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Physiological and biochemical responses of *Vicia faba L.* to folic acid treatments and their relation to *Aphis fabae* infestation

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ABSTRACT

Background: This study investigated the effects of foliar-applied folic acid (FA) on the growth and biochemical responses of faba bean (Vicia faba L.) under black bean aphid (Aphis fabae) infestation.

Objective: This study aimed to evaluate the effect of foliar-applied folic acid on the growth, biochemical composition, and antioxidant defense system of faba bean (Vicia faba L.) and to determine its relationship with black bean aphid (Aphis fabae) infestation.

Methods: Faba bean plants were treated with folic acid at concentrations of 0, 50, 100, and 150 mg/L under greenhouse conditions. Growth parameters, photosynthetic pigments, metabolites, and antioxidant enzyme activities were measured, and aphid infestation trials were conducted to assess plant resistance responses.

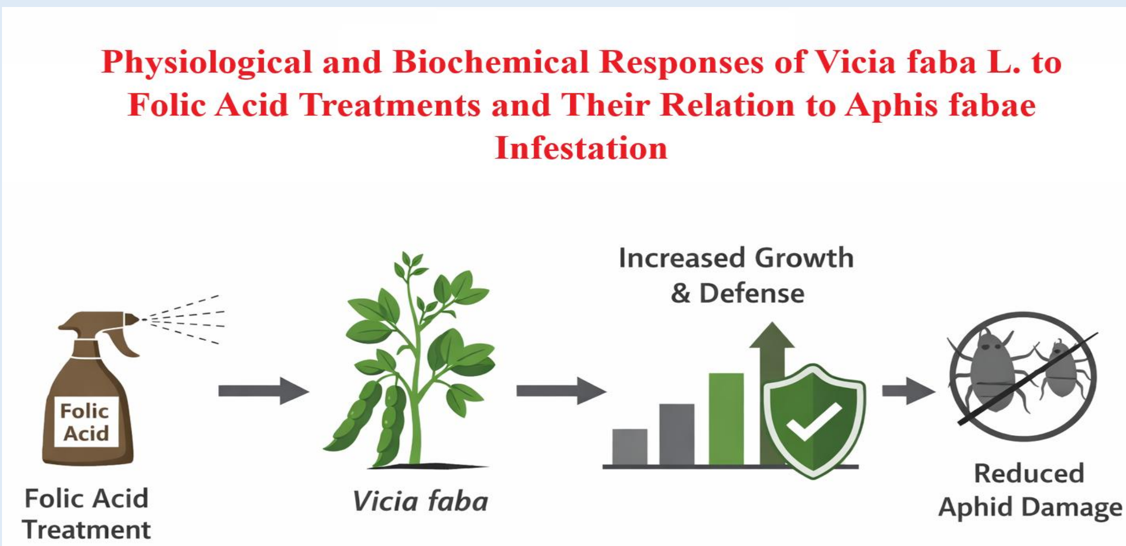
Folic acid significantly improved plant growth, photosynthetic pigments and biochemical composition, with the highest concentration (150 mg/liter) producing the strongest effect. Aphid infestation markedly reduced biomass and pigment content; FA at 100–150 mg/L partially restored these parameters. FA treatments also increased secondary metabolites and antioxidant enzyme activities, infected plants showed a strong defense response, indicating a priming effect against biotic stress.

Results: Folic acid application significantly improved growth parameters of both healthy and aphid-infested green bean plants, with 150 mg/L producing the highest fresh and dry weights. It partially alleviated aphid-induced stress by restoring growth performance compared with untreated infested plants. Treatment also enhanced plant water status and height, indicating improved physiological activity and hormone-like effects. These findings support the potential of folic acid as a biostimulant for sustainable crop productivity and stress tolerance.

Novelty of the study: This study demonstrates a dual role of folic acid as both a growth-promoting biostimulant and defense-promoting agent against Aphis fabae in Vicia faba. Unlike previous studies focused primarily on abiotic stress, this work integrates physiological, biochemical and insect-related responses, supporting folic acid as a sustainable tool to increase crop productivity and resistance under biotic stress.

Conclusion: Folic acid acts as an effective biostimulant that improves growth, photosynthetic efficiency and antioxidant defense in faba bean while reducing aphid-induced damage. These findings support its use as a sustainable strategy to enhance pest tolerance and increase crop productivity.

Keywords: Vicia faba, folic acid, Aphis fabae, antioxidant enzymes, metabolites, plant resistance.



Graphical Abstract: A self-contained graphical abstract has been prepared and submitted as a separate file.

INTRODUCTION

The rapid increase in the global population has created an urgent need for higher food production. Meeting this demand is becoming challenging due to limited arable land, adverse climatic conditions, declining soil fertility and health, and the emergence or re-emergence of crop diseases [1].

Legumes play an important role in the global diet and provide many agronomic and ecological benefits. They improve soil fertility, improve cropping systems and contribute to poverty reduction, food security, nutrition, human health and conservation of natural resources [2-3]. Among legumes, faba bean (Vicia faba L.) is one of the most important species for both human consumption and animal feed due to its high nutritional content of protein, starch and fiber [4- 5]. Faba bean is an important winter crop in many regions [6] and contributes significantly to soil improvement through its ability to fix atmospheric nitrogen [7-8].

Despite its value, faba bean production faces serious constraints, particularly from pests, which cause significant yield and quality losses. Globally, insect pests cause crop losses of up to 60–70% in some tropical regions [9-10]. Although most insect species are harmless, some cause serious damage to humans, livestock and crops [11-12]. Aphids are among the most devastating pests of field crops, especially when populations reach moderate to high levels. They damage plants through direct feeding, formation of dense colonies, honeydew secretion and, most importantly, transmission of plant viruses [13]. Crop loss is particularly serious when infection occurs early, for example at harvest establishment [14].

Black bean aphid (Aphis fabae Scop.; Hemiptera: Aphididae) is the most damaging pest of faba bean worldwide, causing significant economic losses [15-16]. The severity of the infection varies from year to year, influenced by abiotic and biotic factors that shape population dynamics [17-18]. A. Fabe causes damage both directly and indirectly. Direct, stylet penetration into the phloem leads to depletion of essential nutrients, while release of honeydew promotes the growth of sooty fungi, thereby reducing light penetration and photosynthesis [18-19]. Prolonged feeding causes physical stress, curling of leaves, yellowing and stunted growth. Indirectly, A. faba acts as a vector for many pathogenic plant viruses, causing significant yield reduction. In case of severe infection, yield loss in faba beans can exceed 50% [20-21].

The aim of this study is to evaluate the effect of folic acid on the growth, chemical composition and antioxidant defense of faba bean (Vicia faba L.) and investigate its association with infection by black bean aphid (Aphis fabae). Research focuses on whether folic acid improves plant performance and resistance while improving crop quality.

MATERIALS AND METHODS

Plant Material and Growth Conditions: Pure Vicia faba L. seeds were obtained from agricultural fields in Iraq during the 2024 growing season. The seeds were surface sterilized using 1% (v/v) sodium hypochlorite for 3 minutes, rinsed thoroughly with distilled water, and sown in plastic pots (30 cm diameter) filled with a soil mixture of clay and sand. The plants were grown in a greenhouse with 22–25 °C, 16/8 h light/dark photoperiod, and 60–70% relative humidity.

Folic acid (FA; $C_{19}H_{19}N_7O_6$) was obtained commercially (analytical grade, ≥ 98% purity). Foliar sprays in the form of aqueous solutions of FA at concentrations of 0 (control), 50, 100, and 150 mg/L were applied in the present study. The experiment was arranged in a randomized complete block design (RCBD) with three replicates for each treatment.

Collection and Rearing of Aphis fabae: Aphis fabae (black bean aphid) individuals were collected from naturally infested Vicia faba plants in agricultural fields, Iraq, during the early vegetative stage. Infested leaves and shoots were excised with sterilized scissors and transferred in aerated plastic containers (20 × 30 cm) lined with moistened filter paper to maintain humidity, and samples were transported to the laboratory within 2–3 h to minimize stress and mortality. Colonies were carefully examined under a stereomicroscope to exclude predators or parasitoids, and only healthy adults and nymphs were selected for rearing. For colony establishment, aphids were transferred onto healthy potted V. faba seedlings maintained individually in fine mesh insect-proof cages (40 × 40 × 60 cm) under controlled growth chamber conditions (22 ± 2 °C, 65–70% RH, and 16:8 h light: dark photoperiod) [22]. Aphids were allowed to reproduce for 7–10 days, and colonies were sustained by transferring individuals to fresh plants every 5–7 days to ensure a continuous supply of vigorous insects. For experimental infestation, apterous adult aphids were gently collected from the laboratory colony with a fine camel-hair brush, counted under a stereomicroscope, and approximately 30 individuals were released onto the lower surface of leaves of each test plant with minimal tissue disturbance [23].

Time course of the experiment: The experiment was conducted over a period of eight weeks during the 2024 growing season. Vicia faba seedlings were first grown for two weeks under controlled greenhouse conditions before applying folic acid treatments. After foliar application, plants were maintained for six weeks, during which growth parameters, chemical composition, and antioxidant enzyme activities were measured at two-week intervals. Aphid infestation trials were initiated in the third week post-treatment by introducing apterous adult Aphis fabae to designated plants, and population development was monitored for four consecutive weeks. At the end of the experiment, both plant physiological responses and aphid population dynamics were assessed to determine the effects of folic acid.

Physiological and Biochemical Analysis

Growth parameters: After 30 days of treatment, fresh and dry biomass were recorded. Relative water content (RWC) and membrane stability index (MSI) were also determined according to the method depicted by Weatherley (1950) and Sairam et al. (1997)

$$RWC\ (\%) = [(FW - DW) / FW] \times 100$$

$$MSI = [(EC1/EC2) - 1] \times 100$$

Where, FW = fresh weight, DW = dry weight, EC1 and EC2 are the electrical conductivities measured after mild and high temperature exposure, respectively.

Estimation of photosynthetic pigments: Photosynthetic pigments, including chlorophyll a, chlorophyll b, and carotenoids, were extracted from fresh leaf tissue and quantified spectrophotometrically. Chlorophylls were estimated following the method of Arnon (1949), while carotenoids were measured according to Horváth et al. (1972), as modified and adopted by Kissimon (1999), using specific absorbance at characteristic wavelengths.

$$\text{Chlorophyll a} = 10.3\ E663 - 0.918\ E644$$

$$\text{Chlorophyll b} = 19.7\ E644 - 3.87\ E663 \quad \text{Carotenoids} = 5.02\ E480$$

Estimation of some metabolites: Carbohydrates content was analyzed by the method of Hedge and Hosreiter (1962). The total nitrogen was determined by the conventional semi-micropropagation of Kjeldahl method of Rees and Williams (1943). Protein content in the plant extract was estimated by using spectrophotometer according to Bradford (1976). Crude fibre and fats were estimated according to AOAC (1990). Total phenols could be estimated by the Folin- Ciocalteau reagent [24]. Quantitative determination of alkaloid was performed according to the methodology by Harborne (1973).

Extraction and estimation of antioxidant enzymes activity: For polyphenol oxidase (PPO), peroxidase (POX) and catalase (CAT), the extraction medium was 0.1 M phosphate buffer at pH 6.8 was used where all the operations were carried out at 4°C (Agarwal and Shaheen, 2007). PPO and POX activities were assayed according to Devi (2002) while the catalase activity was assayed by the method of Sinha (1972) as modified by Gopalakrishnan and Starlin (2013).

Statistical Analysis: One-way ANOVA was used to examine the data, and Duncan's multiple range test was used to compare the means at a significance level of $p < 0.05$. SPSS software was used to conduct statistical analysis (version 20).

RESULTS AND DISCUSSION

Growth parameters: The study showed that folic acid significantly improved growth parameters in both healthy and aphid-infested green bean plants. In fresh samples, application of 150 mg/L folic acid resulted in the highest fresh weight (52.00 g/plant) and dry weight (10.66 g/plant), indicating its role as a growth stimulant, possibly by improving metabolic processes such as photosynthesis and nutrient uptake [25]. Aphid-infested plants showed an approximately 30% reduction in fresh weight compared to healthy controls, but folic acid at a 100–150 mg/L partially restored growth, suggesting its ability to lessen insect-triggered stress [26]. In addition, dealt with flowers retained higher water percentage (~81%), which is consistent with findings that foliar applications improve water retention via regulating stomatal conductance [27]. There was a substantial increase in plant height with folic acid, specifically at 150 mg/L, that is steady with studies linking folic acid to auxin-like effects that promote cell growth [28]. The observed mitigation of aphid-associated damage supports the hypothesis that folic acid complements plant immunity, potentially by regulating defense-related genes [29-30].

These findings are in line with current reviews highlighting the role of biostimulants in sustainable crop productivity and useful food-oriented agriculture [36-37].

Table 1. Effect of different concentrations of folic acid on growth parameters of healthy and infested samples of faba bean shoot.

Group	Treatments	Conc.	Fresh weight	Dry weight (g/plant)	Water percentage	Plant height(cm)
			Healthy samples			
1	**Control**		**40.00 ± 0.59^{d}**	**8.85 ± 0.38^{d}**	**80.50 ± 0.52^{ab}**	**65.0 ± 1.2^{d}**
		50	46.00 ± 0.72^{bc}	9.70 ± 0.22^{c}	81.38 ± 0.66^{a}	75.0 ± 1.6^{bc}
2	**Folic acid spray**	100	50.00 ± 0.65^{b}	10.45 ± 0.48^{ab}	81.38 ± 0.76^{a}	82.0 ± 1.9^{a}
		150	52.00 ± 0.81^{a}	10.66 ± 0.49^{ab}	81.77 ± 0.77^{a}	84.5 ± 2.1^{a}
			Infested samples			
3	**Control (aphid)**		**28.00 ± 0.57^{e}**	**7.29 ± 0.44^{e}**	**78.54 ± 0.59^{bc}**	**50.0 ± 1.0^{e}**
	Folic acid spray	50	37.00 ± 0.89^{de}	8.91 ± 0.33^{d}	78.12 ± 0.95^{c}	60.0 ± 1.5^{de}
4	**+**	100	41.00 ± 0.60^{d}	9.61 ± 0.24^{c}	78.67 ± 0.80^{bc}	70.0 ± 1.8^{c}
	Aphid	150	43.00 ± 0.76^{cd}	9.22 ± 0.35^{cd}	81.15 ± 0.96^{ab}	74.5 ± 1.9^{bc}
	LSD0.05		**2.5**	**0.8**	**2.5**	**3.5**

Values are mean (n = 3), different superscript letters within each treatment (column) express significant differences at a probability level of 0.05 (Duncan's test).

Photosynthetic pigments: The study highlights the vital function folic acid has in growing the content of photosynthetic pigments in both healthy and aphid-infested inexperienced bean vegetation. In healthy plants, application of 150 mg/l folic acid resulted inside the highest degrees of chlorophyll a (0.668 mg/g FW), chlorophyll b (0.259 mg/g FW) and total pigments (1.A hundred and seventy mg/g FW), demonstrating its effectiveness in selling photosynthetic efficiency. These findings are constant with the research of Alsamadani et al. (2022), who pronounced that foliar application of nutrients,including folic acid, can stimulate chlorophyll biosynthesis by using upregulating key enzymes concerned in pigment synthesis.

Table 2. Effect of different concentrations of humic acid and folic acid on growth parameters of healthy and infested samples of faba bean shoot.

Group	Treatment	Conc. (mg/L)	Chl. a	Chl. b	Chl. a + Chl. b	Chl. a / Chl. b	Carotenoids	Total Pigments
Healthy								
1	Control	0	0.625 ± 0.003a	0.242 ± 0.001a	0.867 ± 0.004a	2.58 ± 0.02a	0.251 ± 0.001a	1.098 ± 0.005a
2	Folic acid spray	50	0.652 ± 0.005c	0.253 ± 0.002c	0.905 ± 0.007c	2.58 ± 0.02a	0.239 ± 0.001c	1.144 ± 0.008c
		100	0.660 ± 0.006cd	0.256 ± 0.003cd	0.916 ± 0.008cd	2.58 ± 0.02a	0.271 ± 0.001cd	1.157 ± 0.009cd
		150	0.668 ± 0.006d	0.259 ± 0.003d	0.927 ± 0.009d	2.58 ± 0.02a	0.283 ± 0.001d	1.170 ± 0.010d
Infested								
3	Control	0	0.598 ± 0.003e	0.232 ± 0.001e	0.830 ± 0.004e	2.58 ± 0.02a	0.225 ± 0.001e	1.055 ± 0.004e
4	Folic acid spray + Aphid	50	0.635 ± 0.005b	0.246 ± 0.002b	0.881 ± 0.007b	2.58 ± 0.02a	0.254 ± 0.001b	1.115 ± 0.008b
		100	0.648 ± 0.005cd	0.251 ± 0.002cd	0.899 ± 0.007cd	2.58 ± 0.02a	0.258 ± 0.001cd	1.137 ± 0.008cd
		150	0.656 ± 0.006cd	0.254 ± 0.003cd	0.910 ± 0.008cd	2.58 ± 0.02a	0.260 ± 0.001cd	1.150 ± 0.009cd
LSD 0.05			**0.012**	**0.008**	**0.005**	**NS**	**0.016**	**0.025**

Values are average (n = 3), Different superscript letters within each treatment (column) express significant variation at a probability level of 0.05 (Duncan's test).

Aphid infestation reduced the content of chlorophyll a and b by approx. 4.3% and 4.1% compared to healthy controls, possibly due to insect-induced stress disrupting chloroplast integrity (Tan et al., 2025). However, folic acid at 150 mg/L restored the chlorophyll level in infected plants to near healthy values (0.656 mg/g FW for Chl. b and 0.254 mg/g FW for Chl. b), suggesting its potential as a biotic stress reducer. This is consistent with the study by Boubakri et al. (2016), who found that foliar vitamins can increase plant resilience by maintaining photosynthetic machinery under insect pressure.

In particular, carotenoid content responded differently, with a slight decrease observed at low folic acid concentrations (50 mg/L) in healthy plants, but peaking at 150 mg/L (0.283 mg/g FW). In infected plants, the carotenoids increased with folic acid treatment, possibly due to their role as antioxidants under stress) [31]. The stability of Chl. a/Chl. b ratio (2.58 in all treatments) suggests that folic acid maintains the structural balance of photosynthetic complexes, as observed in other legumes under nutrient supplementation [32].

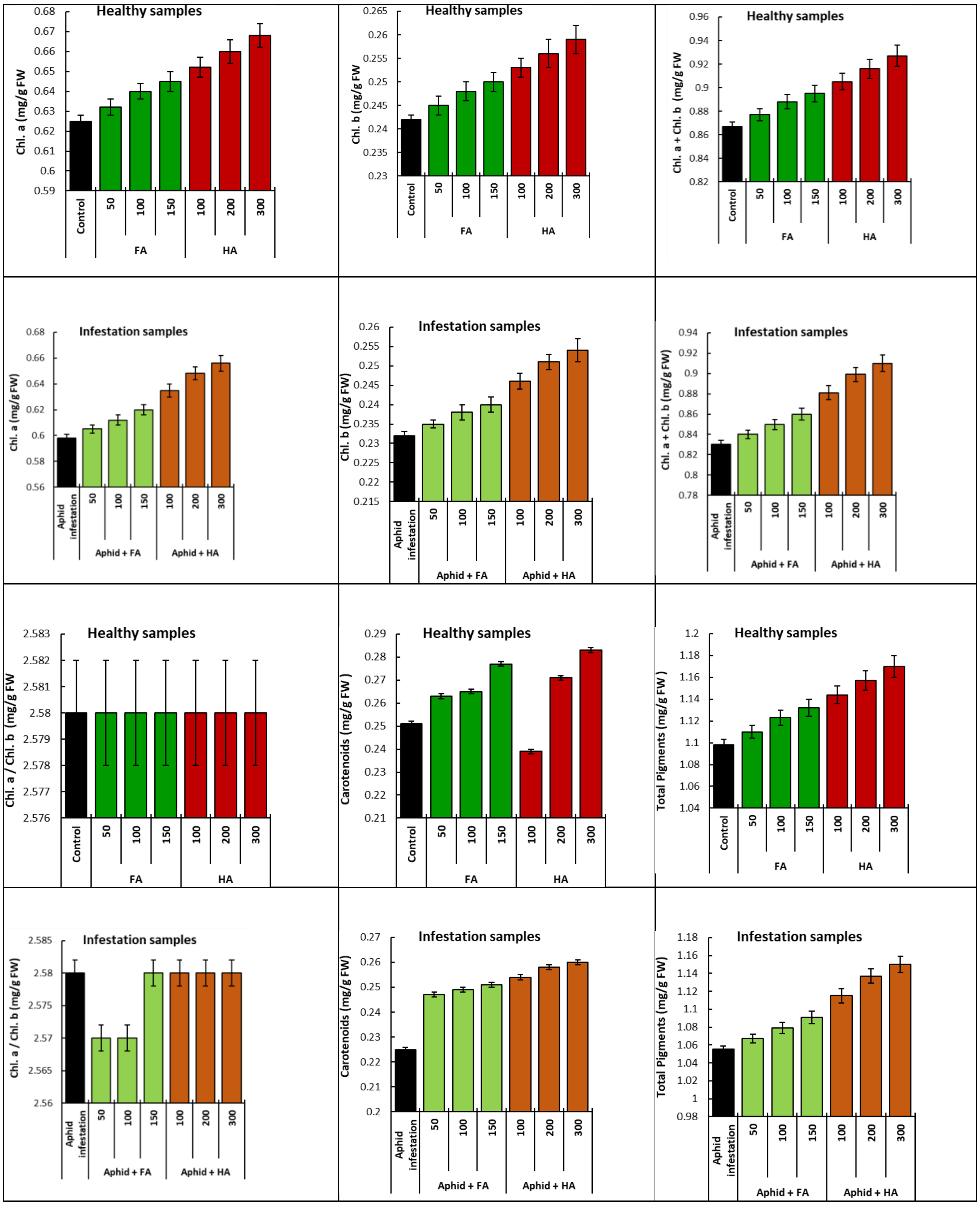


Figure 1. Effect of different concentrations of humic acid and folic acid on Chl. a, Chl. b and Chl. a + Chl. b of healthy and infested samples of faba bean shoot**.**

Estimation of some metabolites: The study showed that folic acid significantly influenced the proximal structure of faba bean shoots, with different responses observed between healthy and aphid-infected plants. In healthy plant life, utility of folic acid at 150 mg/L accelerated protein content to fourteen.76 g/100 g, which is almost double the values found at decrease concentrations, possibly because of extended nitrogen absorption and amino acid synthesis [33]. In assessment, aphid-inflamed flowers confirmed increased tiers of carbohydrates (72.99 g/100 g in the control), indicating a stress-induced shift in the direction of power storage, which become partly reversed through folic acid remedy (63.19 g/100 g at 150 mg/L). The maximum concentration of fiber content found in healthy plants was 150 mg/liter (6.63 g/hundred grams), while a sharp reduction was observed in infected plants at the same concentration (2.8 g/100 g), indicating possible disruption of fiber synthesis pathways under insect pressure. The degree of fat allowed to be repaired. 100 mg/L is required. (4.84 g/100 g), consistent with the conclusion that leaf spray can repair lipid membranes damaged by biological stress) [35]. These results emphasize the dose-dependent and plant health-specific response of folic acid, highlighting its dual role in promoting uptake and reducing stress.

Such metabolic improvements support the emerging concept that agricultural biostimulants contribute directly to the functional value of crops in food systems [38-39].

Table 4. Effect of different concentrations of humic acid and folic acid on proximate composition of healthy and infested samples of faba bean shoot.

Group	Treatments	Conc. (mg/L)	Fibers (g/100g fresh wt.)	Fat (g/100g fresh wt.)	Total nitrogen (g/100g)	Protein (g/100g fresh wt.)	Total carbohydrates (g/100g)
	Healthy samples						
1	Control	–	6.03 ± 0.22^{b}	1.70 ± 0.16^{e}	1.86 ± 0.04^{g}	12.15 ± 0.12^{c}	65.62 ± 0.79^{e}
2	Folic acid spray	50	6.03 ± 0.29^{b}	4.99 ± —	2.36 ± 0.03^{d}	8.46 ± 0.09^{g}	62.34 ± 1.32^{g}
		100	5.12 ± 0.20^{c}	3.44 ± —	2.08 ± 0.02^{f}	14.64 ± 0.30^{a}	62.34 ± 0.77^{g}
		150	6.63 ± 0.15^{a}	4.19 ± 0.10^{a}	2.72 ± 0.04^{b}	14.76 ± 0.21^{a}	60.87 ± 0.83^{h}
	Infested samples						
3	Control	–	5.12 ± 0.19^{c}	4.81 ± —	1.78 ± 0.02^{h}	13.66 ± 0.14^{b}	72.99 ± 0.54^{b}
4	Folic acid spray + Aphid	50	7.26 ± 0.23^{a}	1.95 ± 0.10^{e}	3.07 ± 0.06^{a}	11.08 ± 0.18^{d}	74.55 ± 0.91^{a}
		100	4.42 ± 0.15^{d}	4.84 ± —	1.90 ± 0.02^{g}	8.85 ± 0.17^{f}	72.49 ± 1.07^{b}
		150	2.80 ± 0.12^{e}	3.62 ± —	2.53 ± 0.03^{c}	11.47 ± 0.11^{d}	63.19 ± 0.91^{f}
	$LSD_{0.05}$		**0.58**	**0.35**	**0.09**	**0.47**	**2.43**

Values are means (n = 3). Different superscript letters within each treatment (column) indicate significant differences at a probability level of 0.05 (Duncan's test).

Application of folic acid significantly increased the production of secondary metabolites in faba bean shoots, an effect that was particularly pronounced in aphid-infested plants. In healthy plants, folic acid at 150 mg/L increased the phenolic content by 49.5% (232.0 mg/g vs. 155.2 mg/g in the control), flavonoids by 63.3% (46.7 mg/g vs. 28.6 mg/g) and tannins by 749.1 mg/g (vs. 349.1 mg/g), a clear improvement. Shows a dose-dependent response. These results are consistent with the established role of foliar-applied vitamins in stimulation of the phenylpropanoid pathway [36]. Remarkably, aphid-infected plants showed an even greater increase, with 150 mg/liter folic acid increasing the phenolics to 288.3 mg/g (62.3% increase over the metabolic response of the infected plant), indicating potent metabolic responses. This enhanced effect supports the defense priming phenomenon, where initial stress sensitizes plants to subsequent triggers [37]. The differential response between healthy and stressed plants may reflect altered resource allocation strategies, where infected plants prioritize the production of defense compounds [38]. The particularly strong induction of 5% infected plants is infected plants (73.) ecologically important given their established role in insect deterrence [39]. These findings suggest that folic acid acts upstream in the shikimate pathway, as well as increasing several defense-related metabolites. While the results show the potential of folic acid as a plant defense inducer, further studies under field conditions are needed to confirm efficacy and optimize application rates.

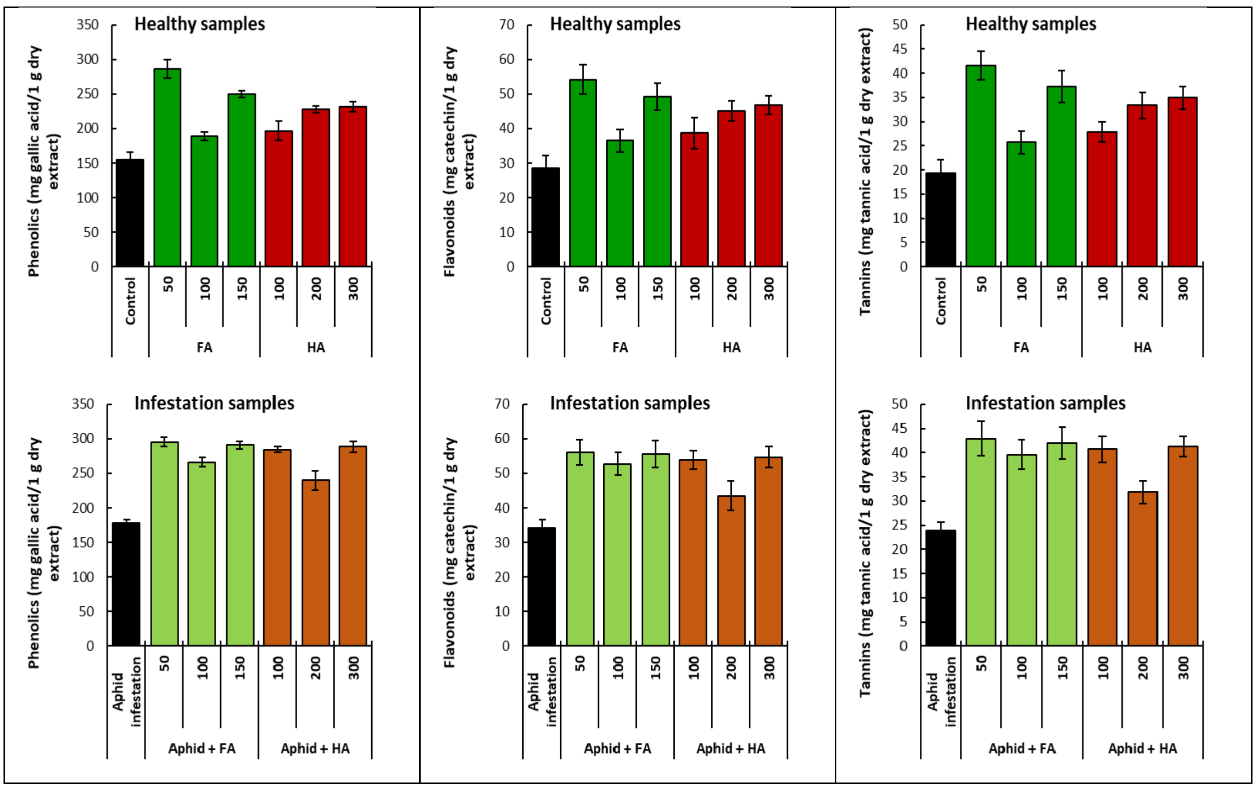


Figure 2. Effect of different concentrations of folic acid on secondary compounds of healthy and infested samples of faba bean shoot.

Antioxidant enzymes activity: The study showed that folic acid significantly enhanced antioxidant enzyme activities in faba bean shoots, with different responses between healthy and aphid-infested plants. In healthy plants, 150 mg/L folic acid increased the activities of PPO, POX and CAT by 69.1%, 76.3% and 105.5%, respectively, compared to the control, indicating dose-dependent stimulation of oxidative defense system. Aphid infection initially suppressed enzyme activities (PPO reduced by 20.6% in infected controls), consistent with stress-induced metabolic disruption [40]. However, application of folic acid at 150 mg/L not only reversed this suppression but also increased PPO, POX, and CAT activities to levels higher than healthy controls by 102.1%, 95.9%, and 133.2%, respectively. This stronger response in infected plants suggests that biotic stress amplifies the effect of folic acid inducing, possibly through priming of defense-related pathways. The coordinated upregulation of all three enzymes suggests systemic activation of the ROS scavenging network rather than stimulation of a single pathway. Of particular note is the approximately twofold increase in CAT activity, which improves H_2O_2 detoxification capacity – a critical process for maintaining redox homeostasis under stress. These findings present folic acid as a promising biostimulant for integrated pest management, although field validation of its protective effect against aphid damage is needed.

Table 5. Effect of different concentrations of folic acid on antioxidant enzymes activity of healthy and infested samples of faba bean shoot.

Group	Treatments	Conc. (mg/L)	PPO (µg/g FW/min)	POX (µg/g FW/min)	CAT (µmol H_2O_2/min/mg protein)
	Healthy samples				
1	Control	–	15.32 ± 0.41^{c}	22.45 ± 0.57^{d}	10.18 ± 0.33^{d}
3	Folic acid spray	50	19.84 ± 0.52^{bc}	28.14 ± 0.62^{c}	13.17 ± 0.41^{c}
		100	23.12 ± 0.57^{ab}	34.82 ± 0.71^{ab}	17.26 ± 0.47^{b}
		150	25.91 ± 0.63^{a}	39.57 ± 0.76^{a}	20.93 ± 0.53^{a}
	Infested samples				
4	Control	–	12.16 ± 0.37^{d}	18.45 ± 0.55^{e}	8.31 ± 0.28^{e}
6	Folic acid spray + Aphid	50	16.18 ± 0.47^{c}	24.92 ± 0.63^{d}	12.04 ± 0.39^{d}
		100	20.73 ± 0.55^{b}	30.47 ± 0.69^{bc}	15.72 ± 0.44^{c}
		150	24.58 ± 0.62^{a}	36.15 ± 0.75^{a}	19.38 ± 0.52^{ab}
	$LSD_{0.05}$		**1.27**	**1.65**	**1.12**

Values are average (n = 3), Different superscript letters within each treatment (column) express significant variation at a probability level of 0.05 (Duncan's test).

Alignment with the FFC 17-Step Model: According to the Functional Food Center's 17-step Functional Food Product Development Model, the present study primarily supports Steps 1–3, which involve the identification of bioactive compounds, evaluation of their biological effects, and validation of their functional roles. By demonstrating the biostimulant and defense-priming effects of folic acid in Vicia faba L. under aphid stress, this research provides foundational evidence supporting folic acid as a functional bioactive agent in sustainable agriculture and functional food systems and motivates subsequent steps, including dose optimization, safety evaluation, and field-scale validation [39-40].

CONCLUSIONS

This study demonstrated that foliar application of folic acid enhances the growth, physiological traits, and defense capacity of faba bean (*Vicia faba* L.). The highest concentration (150 mg/L) significantly improved biomass (52.0 g/plant vs. 40.0 g in controls), chlorophyll a (0.668 mg/g FW), and protein content (14.76 g/100 g). In aphid-infested plants, folic acid reduced stress- induced carbohydrate accumulation and boosted antioxidant enzyme activities, notably catalase (19.38 µmol H_2O_2/min/mg protein). These results indicate that folic acid functions as a biostimulant, improving plant productivity and tolerance to Aphis fabae, offering a sustainable approach to pest management and yield improvement.

Abbreviations: FA: foliar-applied folic acid; RCBD: randomized complete block design; PPO: polyphenol oxidase, POX: peroxidase; CAT: catalase; RWC: Relative water content, and MSI: membrane stability index.

Conflicts of Interest: The authors declare no conflicts of interest.

Author Contributions: All authors contributed equally to this work.

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