA for Nubaria 5, whereas Giza 716 and Nubaria 4 varieties produced the highest pods yield at 0.8 mM.

Effects of AcSA concentrations and faba bean varieties on *O. crenata* parasite

At 135 DAP, the attached *Orobanche* was collected from each pot and divided depending on the stage of development into tubercles (S_2-S_3) and spikes (S_4-S_5).

Mean effects of AcSA concentrations

Data presented in Tables 7 showed that *Orobanche* attachment with faba bean plants varied in their response to AcSA treatments. Usage of AcSA at relatively low concentrations (0.4-1.2 mM) greatly increased the number and weight of tubercles compared with control. Such increase in mean values of number of tubercles and their weights ranged between 133- 196 % and 218-424%, respectively, relative to those of infected plants. On the contrary, the highest AcSA concentration (1.6 mM) completely inhibited tubercle formation in the host plants. As for spike formation, which is considered the most severe stage of *Orobanche* parasite, its number and weight showed variations in their effects by AcSA treatments. Soaking faba bean seeds in AcSA at 0.4 or 0.8 mM mM increased mean values of number of spikes by 15.7% and 32.8% and their weights by 42.8% and 65.8%, respectively, relative to those of control. The superiority of the highest AcSA concentration (1.6mM) was also noticed; it completely inhibited spike formation on the host plants.

Mean effects of faba bean varieties

Among tested faba bean varieties, Nubaria 4 plants showed the lowest number and weight of attached tubercles, whereas Giza 716 plants showed the lowest number and weight of spikes (Table 7). On the contrary, the highest number and weight of both tubercles and spikes was recorded for Nubaria 5 plants.

Interaction effects of AcSA concentrations and faba bean varieties on attached *O. crenata* parasite

Among the three faba bean varieties, Nubaria 4 plants were considered the most sensitive variety to *Orobanche* infestation since plants of this variety had the highest number and weight of both tubercles and spikes per pot (Table 8). A tendency of low AcSA concentrations (0.4 and 1.2 mM) on increasing number and weight of tubercles was noticed for the three tested varieties compared with those attached with control. On the other hand, the application of AcSA at 1.6 mM showed complete inhibition of tubercle formation in the three tested varieties. Except for the significant increase observed for Nubaria 4 at 0.4 and 0.8 mM, all AcSA concentrations tended to reduce the number and weight of spikes/pot as compared with control plants. A complete inhibition in spike formation on the three tested varieties was observed for AcSA at 1.6 mM treatment.

Table 7 Mean effects of AcSA concentrations and faba bean varieties on *O. crenata* growth parameters, 135 days after planting.

Treatments	Tubercles (S_2-S_3)/pot		Tubercles (S_2-S_3)/pot	
	Number	Weight (g)	Number	Weight (g)
AcSA concentrations				
0.4 mM	1.27 a	6.29 a	1.55 ab	37.03 a
0.8 mM	1.33 a	3.91 b	1.78 a	42.98 a
1.2 mM	1.05 b	6.45 a	1.22 b	12.07 c
1.6 mM	0.00 d	0.00 d	0.00 c	0.00 d
Control	0.45 c	1.23 c	1.34 b	25.93 b
Varieties				
Nubaria 5	1.03 a	5.69 a	1.60 a	34.37 a
Giza 716	0.83 ab	3.47 b	0.93 b	9.14 c
Nubaria 4	0.60 b	1.57 c	1.00 b	27.3 b

Means followed by the same letter (s) within the same column are insignificantly different at 0.05 level of probability.

Table 8 Interactions effects between AcSA and variety treatments on growth of *O. crenata* parasite, 135 days after planting.

Variety	AcSA Treatments	Tubercles (S_2-S_3)/pot		Spikes(S_4-S_5)/pot	
		Number	Weight (g)	Number	Weight (g)
Nubaria 5	0.4 mM	1.33	7.2	3.00	51.20
	0.8 mM	1.67	4.3	4.33	103.33
	1.2 mM	1.80	15.9	0.00	0.00
	1.6 mM	0.00	0.00	0.00	0.00
	Control	0.34	1.03	0.67	17.30
Giza 716	0.4 mM	1.80	10.83	0.33	14.67
	0.8 mM	1.33	3.93	0.33	8.33
	1.2 mM	0.67	1.63	2.33	12.20
	1.6 mM	0.00	0.00	0.00	0.00
	Control	0.33	0.98	1.67	10.50
Nubaria 4	0.4 mM	0.67	0.83	1.33	45.23
	0.8 mM	1.00	3.50	0.67	17.27
	1.2 mM	0.67	1.83	1.33	24.00
	1.6 mM	0.00	0.00	0.00	0.00
	Control	0.67	1.67	1.67	50.00
$LSD_{5\%}$		0.55	0.84	0.63	4.04

Discussion

In the pathosystem faba bean, *Orobanche* emergence is observed late during the life cycle of the host plant. As a general rule, no parasite attachments were observed before flowering of the host plant, and the parasite emerged when the crop had already produced pods (Abbes *et al*., 2007). Consequently, at this early sampling time (40 DAP), the growth parameters of tested varieties were not affected by *Orobanche* parasite but were mainly affected by AcSA treatments. According to our results, AcSA treatments varied in their effects on growth parameters depending on their concentration. Since vegetative growth parameters of all tested varieties tended to decrease by 0.4 mM AcSA and showed increases by 1.2 mM, in most cases. In line with these results, Li *et al*. (2022) reported that different SA concentrations have either promoting or inhibiting effects on plant growth in different plant species. An enhancement effect of soaking faba bean seeds in AcSA (0.5-1.5 mM) on plant viability, height, fresh weight, dry weight and with less extended number of leaves was observed recently by El-Mergawi *et al*. (2022). It has been suggested that the growth-promoting effects of SA could be related to or to improving some physiological processes such as photosynthesis, transpiration, and stomatal conductance (Stevens *et al*., 2006). Contrary to our results, Ozpinar *et al*. (2017) found that SA reduced the growth of five plant species at high doses, while it accelerated the growth of these plants at low doses. These contrasting findings about the effects of different AcSA concentrations on plant growth led to suppose that SA has a complex role in plant growth (Lie *et al*., 2022).

The effects of ASA treatments on chlorophyll and total phenolic contents and antioxidant activity in faba bean leaves were determined at the first sampling date (40 DAP). The significant increase in chlorophyll a, b, and total chlorophyll was observed only for the highest AcSA concentration (1.6 mM) compared with the control and other AcSA treatments. In line with our results, Canakci and Munzuuroglu (2007) found that cucumber seeds exposed to AcSA at 0.01 - 5 mM varied in their effects on chlorophyll content, and the highest pigment content was recorded for the highest concentration. The positive effects of AcSA on phenolic content and antioxidant activity that were observed in our study are in accordance with those reported by Briache *et al*. (2020) and Madany *et al*. (2020).

Also, War *et al*. (2011) found that a higher induction in phenolic content was recorded in plants treated with SA at 1.5 mM. The great increase in two antioxidant agents produced by 1.6 mM AcSA may be related to the effectiveness of this treatment in protecting faba bean from *O*. *crenata* parasitism, as shown in Tables 5-6. In line with this suggestion, Madany *et al*. (2020) showed that priming tomato seeds in 1 mM SA can induce resistance to *Orobanche* parasitism via enhancing host antioxidant status. Moreover, Briache *et al*. (2020) mentioned that faba bean resistance against *O*. *crenata* could be a result of the involvement of phenolic compounds after elicitation by SA. Moreover, variation in phenolic contents between faba bean varieties was previously observed by Briache *et al*. (2020).

The significant reduction in faba bean growth and yield as affected by *O*. *crenata* infestation was previously mentioned by many investigators (Ennami *et al*., 2020; El-Mergawi *et al*., 2022). In this concern, Rubiales *et al.* (2003) found that the yield loss by *Orobanche* infection can reach 100%. The holoparasite infection has a negatively influence on the host biomass and yield by acting as a competing sink for assimilation and reducing the photosynthetic efficiency of the host plant (Briache *et al*., 2020). At a budding stage, partitioning of assimilates between the parasite and pods occurs, then the below-ground sink (parasite) suppresses the formation of the above-ground sink (pods) (Manschadi *et al*., 1997).

The promotion effects on biomass and pods yield of faba bean infected plants by 0.8-1.6 mM AcSA are consistent with the results obtained by Abbes *et al*. (2014); Triki *et al*. (2018) and El-Mergawi *et al*. (2022). In this concern, Hayat *et al*. (2009) suggested that exogenous application of SA modified physiological, biochemical, and molecular processes in plants, including anti-oxidative enzyme activities, and regulated the components of its signaling pathway besides getting overlapped with other pathways mediating resistance. The great increase in yield of infected host plants when treated with the highest AcSA dose (1.6 mM) may be related to its effectiveness in the prevention *Orobanche* attack, as shown in Table 5. The increase in host resistance against *Orobanche* stress conditions may be due to activation SAR in plants, which increases the induction of pathogenesis-related proteins and diminishes the damage caused by *Orobanche* spp. (Perez-de-Luque *et al*., 2004; Kusumoto *et al*., 2007; Sillero *et al*., 2012). The observed variations between tested varieties in their biomass accumulation and pods yield may be related to their variations in genetic resources (Abbes *et al*., 2007) or to variations in their resistances to *Orobanche* infection (Ennami *et al*., 2020).

The results propose a potential role of the highest AcSA concentration (1.6 mM) as seed soaking in protecting faba bean plants against *O*. *crenata* infestation. These results are in agreement with the results obtained by Sillero *et al*. (2012), Abbes *et al*. (2014), and Madany *et al*. (2020). The natural resistance of plants to pathogens and parasitic weeds is based on the combined effects induced mechanisms, among which is the systemic acquired resistance (SAR) (Yang *et al*., 2010). The chemical inducers, including SA and its analogues benzothiadiazole (BTH) and AcSA were reported to activate the SAR in plants by increasing the induction of pathogenesis-related proteins, therefore diminishing the damage caused by *Orobanche* species (Pérezde-Luque *et al*., 2004; Kusumoto *et al.*, 2007). Moreover, it can be supposed that this high AcSA dose reduces *Orobanche* infestation by activation of defense responses in the host root, including lignification of the endodermis or by inhibiting elongation of parasite radicles (Kusumoto *et al*., 2007). Lignification has been reported as a defense reaction against *Orobanche* penetration connection to the vascular system and tuber development; it helped the plant the cell wall against physical and enzymatic breakdown (Yang *et al*., 2010). The inhibiting effect on the parasite by 1.6 mM AcSA may be related to its higher induction in phenolic content and antioxidant activity, as shown in Tables 7-8. Phenolics may impede parasite infestation by inhibiting *Orobanche* seed germination via reducing the germination stimulants or increasing inhibitors released by the host plant root (Abbes *et al*., 2014) or increasing the mechanical strength of the host cell wall (Briache *et al*. (2020). On the other side, the great promotion of presoaking seeds in AcSA at less than 1 mM on tubercles and spikes of *Orobanche* may be due to the enhancement effect on *Orobanche* seeds germination and their attachments to the host roots. An increase in *O. crenata* infection on pea plants by 1mM SA was previously observed y Perez-de-Luqe et al. (2004). These results are not confirmed with our previous findings by El-Mergawi *et al*. (2022). Who reported that soaking faba bean seeds (Sakha 4 variety) in o.5-1.5 mM AcSA greatly reduced *Orobanche* infestation, and a complete inhibition of tubercles and emerged spikes occurred by 0.5 mM. The contrasting results of SA were previously reported, whereas 1 mM SA was not effectively sufficient on *O. crenata* grown on pea plant (Perez-de-Luque *et al*., 2004), but this concentration reduced the infection of *O. Cumana* in sunflower (Buschmann *et al*., 2005). The discrepant results of AcSA treatments suggest that SA activates different resistance mechanisms against *Orobanche* (Briache *et al*., 2020).

The number of emerged spikes per host plant is considered the best tolerance index (Rubiales *et al*., 2003). Consequently, in this study, depending on spike number and weight of the spike, we found that the Giza 716 variety was considered the most resistant variety. It is characterized by less number of spikes and the least amount of biomass accumulated by the parasite. Variations between faba bean varieties in their resistance to *Orobanche* infection were previously observed by Zeid and Hemeid (2019). They mentioned that the breeding program in Egypt succeeded in selecting a new source of resistance, from which resistance varieties were developed.

Conclusions

In conclusion, this study reveals that the presoaking of faba bean seeds with different AcSA concentrations varied in their effects on growth and yield of host plants as well as on growth and development *O. crenata* attachment. These effects depended on AcSA concentrations and faba bean varieties. Presoaking in AcSA solution at less than 1 mM enhanced the growth and development of the attached parasite. Our results proposing a potential role of 1.6 mM AcSA as seed soaking in protecting faba bean plants against *O. crenata* infestation. This treatment could contribute to improving plant growth and yield in the Orobanche-infected areas.

Due to the discrepant effects of AcSA treatments, more research and studies by a different AcSA dose in field trials are necessary in order to reduce the danger of growth reduction in faba bean and to increase the efficacy of *O. crenata* control.

Conflict of interest

R. El-Mergawi and M. El-Dabaa declare that they have no conflict of interest.

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کنترل گل جالیز *Orobanche crenata* در سه گونه باقلا با خیساندن بذرها در محلول‌های استیل‌سالیسیلات

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چکیده: شیوع گل جالیز *Orobanche crenata* در مزارع باقلا در منطقه مدیترانه یک مشکل جدی است. القاکننده‌های شیمیایی، ازجمله اسید سالیسیلیک و آنالوگ‌های آن، گزارش شده‌اند که مقاومت سیستمیک اکتسابی را در گیاهان در برابر حملات بعدی پاتوژن‌ها فعال می‌کنند. اثر غلظت‌های مختلف استیل‌سالیسیلات (AcSA)به‌عنوان تیمار پیش‌خیساندن بر گیاهان باقلا آلوده به گل جالیز در سه گونه، در یک آزمایش گلدانی تحت شرایط کنترل شده بررسی شد. در مرحله اولیه، پیش از تثبیت گل جالیز، خیساندن بذرها در محلول‌های AcSA با غلظت‌های ۰/۴، ۰/۸، ۱/۲ و ۱/۶ میلی‌مولار بسته به غلظت‌ها و گونه‌های آزمایش شده اثرات متفاوتی بر رشد باقلا داشت. غلظت‌های پایین‌تر تمایل به کاهش ارتفاع گیاه و وزن تازه و خشک داشتند، اما این پارامترهای رشد در غلظت ۱/۲ میلی‌مولار افزایش قابل‌توجهی نشان دادند. در مرحله غنچه‌زنی، آلودگی به گل جالیز به‌طور قابل‌توجهی زیست‌توده گیاه و عملکرد غلاف را در تمام گونه‌های آزمایش شده کاهش داد. استفاده از AcSA در غلظت‌های ۰/۸ تا ۱/۶ میلی‌مولار افزایش قابل‌توجهی در زیست توده تازه و خشک و عملکرد غلاف گیاهان باقلا نشان داد AcSA.در غلظت‌های کم‌تر از ۱ میلی‌مولار اثر تقویت‌کننده‌ای بر گل جالیز متصل داشت و تعداد و وزن غده‌ها و خوشه‌های متصل را افزایش داد. بالاترین غلظت AcSA (۱/۶ میلی‌مولار) به‌طور کامل رشد و توسعه گل جالیز را در تمام گونه‌های آزمایش شده مهار کرد و افزایش‌هایی در زیست توده میزبان و عملکرد غلاف ایجاد نمود. علاوه براین، این تیمار برگ‌هایی با بالاترین سطوح کلروفیل، فنولیک‌ها و فعالیت آنتی‌اکسیدانی در مقایسه با سایر تیمارها تولید کرد. مطالعات بیش‌تر با استفاده از نرخ‌های نسبتاً بالای AcSA در آزمایش‌های میدانی برای اعتبارسنجی کاربرد عملی آن‌ها ضروری است.

واژگان کلیدی: گل جالیز، باقلا، هولوپارازیت، استیل‌سالیسیلات، رشد، عملکرد