

The stimulatory effect of humic and fulvic acid compound and sowing date on the germination traits of *Vigna unguiculata L.* seeds (cowpea)

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Abstract

An experiment was conducted on the cowpea crop (*Vigna unguiculata L.*) inside a greenhouse belonging to the Horticulture and Landscape Engineering Research Station, College of Agriculture – University of Diyala. Seeds of the variety ‘Ramshorn’ were sown in cork trays with a capacity of 196 seedlings, using German-origin peat moss as the germination medium. The experiment followed a Completely Randomised Design (CRD) with three replications. The study included two factors: Planting dates (26 February and 7 March), and Organic compound (humic and fulvic acids) applied in three treatments: seed soaking in the organic compound at a concentration of 5 ml L⁻¹ for 24 hours, mixing peat moss with the compound at a rate of 5 ml L⁻¹ kg⁻¹ peat moss, and a control treatment without any application. For the second and third treatments, seeds were soaked in water only.

The experimental design included six treatments, with three replications per treatment, giving a total of 18 experimental units. The trays were placed in an unheated plastic greenhouse, consistent with the local method used for seedling production. Data collection began after the emergence of the first seedling and continued daily until germination ceased in all treatments.

The results indicated that the treatments of seed soaking and soil mixing at the second planting date recorded the shortest germination period (6.3 days each). The treatment of seed soaking at the first planting date achieved the highest germination percentage (97.3%), with no significant difference compared to soil mixing at the second date (97.1%). Seed soaking at both planting dates resulted in the highest germination speed (4.99 and 5.46 days seed⁻¹, respectively). The seed soaking treatment at the first planting date gave the highest germination uniformity (10.52 seeds day⁻¹), which did not differ significantly from seed soaking at the second date and soil mixing at both dates (10.49, 10.02, and 9.48 seeds day⁻¹, respectively). The seed soaking treatment at the first date also achieved the highest germination vigour (5.37 g fresh weight seedling⁻¹), with no significant difference from soil mixing at the same date and seed soaking at the second date (4.56 and 4.46 g fresh weight seedling⁻¹, respectively). Seed soaking at both planting dates produced the longest shoot lengths (11.3 and 10.7 cm, respectively) and longest root lengths (8.7 cm each), with no significant difference from soil mixing treatments (5.3 and 5.7 cm, respectively). The seed soaking treatment at the first planting date recorded the highest number of leaves (8.7 leaves seedling⁻¹), not significantly different from soil mixing at the first

date (8.3 leaves seedling⁻¹) and from soil mixing and seed soaking at the second date (7.0 and 8.3 leaves seedling⁻¹, respectively) had no significant effects on the values of germination, speed, uniformity, , or shoot and root length.

Keywords: Cowpea (*Vigna unguiculata*), Humic and Fulvic acid compound, Planting date, Germination characteristics.

Introduction

Modern agricultural systems are increasingly facing challenges due to climate change, declining soil fertility, and water scarcity,. addition to, the urgent need to achieve food security and enhance of agricultural productivity. In this context, focusing on leguminous crops as one of the main pillars of sustainable agriculture has become increasingly important, given their multiple nutritional, economic, and environmental benefits. Among these crops, cowpea (*Vigna unguiculata* L.) is of particular significance, as it represents a rich source of plant protein and essential minerals such as iron, calcium, and magnesium. Furthermore, it contains antioxidant compounds that contribute to promoting of human health [4]. In addition to, its nutritional value, cowpea plays an important role in improving soil properties through its ability to fix atmospheric nitrogen in association with rhizobial bacteria, thereby reducing on costly and environmentally harmful chemical fertilisers. However, these advantages, the productivity of this vital crop remains below the desired level in many regions due to several constraints, including unfavourable climatic conditions, physiological stresses, and inefficient traditional farming practices[17].

The planting date is considered one of the most influential environmental factors affecting the growth and productivity of cowpea. sowing date interacts with climatic

parameters such as temperature, humidity, and light intensity, which in turn influence the physiological processes of seeds inside the greenhouse—such as germination duration, germination percentage, uniformity, speed, and vigor—as well as certain seedling traits, including stem length, root length, and leaf number per seedling.sowing at an inappropriate time can disrupt the plant's growth cycle and reduce seed quality[14] Therefore, determining the optimum planting date is a crucial component of effective agricultural management strategies, particularly when integrated with transplanting techniques and the use of organic growth-promoting compounds.

In recent years, there has been growing interest in the use of natural organic acids, such as humic acid and fulvic acid, as eco-friendly alternatives to conventional fertilisers. These compounds, derived from decomposed organic matter (e.g. humus and compost), act as biostimulants that enhance nutrient uptake, improve the physical structure of soil, and increase the efficiency of photosynthesis and plant enzyme activity [6]

Humic acid is widely recognized for its capacity to enhance cation exchange capacity and to stimulate root development. and increase plant tolerance to environmental stresses such as drought and salinity. Meanwhile, fulvic acid, due to its smaller molecular size compared to humic acid, can penetrate plant cell membranes

more easily, enhancing metabolic transport processes and stimulating enzyme activity, as well as improving the uptake of micronutrients such as iron, zinc, and manganese [10] Stimulating the germination of cowpea seeds through the application of humic and fulvic acids in conjunction with

Materials and Methods

An experiment was carried out on the cowpea in the greenhouse belonging to the research station of the Department of Horticulture and Landscape Engineering at the College of Agriculture - University of Diyala, Baquba, located at longitude 45° and latitude 33°.

To evaluate the response of cowpea to the transplanting technique and determine its success, as well as to assess its response to the humic and fulvic acid organic compound, seeds of the 'Ramshorn' variety were sown in cork trays (196 seedlings) using German-origin peat moss as the growing medium for germination inside the greenhouse (Figure 1).The experiment followed a Completely Randomised Design (CRD) with three replications. It included two factors:Planting dates (26 February and 7 March) , Organic compound humic and fulvic acids(Soaking seeds in the organic compound at a concentration of 5 ml L⁻¹ for 24 hours , Mixing peat moss with the organic compound at a concentration of 5 ml L⁻¹ kg⁻¹ peat moss, Control treatment without any organic application).For the second and third treatments, the seeds were soaked in water only.

The experiment therefore comprised six treatments with three replications, resulting in 18 experimental units.The trays were placed inside an unheated plastic greenhouse, following the local standard

the appropriate planting date aims to achieve superior seed germination characteristics and vigorous seedling growth, which may later reflect positively on transplant quality, ultimately leading to higher yield and improved crop quality in the permanent field.

method for seedling production [2] Data collection began after the emergence of the first seedling and continued daily until germination had completely ceased in all treatments.

Studied Traits

- Germination Tests

The following germination parameters were measured:

1. Germination period (days): the number of days required for seed germination. Germinated seeds were counted daily starting from the sixth day after sowing (when the first seedling appeared) until germination ceased on the eighteenth day after sowing.

2. Germination percentage (%): Calculated according to the following equation:

$$\text{Germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

Repeat the writing of formula . please ?

3. Germination speed (days seed⁻¹): Calculated according to the following equation:

Total (number of sprouted seeds each day X day number)

$$\frac{\text{Total number of germinated seeds at the end of the germination period}}{\text{Germination speed}}$$

4. Germination uniformity (seeds day⁻¹):

This is an important seedling trait; the more uniform the germination, the more homogeneous the seedlings in height, thickness, and vigour, resulting in a higher proportion of transplants ready for permanent field planting at the same time (Figure 2). Germination uniformity was calculated according to [11] as follows:

$$\frac{\text{Total number of germinated seeds at the end of the germination period}}{\text{Number of days from the start of sowing the seeds until germination stops}}$$
 Germination uniformity

5. Germination vigour (g fresh weight seedling⁻¹): This test was used to distinguish vigorous seeds from weak, less viable seeds (Figure 3). Germination vigour represents the ability of the seed to produce a strong seedling under favourable conditions. It was determined by measuring the average fresh weight of seedlings before transplanting to the field [11].

- Vegetative Characteristics:

Before transplanting the seedlings into the permanent field (Figure 4), the following vegetative traits were recorded:

1. shoot length (cm): Measured from the point of contact between the stem and the soil surface to the top of the main stem using a graduated ruler.

2. Root length (cm): Measured after gently removing all peat moss from the roots and placing them on a flat surface, then measuring with a graduated ruler.

3. Number of leaves: The total number of leaves was counted for the same seedlings used to measure stem and root length.

The data obtained were statistically analyzed according to the analysis of variance method for a Completely Randomized Design (CRD) using the SAS statistical software [15]. The Least Significant Difference (LSD) test was chosen to differentiate between the means of the treatments for significant differences at a probability level of 5% [16].



Figure 1. Cork trays used in greenhouse experiment treatments



Figure 2. Variation in germination uniformity of cowpea seeds with different treatments of humic and fulvic compounds.



Figure 3. Germination strength of cowpea seeds as a result of treatment with humic and

fulvic acid compound at the first planting date.



Figure 4. Cowpea seedlings before transplanting to the permanent field

Results and Discussion:

Germination Period (Days):

The results presented in Table 1 showed significant effects of the humic and

fulvic acid compound, the sowing date, and the interaction between them on the mean germination period. Soaking the seeds in the humic and fulvic acid compound resulted in the shortest germination period (7.7 days), followed by the treatment of mixing peat moss with the compound (8.5 days), compared with the control treatment which recorded 12.2 days. Regarding the sowing date, planting on the second date (March 7) resulted in a shorter germination period (7.9 days) compared to the first date (February 26) which recorded 11.0 days.

As for the interaction between of the seed soaking and soil mixing with the compound at the second sowing date achieved the lowest germination period (6.3 days) for both, compared with the control treatment at the same date, which recorded 11.0 days.

The shortened germination period observed at the second sowing date is likely attributable to the elevated temperatures prevailing in March. as opposed to February which is characterized by lower temperatures that slow down the metabolic reactions inside seeds, thus delaying seedling emergence. The higher temperatures during the second date provided a more favorable environment for water absorption and enzyme activation, particularly those responsible for the hydrolysis of stored materials in the seeds, which led to a shorter germination period. These findings are consistent with those reported by [3,18] who emphasized the importance of synchronizing the sowing date with optimal environmental conditions to enhance germination.

The reduction in germination period when using the humic and fulvic acid compound could be due to the fact that

humic substances act as biostimulants, improving seed coat permeability to water and increasing its absorption efficiency. Moreover, they enhance the activity of enzymes such as amylase and protease, which catalyze the breakdown of starches and proteins into simple sugars and amino acids readily available for early growth. This

agrees [5] , who reported that humic acids stimulate metabolic reactions and increase ATP energy production during the early stages of germination. Similar findings were confirmed in a study on maize, where soaking seeds in synthetic humates significantly reduced germination duration [12] .

Table 1. Effect of humic and fulvic acid compound, sowing date, and their interaction on cowpea seed germination period (days).

Planting Date	Seed Soaking	Peat Moss Mixing	Control	Date Mean
26 February	9	10.7	13.3	11.0
7 March	6.3	6.3	11.0	7.9
Compound Mean	7.7	8.5	12.2	
LSD (0.05)	Compound = 0.66	Date = 0.54	Interaction = 0.93	

Seed Germination Percentage (%):

The results presented in Table 2 showed that the humic and fulvic acid compound and its interaction with the sowing date had significant effects on the germination percentage, while the sowing date alone had no significant effect. The treatment of seed soaking in humic and fulvic acid recorded the highest germination percentage (97.4%), followed by peat moss mixing (94.2%), compared with the control treatment, which recorded 89.2%. Regarding the interaction, the treatment of seed soaking at the first sowing date highest germination rate (97.3%), which did not

differ significantly from the peat moss mixing treatment at the second sowing date (97.1%), Compared to the control treatments for the first and second planting dates, which reached 89.8% and 88.5% respectively.

The clear improvement in germination percentage may be attributed to the fact that humic and fulvic acids enhance soil properties, increase seed coat permeability to water, and stimulate the activity of enzymes responsible for germination. In addition, they promote cell division and the formation of embryonic roots, which leads to higher germination rates [6,19] .

Table 2. Effect of humic and fulvic acid compound and sowing date on the germination percentage (%) of cowpea seeds.

sowing date	Seed Soaking	Peat Moss Mixing	Control	Date Mean
26 February	97.3	94.0	89.8	93.7
7 March	97.1	94.3	88.5	93.3
Compound Mean	97.2	94.2	89.2	
LSD (0.05)	Compound = 1.33	Date = NS	Interaction = 1.09	

Seed Germination Rate (Days per Seed):

The results presented in Table 3 showed significant effects of the humic and fulvic acid compound, as well as the interaction between the compound and sowing date, on the seed germination rate, while no significant differences were observed between sowing dates alone. The seed soaking treatment recorded the fastest germination rate (5.23 days per seed), followed by the peat moss mixing treatment (6.79 days per seed), compared with the control treatment, which recorded the slowest rate (8.41 days per seed). Regarding the interaction, seed soaking at both sowing dates achieved the fastest germination rates of 4.99 and 5.46 days per seed, respectively, compared with the control treatments at the second and first sowing dates, which

recorded the slowest rates of 8.91 and 7.90 days per seed, respectively.

This improvement can be explained by the fact that humic and fulvic acids act as biostimulants, enhancing metabolic activity within the seeds and stimulating enzymes responsible for carbohydrate and protein hydrolysis, providing energy required for the embryo [6,19]. Additionally, these acids improve water and mineral ion absorption, increasing tissue swelling and accelerating germination. The slower germination rate observed in some soaking treatments may be due to over-saturation of seeds with water, which affects oxygen balance [9]. Furthermore, the superiority of the first sowing date confirms that moderate temperatures to increase enzymatic activity and accelerate metabolism [1,18].

Table 3. Effect of humic and fulvic acid compound, sowing date, and their interaction on cowpea seed germination rate (days per seed).

sowing date	Seed Soaking	Peat Moss Mixing	Control	Date Mean
26 February	4.99	6.78	7.90	6.56
7 March	5.46	6.80	8.91	7.06
Compound Mean	5.23	6.79	8.41	
LSD (0.05)	Compound =0.772	Date = NS	Interaction =1.092	

Germination Uniformity (Seeds per Day):

The Table 4 showed significant effects of the humic and fulvic acid compound and its interaction with sowing date on germination uniformity, while no significant differences were observed between sowing dates alone. The seed soaking treatment achieved the highest germination uniformity, reaching 10.51 seeds/day, followed by the peat moss mixing treatment (9.75 seeds/day), compared with the control treatment which recorded the lowest uniformity of 8.86 seeds/day. Regarding the interaction, seed soaking at the first sowing date achieved the highest uniformity (10.52 seeds/day), which did not differ significantly from seed soaking at the second sowing date and peat moss mixing at the first and second

sowing dates (10.49, 10.02, and 9.48 seeds/day, respectively), compared with the control treatments at both sowing dates (8.77 and 8.95 seeds/day, respectively).

These results indicate that the use of the humic and fulvic acid compound, especially when mixed with peat moss, contributed to increasing germination uniformity and enhancing seed vigour, while no significant differences were observed between sowing dates. The improvement in germination uniformity can be explained by the ability of humic and fulvic acids to activate germination-stimulating enzymes and regulate water and nutrient uptake, which reduces variability among seedlings and increases germination [6,7] .

Table 4. Effect of humic and fulvic acid compound, sowing date, and their interaction on cowpea seed germination uniformity (seeds per day).

sowing date	Seed Soaking	Peat Moss Mixing	Control	Date Mean
26 February	10.52	10.02	8.77	9.77
7 March	10.49	9.48	8.95	9.64
Compound Mean	10.51	9.75	8.86	
LSD (0.05)	Compound = 0.8208		Date = NS	Interaction = 1.1608

Seedling Vigour (g Fresh Weight per Seedling):

The Table 5 indicated significant effects of the humic and fulvic acid compound and its interaction with sowing date on seedling vigour, while no significant differences were observed between sowing dates alone. The seed soaking treatment recorded the highest seedling vigour (4.92 g fresh weight per seedling), followed by the

peat moss mixing treatment (4.27 g fresh weight per seedling), compared with the control treatment, which recorded the lowest vigour (3.07 g fresh weight per seedling). Regarding the interaction, seed soaking at the first sowing date achieved the highest vigour (5.37 g fresh weight per seedling), which did not differ significantly from peat moss mixing at the same date and seed soaking at the second sowing date (4.56 and

4.46 g fresh weight per seedling, respectively), compared with the control treatments at the second and first sowing dates (2.93 and 3.20 g fresh weight per seedling, respectively).

The improvement in seedling vigour may be attributed to the effect of humic and fulvic acids in enhancing nutrient and water uptake and activating enzymes responsible for early growth [6,10] .

Table 5. Effect of humic and fulvic acid compound and sowing date on cowpea seedling vigour (g fresh weight per seedling).

Planting Date	Seed Soaking	Peat Moss Mixing	Control	Date Mean
26 February	5.37	4.56	3.20	4.38
7 March	4.46	3.97	2.93	3.79
Compound Mean	4.92	4.27	3.07	
LSD (0.05)	Compound = 1.282	Date = NS	Interaction = 1.813	

Stem Length (cm):

The results presented in Table 6 showed significant effects of the humic and fulvic acid compound and its interaction with sowing date on mean stem length, while no significant differences were observed between sowing dates alone. The seed soaking treatment resulted in the highest stem length (11.0 cm), followed by the peat moss mixing treatment (8.4 cm), compared with the control treatment which recorded the lowest stem length (7.2 cm).

Regarding the interaction, seed soaking at the first and second sowing dates achieved the highest stem lengths (11.3 and 10.7 cm, respectively), compared with the control treatments at the second and first sowing dates, which recorded stem lengths of 7.0 and 7.3 cm, respectively.

The increase in stem length under the influence of the compound may be attributed to the activation of enzymes responsible for cell division and elongation and the stimulation of nutrient uptake [6,7] .

Table 6. Effect of humic and fulvic acid compound, sowing date, and their interaction on mean stem length (cm) of cowpea seedlings.

Planting Date	Seed Soaking	Peat Moss Mixing	Control	Date Mean
26 February	11.3	8.7	7.3	9.1
7 March	10.7	8.0	7.0	8.6
Compound Mean	11.0	8.4	7.2	
LSD (0.05)	Compound = 0.93	Date = NS	Interaction = 1.32	

Root Length (cm):

The results presented in Table 7 indicated significant effects of the humic and fulvic acid compound and its interaction with sowing date on mean root length, while no significant differences were observed between sowing dates alone. The seed soaking treatment resulted in the highest root length (8.7 cm), followed by the peat

moss mixing treatment (7.9 cm), compared with the control treatment which recorded the lowest root length (5.5 cm). Regarding the interaction, seed soaking at the first and second sowing dates achieved the highest root length (8.7 cm each), which did not differ significantly from peat moss mixing at the two sowing dates, which recorded 5.3 and 5.7 cm, respectively.

This effect may be attributed to the ability of the compound to enhance nutrient and water uptake and stimulate root activity [6,13] .

Table 7. Effect of humic and fulvic acid compound, sowing date, and their interaction on mean root length (cm) of cowpea seedlings.

Planting Date	Seed Soaking	Peat Moss Mixing	Control	Date Mean
26 February	8.7	8.0	5.3	7.3
7 March	8.7	7.7	5.7	7.4
Compound Mean	8.7	7.9	5.5	
LSD (0.05)	Compound = 1.59	Date = NS	Interaction = 2.25	

Number of Leaves per Seedling:

The results presented in Table 8 indicated significant effects of the humic and fulvic acid compound, sowing date, and their interaction on the mean number of leaves per seedling. The seed soaking treatment produced the highest leaf number (8.2 leaves per seedling), followed by the peat moss mixing treatment (7.7 leaves per seedling), compared with the control treatment, which recorded the lowest number (5.5 leaves per seedling). Regarding sowing date, the first sowing date resulted in higher leaf number (7.6 leaves per seedling) compared with the second sowing date (6.7

leaves per seedling). For the interaction, seed soaking at the first sowing date achieved the highest number of leaves (8.7 leaves per seedling), which did not differ significantly from peat moss mixing at the first sowing date (8.3 leaves per seedling) and peat moss mixing and seed soaking at the second sowing date (7.0 and 8.3 leaves per seedling, respectively).

The increase in leaf number may be attributed to the ability of the compound to enhance nutrient uptake and stimulate metabolic processes and early vegetative growth, reflecting the plant’s health and vigour [6,13] .

Table 8. Effect of humic and fulvic acid compound, sowing date, and their interaction on mean number of leaves per cowpea seedling.

sowing date	Seed Soaking	Peat Moss Mixing	Control	Date Mean
26 February	8.7	8.3	5.7	7.6
7 March	7.7	7.0	5.3	6.7
Compound Mean	8.2	7.7	5.5	
LSD (0.05)	Compound = 0.66	Date = 0.54	Interaction= 0.93	

From the current experiment, it can be concluded that the use of the humic and fulvic acid compound in combination with sowing date had significant effects on most germination traits of cowpea seeds as well as certain seedling vegetative traits under protected environment conditions. It is recommended to apply the humic and fulvic acid compound, either as seed soaking or soil mixing, at both sowing dates, as it resulted in the shortest germination duration, the highest germination percentage, and the greatest germination rate, uniformity, and vigour, as well as the highest stem and root length and the largest number of leaves per seedling.

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