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Effect of of Humic Acid Spraying at Different Flowering Stages on Seed Yield and Its Components of Berseem (*Trifolium alexandrinum* L.)

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ABSTRACT: This experiment was conducted in an agricultural field in the Musayib district, located 37 km from the city center of Babil Governorate, Iraq, during the period from mid-October 2024 to mid-June 2025, in order to study the effect of humic acid concentrations (0, 2, 4, 6 g L⁻¹) and three spraying stages: (10% of flower buds open, 50% of flower buds open, and 75% of flower buds open) The local variety of alfalfa was used in this study. The experiment was conducted using a randomized complete block design (R.C.B.D.) with split-plot arrangement and three replications. The spraying stages constituted the main treatments, while the humic acid concentrations constituted the secondary treatments. The following traits were studied (number of seeds per inflorescence, 1,000-seed weight, seed yield, biological yield, and harvest index). The results showed that spraying humic acid led to a significant increase in the number of seeds per inflorescence, seed yield, and biological yield. Spraying with humic acid at concentrations of 4 and 6 g L⁻¹ yielded the highest average number of seeds per inflorescence, reaching 48.1 and 50.53 seeds per inflorescence, respectively, as well as the highest average seed yield of 473.47 and 477.83 kg ha⁻¹, respectively, and also yielded the highest average biological yield of 3.36 and 3.354 tons ha⁻¹, respectively, with no significant difference between them. The spraying stage at 10% flowering of the inflorescences outperformed the others in terms of the average number of seeds per inflorescence (49.7 seeds per inflorescence), seed yield (486.77 kg ha⁻¹), and biological yield (3.6 tons ha⁻¹). There was a significant interaction between humic acid concentrations and application stages for both the number of seeds per inflorescence, seed yield, biological yield, and harvest index. The results indicated that there was no statistical evidence that the weight of 1,000 seeds was affected by either factor, and the harvest index was not affected by the spraying concentrations of humic acid.

INTRODUCTION

Berseem is one of the most important leguminous forage crops in many countries worldwide and ranks second in central and southern Iraq after sorghum in terms of importance and cultivated area, as it is characterized by high green forage yield and high nutritional value compared to many other forage crops (1). Berseem is widely cultivated in various regions such as the Nile Valley, the Indian subcontinent, Turkey, and parts of the United States, as it has the ability to adapt to different environmental conditions and yields high levels of dry matter and crude protein, making it one of the primary sources of nutrition for ruminants. In addition, it can be grown as a stand-alone crop or in forage mixtures with grass crops, thereby contributing to improved forage productivity and nutritional quality (2). Berseem has the advantage of being a legume capable of fixing atmospheric nitrogen through its symbiotic relationship with rhizobia bacteria, which helps improve soil fertility and

increase its nutrient content (3). Berseem is distinguished by producing multiple harvests per season, which helps meet livestock's need for green forage during the winter when grass growth ceases. Consequently, demand for this crop has increased, particularly following the expansion of livestock projects, leading to a notable rise in seed prices. Consequently, specialists in this field have sought to develop agricultural techniques to increase the production of this crop's seeds, which requires the use of modern agricultural methods that can improve plant growth and boost productivity. Among these methods is the use of humic substances, the most important of which is humic acid, as it is one of the main components of organic matter formed as a result of the decomposition of plant and animal residues in the soil. Consequently, it is characterized by its ability to improve soil properties and increase the availability of nutrients to plants by forming chelate complexes with mineral elements, which facilitates their absorption by the roots and enhances plant growth and productivity (4). Humic acid plays an important role in regulating many physiological processes within the plant, as it stimulates the plant's hormonal activity, including increasing levels of auxins and cytokinins, thereby stimulating cell division and increasing the growth of roots and vegetative parts, and consequently increasing total yield (5). In addition, humic acid helps increase the permeability of cell membranes and activate numerous enzymes responsible for metabolic processes. It also improves the efficiency of photosynthesis and increases the production of chlorophyll, sugars, and amino acids within the plant, which has a positive impact on plant growth and yield (6). The effectiveness of humic acid in improving plant growth may vary depending on the method and timing of application during the plant's different growth stages, as the timing of agricultural treatments—particularly foliar spraying with growth-promoting substances—is one of the most important factors determining the plant's response to these treatments, as it is linked to the plant's physiological state during its various growth stages. The flowering stage is considered one of the most critical phases in the plant life cycle, as it involves a significant physiological shift from vegetative growth to reproductive growth(22), leading to the redistribution of photosynthetic products and nutrients toward flower and seed formation (7). Therefore, it is crucial to identify the appropriate stage of flowering for foliar application of humic acid, as applying it at the right physiological stage improves flower formation efficiency, increases fruit set and seed formation, and consequently boosts seed yield and seed composition in the plant (8). Although there are many studies that have addressed the effect of humic acid on the growth of various plants, information regarding the determination of the most appropriate physiological stage for spraying with humic acid during the different flowering stages of Berseem remains limited. Therefore, this study was conducted to determine the appropriate concentration and stage for spraying humic acid on the crop while achieving the highest seed yield in this crop.

MATERIALS AND METHODS

A field experiment was conducted in one of the agricultural fields in the Musayib district on loamy-clay soil during the 2024-2025 growing season. Soil preparation operations were carried out, including two perpendicular plowings (using a disc plow), leveling (using disc harrows), and grading using a grader, followed by division into several experimental units. The area of each experimental unit was 9 m², with a total of 36 experimental units, comprising three replicates, each containing 12 experimental units. The seeds were then broadcast sown on October 26, 2025, at a rate of 20 kg/ha (9). Afterward, the alfalfa seeds were sown and covered with soil, followed by germination irrigation. After the planting process was completed, potassium sulfate fertilizer (K₂SO₄, 42%) was applied at a rate of 50 kg K₂SO₄/ha in three applications. Urea fertilizer (46% N) at a rate of 100 kg N/ha, applied in three doses (after emergence, after the first cutting, and after the second cutting). Triple superphosphate (P₂O₅ 21%) was also applied at a rate of 75 kg P₂O₅/ha, added during soil preparation (before planting) (10). Weeding was performed manually as needed. The plants were pruned manually whenever they reached a height of approximately 40 cm, leaving a height of 6–9 cm above the soil surface, with a total of four pruning sessions (11). The experiment was conducted using a completely randomized block design with split-plot arrangement, with three replicates. The experiment included two factors (the main treatments involved the stages of spraying humic acid (10% flowering stage, 50% flowering stage, and 75% flowering stage), while humic acid concentrations constituted the secondary treatments (0, 2, 4, and 6 g/L). The following traits were measured:

1- Number of seeds per inflorescence (seeds per inflorescence⁻¹): Twenty inflorescences were randomly selected from those counted in paragraph (5), and the number of seeds was counted as the average per inflorescence.

2- Weight of 1,000 seeds (g): 250 seeds were randomly selected from each experimental unit and converted to 1,000 seeds, then weighed using a precision electronic scale.

3- Seed yield (kg ha^{-1}): After examining the flower heads, they were dried, and once the weight stabilized, the yield per plant was determined. Based on this, the total yield per hectare was calculated according to the formula: Total yield = yield per plant $\times$ plant density per hectare, based on plant density per hectare (plants per hectare).

4- Biological yield (ton ha^{-1}): The total weight of the ten plants (seeds + other plant parts) was recorded after drying until the weight stabilized (12), then calculated using the following equation: - Biological yield = dry weight of a single plant $\times$ plant density per hectare

5- Harvest index (%): It was calculated according to the equation mentioned in (13) as follows: Harvest index (%) = seed yield / biological yield $\times$ 100.

RESULTS AND DISCUSSION

1- Number of seeds per inflorescence (seeds per inflorescence $^{-1}$)

Table (1) shows that the number of seeds per inflorescence increased with increasing concentrations of humic acid spray, with the highest mean for this trait recorded at a concentration of 6 g L $^{-1}$ (50.53 seeds per inflorescence), which differed significantly from all other concentrations except for 4 g L $^{-1}$, where the mean number of seeds per inflorescence was 48.1 seeds per inflorescence, while the control treatment recorded the lowest mean for this trait at 42.64 seeds per inflorescence and differed significantly from all sprayed concentrations. The percentage increase in this trait when sprayed with concentrations of 2, 4, 6 g L $^{-1}$ was 9.43%, 12.80%, and 18.50%, respectively, compared to spraying with a concentration of 0 g L $^{-1}$. The increase in the number of seeds per inflorescence with higher humic acid concentrations may be attributed to its role in enhancing pollination and fertilization efficiency and reducing losses in fertilized flowers, as humic acid improves pollen viability and promotes pollen tube growth, in addition to prolonging the functional life of the stigma and ovary. This increases the rate of successful fertilization and the number of ovules that develop into mature seeds (14). Furthermore, improving the plant's nutritional status during the flowering and fruit-filling stages contributes to sustained embryo development and reduces early seed abortion, which directly results in an increase in the number of seeds formed in each flower (15). Table (1) also indicates that spraying at 10% flowering (S1) was statistically superior to spraying at 50% flowering (S2) and spraying at 75% flowering (S3), where the highest mean number of seeds per flower was recorded at 49.7 seeds per flower, while the spraying stage at 50% flowering did not differ significantly from the stage at 75% flowering (S3). This may be due to the fact that spraying at the stage of 10% flowering of the floral inflorescences, after the plant has been established in terms of height and leaf area leads to a division of photosynthetic products between the reproductive and vegetative parts, thereby potentially providing a greater opportunity for the florets within the floral inflorescence in terms of growth and development leading up to pollination and fertilization (16). The results in Table (1) also indicate a significant interaction between humic acid concentrations and spraying stages, which can be explained by differences in the relative response to humic acid concentrations and spraying stages. It is observed that the highest mean number of seeds per inflorescence at a spraying concentration of 6 g L $^{-1}$ and spraying at the 10% flowering stage was 56.12 seeds per inflorescence, while the 0 g/L concentration recorded the lowest mean when sprayed at the 50% flowering stage, at 41.72 seeds per inflorescence.

Table (1) : Effect of humic acid concentrations, spraying stages, and their interaction on the number of seeds per inflorescence (seeds per inflorescence).

spraying stages S	H Concentrations				Mean spraying stages
	H0	H1	H2	H3	
S1	42.29	46.82	53.59	56.12	49.7
S2	41.72	43.57	46.26	49.22	45.19
S3	43.9	49.6	44.47	46.24	46.05
Mean Concentrations	42.64	46.66	48.1	50.53	

LSD 0.05	spraying stages	Concentrations	interaction
	1.791	3.073	4.762

2- Weight of 1,000 seeds (g)

Table (2) shows that there are no significant differences between the corresponding arithmetic means for the humic acid spray concentrations. This result is consistent with the findings of Abdulrazzaq (2024) in a study of the effect of adding humic and fulvic acids on the seed yield of Egyptian alfalfa, which showed that the addition of humic and fulvic acids had no significant effect on the 1000-seed weight. Table (2) indicates that there were no significant differences between the corresponding arithmetic means of the humic acid spraying stages in the 1000-seed weight. The reason for the lack of significant differences between the treatment levels of humic acid application on the 1000-seed weight trait may be attributed to the fact that this trait represents a relatively stable genetic trait rather than a responsive environmental trait, since seed weight is primarily related to the plant's genetic ability to fill the seeds and regulate the accumulation of dry matter within them during the ripening stage, a process that depends largely on the genetic makeup of the variety and the general conditions for seed filling rather than on the timing of spraying in the earlier stages (Rouphael and Colla, 2020). Table (2) also shows that there is no statistical evidence of a significant interaction between the factors (humic acid concentrations and spraying stages).

Table (2): Effect of humic acid concentrations, spraying stages, and their interaction on the weight of 1,000 seeds (g).

spraying stages	H Concentrations				Mean spraying stages
S	H0	H1	H2	H3	
S1	2.847	2.447	2.58	2.647	2.63
S2	2.673	2.537	2.61	2.457	2.569
S3	2.737	2.437	2.587	2.477	2.559
Mean Concentrations	2.752	2.473	2.592	2.527	
LSD 0.05	spraying stages	Concentrations	interaction		
	N.S	N.S	N.S		

3- Seed Yield (kg ha⁻¹)

Table (3) shows that spraying humic acid resulted in a significant increase in seed yield; the high concentration of 6 g L⁻¹ achieved the highest mean seed yield of 477.83 kg ha⁻¹, which differed significantly from all spray concentrations except the 4 g L⁻¹ concentration, which achieved a mean of 473.47 kg ha⁻¹. Meanwhile, spraying at a concentration of 0 g L⁻¹ resulted in the lowest mean seed yield of 402.73 kg ha⁻¹. The increase in seed yield resulting from spraying humic acid at concentrations of 2, 4, and 6 g L⁻¹ compared to the non-sprayed control was 11.10%, 17.57%, and 18.66%, respectively. The increase in seed yield following humic acid application may be attributed to the increase in yield components, such as the number of seeds per inflorescence, as shown in Table (1). Table (3) also illustrates the significant effect of the timing of humic acid spraying on seed yield, as the spraying stage at 10% flowering recorded the highest mean seed yield of 486.77 kg ha⁻¹ and differed significantly from the other spraying stages, whereas the spraying stage at 50% flower bud opening recorded the lowest mean seed yield of 425.84 kg ha⁻¹. The reason for the superior seed yield at the 10% flower bud opening stage may be attributed to its superiority in key yield components, namely the number of seeds per inflorescence (Table 1). This result is consistent with the findings of (18), who studied the effect of spraying stages on seed yield and alfalfa components, demonstrating their impact on seed yield. Table (3) also indicates a significant interaction between humic acid concentrations and spraying stages,

which may be due to the differing relative responses to humic acid concentrations across spraying stages. Spraying at a concentration of 6 g L^{-1} during the 10% flowering stage of the inflorescences, it achieved the highest mean seed yield of $486.77 \text{ kg ha}^{-1}$ whereas spraying at a concentration of 0 g L^{-1} during the 50% flowering stage of the inflorescences resulted in the lowest seed yield of $386.66 \text{ kg ha}^{-1}$.

Table (3): Effect of humic acid concentrations, application stages, and their interaction on seed yield (kg ha^{-1}).

spraying stages S	H Concentrations				spraying Mean stages
	H0	H1	H2	H3	
S1	413.61	486.77	519.56	527.12	486.77
S2	386.66	412.45	446.74	457.49	425.84
S3	407.91	443.08	454.1	448.88	438.49
Mean Concentrations	402.73	447.44	473.47	477.83	
LSD 0.05	spraying stages	Concentrations	interaction		
	3.834	5.824	9.11		

4- Biological Yield (t ha^{-1})

Table 4 shows that spraying with a concentration of 4 g L^{-1} of humic acid recorded the highest mean biological yield of 3.36 t ha^{-1} , which did not differ significantly from the 6 g L^{-1} concentration that achieved a mean of 3.354 t ha^{-1} . Meanwhile, the control treatment recorded the lowest mean for this trait at 2.936 t ha^{-1} , which differed significantly from all spray concentrations. The increase in biomass yield when sprayed with concentrations of 2, 4, and 6 g L^{-1} compared to the control treatment (0 g L^{-1}) was 6.68%, 14.44%, and 14.24%, respectively. The increase in biomass yield may be attributed to the role of humic acid in improving the plant's vegetative growth efficiency and increasing biomass formation by enhancing carbon fixation efficiency and stimulating the uptake of essential nutrients, thereby increasing dry matter accumulation in the plant's vegetative parts, which is reflected in increased overall growth and total biomass yield (19). Table (4) shows that the biological yield was significantly affected by the different stages of humic acid application, where application at the flowering stage (10% of flower buds) recorded the highest mean for this trait at 3.61 t ha^{-1} , which differed significantly from the spraying stage at 50% flowering, with a mean of 2.994 t ha^{-1} ; and the stage at 75% flowering, with a mean of 2.983 t ha^{-1} , which did not differ significantly from one another. This may be due to the fact that spraying at the 10% flowering stage encouraged vegetative growth, thereby increasing the plant's ability to intercept sunlight, which in turn enhances photosynthetic efficiency (20). It is evident that delaying spraying leads to a decrease in the plant's biological yield; the rate of decrease when spraying at the 50% and 75% flowering stages, compared to spraying at the 10% flowering stage, was 17.06% and 17.37%, respectively. Table (4) also shows a significant interaction between humic acid concentrations and spraying stages. This is due to the difference in the relative response to humic acid concentrations across different spraying stages, as spraying at a concentration of 4 g L^{-1} at the 10% flowering stage recorded the highest mean biological yield of 3.907 t ha^{-1} , whereas spraying at a concentration of 0 g L^{-1} at the 75% flowering stage recorded the lowest mean yield of 2.746 kg ha^{-1} .

Table (4): Effect of humic acid concentrations, application stages, and their interaction on biological yield (t ha^{-1}).

spraying stages S	H Concentrations				Mean spraying stages
	H0	H1	H2	H3	
S1	3.227	3.473	3.907	3.833	3.61
S2	2.837	2.977	3.047	3.117	2.994
S3	2.743	2.947	3.127	3.113	2.983

Mean Concentrations	2.936	3.132	3.36	3.354	
LSD 0.05	spraying stages	Concentrations	interaction		
	0.0422	0.0707	0.1098		

5 -Harvest Index (%)

Table (5) shows that there are no significant differences between the corresponding arithmetic means of the humic acid spray concentrations. This may be due to the fact that seed yield values increased with higher spray concentrations. Similarly, regarding the biological yield, when divided, the gap between the harvest index values remained narrow. This may also be explained by the superior grain yield, which positively influenced the increase in the harvest index due to the direct relationship between them (21). Table (5) indicates a significant effect of the stages of humic acid spraying on the harvest index. The spraying stage at 10% flowering of the inflorescences recorded the highest mean for this trait, reaching 17.51% and differed significantly from the spraying stages at 50% and 75% flowering, where the mean yield was 13.562% and 15.923%, respectively. This may be due to the fact that spraying at the 10% flowering stage achieved the highest mean seed yield in Table (3). The results in Table (5) showed a significant interaction between the study factors, and the reason for this interaction is the difference in the relative response to humic acid concentrations depending on the spraying stage. It is noted that spraying at the 10% flowering stage of the inflorescences at a concentration of 4 g/L⁻¹ recorded the highest mean for this trait, reaching 18.44%, while spraying at the 50% flowering stage of the inflorescences at a concentration of 4 g/L recorded the lowest mean for the harvest index, reaching 12.847%.

Table (5): Effect of humic acid concentrations, spraying stages, and their interaction on the harvest index (%).

spraying stages S	H Concentrations				Mean spraying stages
	H0	H1	H2	H3	
S1	16.133	17.243	18.44	18.223	17.51
S2	14.293	13.737	12.847	13.37	13.562
S3	16.44	15.97	15.747	15.537	15.923
Mean Concentrations	15.622	15.65	15.678	15.71	
LSD 0.05	spraying stages	Concentrations	interaction		
	0.0352	N.S	0.1662		

CONCLUSIONS

The application of humic acid is an effective strategy for improving productivity in alfalfa by increasing the number of seeds per flower head and boosting seed and biomass yields, with a clear advantage for high concentrations (4 and 6 g L⁻¹) and early application at the 10% flower opening stage, reflecting the importance of synchronizing the plant's physiological condition with the timing of treatment. The results also confirm an interactive response between humic acid concentration and the spraying stage for most yield traits, compared to relative stability in 1000-seed weight and harvest index, which suggests that the stimulatory effects are primarily focused on yield components without a fundamental change in the genetic structure or the relative distribution of biomass. This is consistent with recent global trends toward the use of biostimulants in sustainable agriculture to increase production efficiency and reduce reliance on chemical inputs.

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