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RESEARCH ARTICLE

Effect of seed treatment with salicylic acid, humic acid and zinc on the growth rate of broad bean seedlings (*Vicia faba* L.)

Azhar Taher Sleibi*

Al-Furat Secondary School for Distinguished Students, Directorate of Education, Baghdad, Al-Karkh I, Iraq

*Correspondence email - azhar.Taher2202p@ihcoedu.uobaghdad.edu.iq

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Abstract

Seed germination and early seedling growth are critical stages that determine successful crop establishment and productivity in grain legumes. However, limited information is available regarding the comparative physiological effects of individual seed treatments with salicylic acid (SA), humic acid (HA) and zinc (Zn) on faba bean seedlings. This study aimed to evaluate the physiological effects of individual seed treatments with SA, HA and Zn on the germination performance and growth of faba bean (Barcino cultivar) seedlings. Seeds were sterilized and soaked in different concentrations of each treatment (SA at 0, 50, 150, 200 mg/L; HA at 0, 100, 200, 300 mg/L; Zn at 0, 200, 300, 400 mg/L). Germination and seedling vigour were assessed using standard indices. The results showed significant superiority of all treatments compared to the control. Salicylic acid at 150 mg/L was the most effective treatment, recording the highest values for the final germination percentage (98.21 %), the lowest average germination time (3.4 days) and the highest germination index (37.21), in addition to its superiority in all vegetative growth traits and vitality indicators. Humic acid followed in performance at 300 mg/L, followed by Zn at 300 mg/L. This study concludes that seed pre-treatment with 150 mg/L SA is a promising approach enhancing germination and early seedling growth in faba bean. The findings highlight the importance of determining the optimal concentration for each biostimulant to avoid potential inhibitory effects at higher doses.

Keywords: germination; humic acid; salicylic acid; seed priming; seedling vigour; *Vicia faba* L.; zinc sulfate

Introduction

As a living entity, seed quality inevitably declines from the time of harvest until it is sown in the field if not managed with appropriate care (1). The application of micronutrients as a seed treatment has proven effective for improving early seedling vigour, crop establishment, growth and final yield. Additionally, this approach helps to correct specific nutrient deficiencies present in the soil (2). A range of methodologies, encompassing both agronomic and genetic techniques, are employed to increase micronutrient levels in various agricultural crops. Agronomic biofortification, which involves the exogenous application of micronutrients to plants, is a widely used strategy to increase nutrient concentrations in edible grains; for example, zinc (Zn) can be supplied as zinc sulfate ($ZnSO_4$) via soil or foliar application, or through seed priming, to improve Zn nutrition in crop plants (3). This approach utilizes micronutrient-rich fertilizers such as $ZnSO_4$, ferrous sulfate ($FeSO_4$) and chelated forms such as Zn -EDTA, which can be applied through several methods to effectively enhance the nutrient content of grains during the biofortification process (4, 5). Techniques including soil and foliar application and seed priming are used to apply micronutrients (6–8). Seed priming is a cost-effective technique for administering fertilizers, particularly under adverse growing conditions (9). This pre-sowing treatment induces a physiological state in seeds that enhances germination performance. The process involves partially hydrating seeds to a point that precludes radicle emergence (10–12). Typically, seeds are immersed in aerated nutrient solutions and subsequently dried back to their original moisture content (2, 13).

Salicylic acid ($C_6H_4(OH)COOH$), a phenolic compound, plays a significant regulatory role in various physiological and developmental processes in plants. These include modulating photosynthetic activity, stomatal conductance, oxidative phosphorylation and mitochondrial electron transport, as well as enhancing innate immunity against oxidative stress (14–17). It also promotes flowering by counteracting the inhibitory effect of amino-oxyacetic acid under poor-nutrition (nutrient-deficiency) stress conditions (18). Furthermore, SA increases the activity of nitrogenase and nitrate reductase enzymes, leading to improved fertilizer use efficiency (19). Its regulatory function extends to flowering through involvement in photoperiodic pathways and to the modulation of senescence-related genes (20, 21). Furthermore, SA modulates the activity of key apoplastic proteins and enzymes, including catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POX), guaiacol peroxidase (GPO), ACC-oxidase and ortho-hydroxycinnamic acid. It also exerts a broader regulatory influence on overall plant growth and developmental processes (22).

Humic acid (HA) is a natural organic compound commonly extracted from lignite or leonardite deposits containing ancient, fossilized organic matter. It offers extensive benefits for both soil and plants. It significantly enhances seed germination through a process called seed priming and promotes robust root development in cuttings by stimulating natural rooting hormones. Acting as a powerful natural chelating agent, it unlocks and makes available essential macro- and micronutrients (such as phosphorus, calcium, iron, zinc and manganese) that are often immobilized in the soil, thereby also helping to alleviate soil salinity and improve problematic soils such as red and calcareous soils. Furthermore, it improves the soil's physical structure by darkening its color to absorb more heat, preventing crusting and cracking to protect roots and enhancing aeration and water retention. Humic acid is predominantly negatively charged, enhancing cation retention and reducing the bioavailability of certain toxic metals, which can indirectly favour beneficial soil microbial activity. Physiologically, it activates vital enzymes and energy-related processes within the plant, leading to stronger roots, increased cell division, improved stress tolerance due to higher osmotic pressure and ultimately earlier production and higher-quality fruits (23).

Zinc is one of the vital micronutrients for plants, as it has many critical functions. It acts as a cofactor for more than 300 enzymes and is also required for the production of tryptophan, which is a precursor of auxin (24). Zinc also plays a key role in maintaining the integrity of biological membranes, protein synthesis, photosynthesis, pollen formation and disease resistance (25). Moreover, more than 3000 proteins contain Zn prosthetic groups in their structure (26).

Treating seeds with Zn has been shown to enhance early seedling development, leading to better crop establishment. This method also serves to alleviate soil Zn deficiency, which contributes to increased yields and enriched Zn content in the grain of numerous crops (27–29), as seeds with low Zn contents showed delayed germination and poor seedling vigour, which negatively affected plant growth and final grain yield (30).

Faba bean (*Vicia faba* L.), also known as broad bean, is an annual cool-season legume (Fabaceae) cultivated widely for its protein-rich edible seeds. Beyond its importance as a food and feed crop, faba bean contributes to soil fertility through symbiotic nitrogen fixation and is often integrated into crop rotations and green-manure systems (31). Broad bean protein contains essential amino acids and high concentrations of niacin, lysine and folic acid, also it has high levels of minerals mainly iron and Zn (32, 33). *Vicia faba* is cultivated mainly for its fresh green seeds (shelled) and mature dry seeds, although the pods may also be consumed at a very young stage. As a legume, it fixes atmospheric nitrogen and is commonly used in rotations, supporting soil fertility and soil health (34). Broad beans grow in temperate and warm climates and the optimum temperature for seed germination ranges from 20–25 °C.

Broad beans are an important crop in Iraq, both nutritionally and economically. However, their production faces challenges that affect cultivated area and seedling production. Therefore, the main objective of this research was to evaluate the physiological effects of individual treatments of organic (HA), mineral (Zn) growth stimulants and plant growth regulator (SA) on the germination and growth rate of broad bean seedlings. This study contributes to bridging the research gap in Iraq, where such advanced studies on vegetable crops, especially broad beans are rare and essential for the development of agricultural practices.

Materials and Methods

Plant material and seed sterilization

Seeds of the Barcino broad bean variety (*V. faba*) were used in this study. Prior to treatment, seeds were surface-sterilized to eliminate microbial contaminants following a previously described protocol (35). Seeds were washed sequentially in 10 % (v/v) neutral detergent, rinsed, briefly immersed in 70 % (v/v) ethanol (30–60 sec) and then treated with 2.5 % (v/v) sodium hypochlorite for 15 min. Finally, seeds were rinsed three times with autoclaved distilled water and blotted dry on sterile filter paper before use (35).

Experimental design and treatment application

The experiment was conducted under controlled laboratory conditions at the University of Baghdad, Iraq in 2024. Three separate completely randomized design (CRD) experiments were conducted (SA, HA and $ZnSO_4$), each with four treatments (including the control) and four replications per treatment. Each replication consisted of 25 seeds. Seeds were treated with one of three bioactive compounds: SA, HA or Zn ($ZnSO_4 \cdot 7H_2O$). For each compound, four concentration levels were tested, including a zero-concentration control.

Salicylic acids treatments

Seeds were soaked for 24 hr in aqueous solutions of 99.5 % pure SA at concentrations of 0 (control), 50, 150 and 200 mg L^{-1}. To facilitate dissolution, the SA was first dissolved in 1 mL of 96 % ethanol before being brought to the final volume with distilled water. An equivalent amount of ethanol (1 mL L^{-1}) was added to the control treatment (0 mg L^{-1}) to account for any solvent effects (36).

Humic acid treatments

Seeds were soaked for 12 hr in aqueous solutions of HA at concentrations of 0 (control), 100, 200 and 300 mg L^{-1} with gentle agitation at 25 °C (37). To prepare the required solutions, 0.5 g KOH was dissolved in 500 mL distilled water. The required amount of HA was then slowly added to the alkaline solution while stirring vigorously for 15–20 min until fully dissolved.

Zinc treatments

Seeds were soaked for 8 hr in $ZnSO_4 \cdot 7H_2O$ (MW = 287.56 g mol^{-1}, purity ≥ 99 %) solutions at concentrations of 0 (control), 200, 300 and 400 mg L^{-1} (ppm). The solutions were prepared by dissolving 0.2, 0.3 and 0.4 g of $ZnSO_4 \cdot 7H_2O$ respectively, in distilled water and making up to a final volume of 1 L.

Following all soaking treatments, seeds were removed from the solutions, rinsed briefly with distilled water and after priming, surface air-dried on sterile filter paper at room temperature (25 ± 2 °C) until no free water remained on the seed surface and they regained their constant initial weight.

Germination bioassay and measured parameters

Twenty five treated and dried seeds from each treatment (with four replications) were placed in 9 cm Petri dishes on two layers of moist germination paper (Germitest), covered with a third layer and moistened with distilled water equivalent to 2.5 times the dry weight of the paper. The Petri dishes were placed in an incubator set at 25 °C with a 16/8-hr light/dark photoperiod and 65 % relative humidity and germination was monitored daily (38).

Germination was counted daily and the final germination percentage (FGP %) was recorded on the eighth day after sowing in accordance with standard seed testing rules and calculated (36) as:

% FGR =

(Total seeds germinated/Total number of seeds) x 100

(Eqn.1)

Mean germination time (MGT) was calculated using the formula (39):

SVI - II =

FGP % x Seedling dry weight (g) (Eqn. 2)

Germination index was also measured as following:

$$GI = \sum (Gt/t)$$

(Eqn. 3)

Where, Gt is the number of germinated seeds on day t (40).

On the 10th day, ten normal seedlings were randomly selected from each replication. Shoot length (SHL) measured from the point of seed attachment to the tip of the plumule and root length (RL) along from the point of seed attachment to tip of the root were measured in cm. Seedlings were then oven-dried at 70 °C for 48 hr to obtain a constant seedling dry weight (SDW).

Finally, seed vigour index I (SVI-I) was calculated as an indicator of overall growth performance (41) and SVI-II as an indicator of biomass accumulation (41).

SVI - I =

FGP % x Total seedling length (cm) (Eqn. 4)

SVI - II =

FGP % x Seedling dry weight (g) (Eqn. 5)

Data analysis

The experiment was arranged in a CRD. The results were subjected to analysis of variance (ANOVA) and the significant differences of all means were compared using the least significant difference test (LSD) at a significance level of $p \leq 0.05$ using SPSS V.21.

Results and Discussion

The results presented in Table 1 clearly demonstrate that treating Barcino broad bean seeds with SA, HA or Zn significantly improved germination parameters compared to the untreated control.

Among all treatments, SA at a concentration of 150 mg/L proved to be the most effective, achieving the highest FGP (98.21 %), the shortest MGT (3.4 days) and the highest GI (37.21). This finding is consistent with previous studies reporting that SA treatment increased the germination percentage in *Arabidopsis* (42). Similarly, a maximum germination percentage of 95.83 % was observed in pea plants treated with 300 mg/L SA (43). The efficacy of SA is likely due to its role as a signalling molecule that promotes key enzymatic activities crucial for breaking seed dormancy and initiating growth (44). It is important to note that the positive effect of SA was concentration-dependent, as a higher dose of 200 mg/L resulted in a significant decline in performance, making it almost similar to the control. This suggests a hormetic response, where low doses are stimulatory, but high doses can become toxic or inhibitory to the seeds.

For HA, the positive effects on germination became more pronounced as the concentration increased. The optimal results were observed at 300 mg/L, which yielded a high FGP (96.42 %), a short MGT (3.41 days) and a strong GI (35.61). The results of this study agreed with a previous study that found an increase in germination percentage in corn seeds treated with HA; however, no significant differences were observed among the doses used. This may be due to the high baseline germination of faba bean (*V. faba*), which could mask HA effects on germination (45). The beneficial impact of HA can be attributed to its multifaceted mechanism of action: it enhances plant respiration and stimulates oxidative phosphorylation, leading to increased ATP production. This energy boost consequently improves the absorption and transport of nutrients and the biosynthesis of compounds essential for growth. Finally, it is important to note that HA particularly affects the germination of different plant species depending on its source and dosage, as an inhibitory effect on germination has been observed at high doses of HA (46).

Similarly, Zn application showed a progressive improvement with increasing concentration. The best results for Zn were achieved at the highest tested concentration of 300 mg/L, with an FGP of 94.21 %, an MGT of 3.59 days and a GI of 33.34. The results of this study agreed with a previous study reporting that spinach seed germination performance and germination speed increased after seed treatment with Zn (47). Additionally, the results of this study were consistent with previous reports showing that $ZnSO_4$ treatment of tobacco seeds enhanced germination potential, germination index and shortened mean germination time (48).

The improvement in germination indicators of Zn-treated seeds is attributed to the stimulation of enzymes such as α-amylase, which accelerates the breakdown of food reserves and enhances energy supply to the growing embryo (10). In addition, Zn plays a

Table 1. Effect of seed treatment with SA, HA or Zn on germination parameters of Barcino broad bean seeds

Treatment	Concentration (mg/L)	Final germination percentage (FGP, %)*	Mean germination time (MGT, days)*	Germination index (GI)*
Control	0	86.65±2.34^{c}	4.6±0.22^{a}	24.98±1.12^{d}
SA	50	93.41±1.98^{b}	3.7±0.15^{b}	33.4±1.32^{b}
SA	150	98.21±1.05^{a}	3.4±0.16^{c}	37.21±0.94^{a}
SA	200	88.05±1.42^{bc}	4.1±0.23^{b}	27.6±1.21^{c}
HA	100	90.01±2.21^{b}	3.84±0.24^{b}	29.54±1.28^{bc}
HA	200	94.35±1.67^{ab}	3.54±0.12^{bc}	33.15±1.08^{b}
HA	300	96.42±1.45^{a}	3.41±0.19^{c}	35.61±0.11^{ab}
Zn	100	86.54±2.01^{bc}	4.05±0.31^{b}	27.74±1.21^{cd}
Zn	200	90.15±1.61^{b}	3.82±0.14^{b}	30.74±0.87^{bc}
Zn	300	94.21±2.12^{a}	3.59±0.24^{c}	33.34±1.23^{b}

*Values are mean ± standard error (n=4). Means within a column followed by different letters are significantly different according LSD test at $p \leq 0.05$. SA: Salicylic acid, HA: Humic acid, Zn: Zinc.

crucial role in the synthesis of DNA, RNA and proteins during seed priming (49).

In conclusion, while all treatments were beneficial, their efficacy and optimal concentrations varied. Zinc showed a positive but generally less pronounced effect compared to the organic biostimulants (SA and HA). Humic acid performed best at a high concentration (300 mg/L), promoting growth through enhanced energy metabolism and nutrient uptake. However, SA at 150 mg/L was the most effective treatment overall, acting as a powerful physiological trigger for germination. The decline in its efficacy at 200 mg/L underscores the critical importance of identifying the precise optimal concentration for each biostimulant to avoid inhibitory effects.

The effect of different seed treatments on the growth and vigour of Barcino broad bean seedlings is presented in Table 2. All treatments (SA, HA and Zn) demonstrated a significant positive effect ($p \leq 0.05$) on all measured growth parameters compared to the untreated control.

Salicylic acid at 150 mg/L showed the highest performance in all studied traits, as it recorded the highest values for stem length (12.87 cm), root length (9.48 cm), dry weight (68.14 mg) and vigour indices (2187 and 6666 for vigour index I and II respectively). This was followed in performance by HA at 300 mg/L, which showed significantly better results than the control but lower than the SA treatment. Zn at 200 mg/L also significantly enhanced all growth metrics compared to the control, though it was the least effective among the treatments for most traits.

The superior performance of the SA treatment aligns with its well-documented role as a plant growth regulator and an elicitor of systemic resistance (19, 42). Seeds germinated in the presence of SA exhibited higher activities of the key glyoxylate cycle enzymes, isocitrate lyase and malate synthase, than those germinated in its absence (22). These two enzymes are key components of the glyoxylate cycle, which is essential for converting storage lipids into carbohydrates during seed germination and early seedling development. The upregulation of this metabolic pathway by SA is indicative of an enhanced transition from a metabolically quiescent state to an active one (49). This elicitation likely facilitates the improved seed vigour observed in the presence of SA. Additionally, the improvement in germination characteristics of primed seeds could be the result of an increased antioxidant profile of treated seeds (10, 35).

Humic acid was next in effectiveness in enhancing broad bean seedling growth and vigour indices, as it had a positive impact on both root and shoot systems. A primary observed effect of HA is the stimulation of overall seedling growth, leading to increased biomass. This finding is in agreement with a previous study which showed that HA was directly correlated with increased shoot length and dry mass in corn seedlings (45). This effect is attributed to HA stimulation of the root system, particularly lateral roots, which is crucial as it expands the root surface area, thereby improving the plant's capacity for water and nutrient uptake (50). Humic acid enhances lateral root development by activating the plasma membrane H^+-ATPase and the H^+ pump in the tonoplast; this acidification of the cell wall and apoplast facilitates cell elongation and root growth (51). This establishes a clear trend in which humic substances act as potent biostimulants for general plant growth. Moreover, the use of HA improves the performance of seedlings by accelerating their emergence speed and enhancing the vigour of the seeds of several species; therefore, the use of such biologically active chemicals may contribute to enhanced seed vigour (52).

Of all the treatments tested, Zn was the least effective in improving seedling growth indicators; however, it was superior to the water-treated control. Zinc is a vital micronutrient fundamental to plant growth, primarily functioning as a cofactor for enzymes critical to several physiological processes. It is integral to the detoxification of reactive oxygen species (ROS), such as the superoxide radical (O_2^{+}) and hydrogen peroxide (H_2O_2), through its role in enzymes like superoxide dismutase (53). Furthermore, Zn is indispensable for protein synthesis, gene expression and the structural and functional integrity of a vast array of proteins, with nearly 10 % of all proteins requiring Zn for their synthesis or activity (54). This underscores its importance from the molecular to the whole-plant level.

The adequacy of Zn seed reserves is particularly crucial for ensuring vigorous germination and strengthening seedling resilience against abiotic stresses during this vulnerable early developmental stage (55). Zinc seed priming has proven effective in enhancing FGP %, seedling growth (shoot and root length) and seed vigour indices (SVI-I and SVI-II). Studies across diverse species, including chickpea (28), maize (56), rice (57) and most recently tobacco (48), have consistently demonstrated that priming with $ZnSO_4$ significantly improves germination rates, seedling growth and vigour indices. This improvement is attributed to the efficient translocation of primed Zn to support early seedling development.

Although Zn priming improved some germination and vigour traits, its overall effect was weaker than that of SA and HA. This may be because the faba bean seeds already had sufficient internal Zn reserves, limiting the benefit of additional Zn. Moreover, Zn has a narrow optimal range and higher concentrations may cause mild osmotic or toxic effects that reduce gains. High baseline germination of the seed lot may also have masked treatment differences.

The enhanced seedling vigour observed in Zn-primed seeds is well-supported mechanistically and is a direct result of Zn's involvement in key developmental processes. The promoted growth is linked to Zn's critical role in radicle development and coleoptile growth, potentially through its influence on auxin synthesis (28, 58). Ultimately, the overarching mechanism behind the efficacy of Zn priming lies in its fundamental contribution to cell

Table 2. Effect of seed treatment with SA, HA or Zn on seedling growth and vigour indices of Barcino broad bean seeds

Treatment	Concentration (mg/L)	Shoot length (cm)*	Root length (cm) *	Seedling dry weight (mg) *	Vigour index I*	Vigour index II*
Control	0	8.6±0.41^{d}	6.1±0.21^{c}	44.82±2.85^{d}	1248^{d}	3822^{d}
SA	150	12.87±0.51^{a}	9.48±0.32^{a}	68.14±2.58^{a}	2187^{a}	6666^{a}
HA	300	11.3±0.28^{b}	8.21±0.21^{b}	60.51±2.41^{b}	2015^{b}	5807^{b}
Zn	200	10.54±0.25^{c}	7.81±0.31^{b}	58.21±1.95^{bc}	1996^{b}	5512^{c}

*Values are mean ± standard error (n=10, for length and weight). Means within a column followed by different letters are significantly different at $p \leq 0.05$. Only the best-performing concentration from each treatment is shown for clarity.

division, cell proliferation, protein synthesis and the maintenance of membrane integrity, which collectively ensure a strong and healthy start for the plant (59).

Conclusion

Based on the strong evidence of its efficacy, pre-treating broad bean seeds with 150 mg/L SA is a highly recommended agricultural practice to significantly boost germination and seedling vigour, which is expected to positively influence final yield. Future studies should validate these results under Iraqi field conditions and quantify their effects on crop productivity and grain quality. The potential synergistic benefits of combined applications of SA, HA and Zn should also be tested. In addition, physiological analyses such as antioxidant responses and reserve mobilization during germination are recommended to clarify mechanisms. Finally, extending this approach to other important legume and vegetable crops in Iraq will help assess its broader applicability.

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Compliance with ethical standards

Conflict of interest: The author do not have any conflict of interest to declare.

Ethical issues: None

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