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Enhancing Linseed Oil Yield using the Synergistic Effect of Seed Priming, Humic Acid and Sulphur under Optimized Irrigation

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ABSTRACT

Background: Linseed, popularly known as flaxseed, is a prominent dual-purpose crop cultivated for both fiber and oilseed crops. It is especially valued for its rich nutritional profile, which includes alpha-linolenic acid, an essential omega-3 fatty acid, along with dietary fiber and lignans, making it more suitable for value addition and sustainable agriculture. Despite its adaptability and uses, it faces challenges such as grain yield followed by oil yield due to moisture stress. In the past few years, it has received special attention due to its industrial application and health benefits. Although the individual impact of seed priming, use of humic acid and sulphur on healthy seedling establishment, crop growth and oil yield under limited water conditions has shown the beneficial impact, their synergistic studies remain to be explored. Therefore, an integrated effort with seed priming, humic acid and sulphur was implemented to mitigate the adverse response to moisture stress.

Methods: An experiment was executed using a split-plot design considering the most popular variety of linseed (LC 2063) over the research farm of Lovely Professional University. The main plot treatments consisted of three levels of irrigation: I_0, I_1 and I_2, that is, no irrigation, moderate irrigation and full irrigation, while subplots treatments comprised seven combinations, including seed priming with KNO_3, humic acid and sulphur used as soil application (C_0 to C_6), whereas 15 mM of KNO_3 was used as seed priming and humic acid was used besal application @ 12 Kg ha^{-1}.

Result: The frequency of irrigations had a significant impact (p=0.05) on the phenological traits in linseed. Out of all the irrigation frequencies, two irrigations (I_2) minimized the days taken to the branching and 50% flowering and maximized the days taken to maturity, number of capsules $plant^{-1}$ and oil yield qt ha^{-1} as compared to single (I_1) and no post-sowing irrigation (I_0). As for the percent increase or decrease over control is concerned, I2 reduced days by 16% and 13% and increased maturity, number of capsules and oil yield by 7%, 23% and 22%, respectively. The combined application of KNO_3 and humic acid (C_5) was the most effective treatment at (p=0.05), significantly reducing days to emergence, branching, 50% flowering and maturity by 47, 18, 13 and 4% respectively and enhancing maturity, number of capsules and oil yield by 4%, 38% and 32%. A negative relationship was detected between the relative water content (RWC%) and proline content (µmol g^{-1}) across the irrigation regimes, wherein I_2 showed the highest RWC and lowest proline. Among the chemical treatments, C_5 maintained the highest RWC% and proline, followed by C_4> C_6> C_3> C_2> C_1> C_0. Most of the parameters studied had a significant interaction effect between the irrigation frequency and chemical treatments at (p=0.05), indicating that the combined application of C_5 with I_2 can markedly enhance oil yield in linseed.

Key words: Humic acid, Irrigation frequency, KNO_3, Proline, Relative water content, Sulphur.

INTRODUCTION

Linseed (*Linum usitatissimum* L.), popularly known as flaxseed, belongs to the *Linaceae* family, mostly grown for oil production. Out of all the linseed oil production, around 80% is used in the food and oil industry for paints, varnishes, coating oils, linoleum, printing and printing ink, and leather finishing (Kaurav *et al.,* 2024). In India, flax is cultivated in an area of 3.3 lakh hectares with a production of 1.7 million tonnes, wherein Madhya Pradesh, Rajasthan, Himachal Pradesh and some districts of Punjab, Bihar and Uttar Pradesh are the leading states (Chaurasiya *et al.,* 2024).

Abiotic stress, including moisture stress, is a challenging situation for the linseed plant to align the morpho-physiological traits necessary for achieving optimum flax and oil yield, even though it is considered a low water requirement crop (2-3 irrigations are enough). Water deficit during the critical stage of linseed can hamper the yield adversely (Sanwal *et al.,* 2025 and Ibrar *et al.,* 2016). Seed priming is a kind of technology wherein seeds are partially hydrated up to a point where the germination processes begin and accelerate synchronous growth at

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an early stage of crop establishment. Potassium nitrate (KNO_3), calcium nitrate [$Ca(NO_3)_2$] and [$Mg(NO_3)_2$] are widely used priming agents known to balance the hormonal imbalance between ABA and GA_3, stimulate the hydrolysis of starch, and trigger the synthesis of chlorophyll (Badkul *et al.,* 2022; Zrig *et al.,* 2023 and Kalangutkar and Siddique, 2023). Sulphur is an essential element for plants to improve their growth, yield and quality parameters. It also plays a significant role in the synthesis of chlorophyll, vitamins and oil content in linseed (Solo *et al.*, 2021 and Singh *et al.,* 2022). Additionally, it accelerates protein synthesis by improving the synthesis of sulfur-containing amino acids such as cystine, cysteine and methionine (Dawar *et al.,* 2023).

Humic acid is widely recognised for its multiple functions as a mediator between stress responses and physiological processes (Chen *et al.,* 2022). It is a complex substance resulting from organic matter decomposition that increases the retention of water in the soil and improves nutrient absorption and permeability of plant membranes. Thus, it has the potential to ameliorate the consequences of stress caused by the scarcity of water (Ampong *et al.,* 2022). Therefore, the synergistic effect of seed priming, humic acid, and sulphur under optimized irrigations was evaluated to ameliorate the impact of oil yield under prevailing conditions.

MATERIALS AND METHODS

A field experiment was laid out during the *Rabi* seasons of 2022-2023 and 2023-24 to evaluate the synergistic effect of seed priming, humic acid and sulphur under optimized irrigations at the Agricultural Research Farm of Lovely Professional University, Phagwara, Punjab, India, which is located at 31.2′N latitude 75.7′E longitude experiencing a subtropical climate characterized by 25-45°C in summer, 25-35°C in monsoon and minimum 4°C in winter eason with annual rainfall of 816 mm.

Execution of experiment

The experiment was carried out in a split-plot design with three replications, considering the plot size of 25 m^2 for $plot^{-1}$. Three levels of irrigation (I_0 to I_2) and seven chemical treatments were comprised, including seed priming with humic acid and sulphur. Seed priming with KNO_3 was used @ 15 mM while humic acid and sulphur were used as basal application @ 12 Kg and 60 Kg ha^{-1}. The linseed variety [LC 2063] was used for experimentation, which was procured from the Punjab Agricultural University. The recommended distance between rows and plants was maintained as 25 × 10 cm and the standard procedure was followed to assess the pH and EC of irrigation water before use, which was 7.4 and 0.9 dS m^{-1}.

Phenological studies

Days to emergence, branching, 50% flowering and days to maturity were considered as phenological traits in the present study. To record the days to emergence, branching, 50% flowering and days to maturity, consider the date when at least 50% of the seed, branches, flowering and maturity are visible over the field. However, the total number of capsules $plant^{-1}$ was considered after taking the average of plants from an area of 1 square meter.

Measurement of relative water content (RWC%)

Relative water content was recorded at two intervals of 45 and 90 DAS, as per the Barrs and Weatherley, (1962) involved several steps wherein the fresh weight, dry weight, and turgid weight of the known samples were the major steps. The dry weight of the samples was measured after placing the samples in a hot air oven at 80°C 48 hours. However, to derive the amount of relative water content (%), the following formula was considered.

$$RWC\ (\%) = \frac{\text{Fresh weight (g) - Dry weight (g)}}{\text{Turgid weight (g) - Dry weight (g)}} \times 100$$

Estimation of proline content

The proline content was estimated using a method described by Bates *et al.* (1973) wherein a known amount of leaf sample was homogenized in 5 mL of 3% Sulphosalicylic acid, followed by a centrifugation process at 10000 rpm for 10 minutes. The sample was placed in a hot water bath after mixing an aliquot, glacial acetic acid and ninhydrin in the test tube. Proline was separated from the solution by using 6 ml of toluene in a separating funnel. The optical density of the sample was measured at 520 nm.

Estimation of oil yield

The estimation of linseed oil content was done by using the Soxhlet, wherein a known amount of dry and ground seeds was placed Soxhlet thimble and extracted with petroleum ether using a 100 ml round-bottom flask for 6-8 hrs. Thereafter, the solvent was separated by using rotary evaporation, while the recovered oil content was cooled, weighed and used to calculate the amount of oil qt ha^{-1}.

Statistical analysis

The data recorded in the present study were subjected to statistical analysis of variance (ANOVA) to determine the significance of the treatment at P=0.05 as described by Gomez and Gomez, (1984), using an online operational statistical tool (OPSTAT) developed by HAU, Hisar, India.

RESULTS AND DISCUSSION

The synergistic effect of seed priming, humic acid and sulphur under a pre-defined irrigation schedule was evaluated based on the studies of phenological, biochemical, and oil yield traits to optimize the response of treatments for enhancing the oil yield of linseed.

Phenological traits and oil yield

Data depicted in Table 1 revealed that the irrigation schedule and chemical treatments had a significant impact on most phenological traits at p=0.05, except for the days taken for the emergence. A significant interaction between

irrigation schedule and chemical treatments was also noticed for most of the traits, except for the days taken for branching. Among the irrigation schedules, two irrigation (I_2) resulted in the shortest duration for the emergence, branching and 50% flowering, 8.63, 39.25 and 96.62 days, respectively, compared to the zero irrigation (I_0). As for the reduction of days due to the irrigation schedule in percent is concerned, the maximum reduction was noticed in I_2 for days to branching (16%) and days to 50% flowering (13%) (Fig 2).

Among the chemical treatments, the most effective treatment was C_5, which is a combination of KNO_3+Humic acid (Table 1). In terms of the percent reduction due to the chemical treatments, C_5 showed the highest reduction of days due to chemical treatment in percent is concerned, with the maximum reduction in days to emergence (47%), days to branching (18%) and days to 50% flowering (13%). Conversely, days to maturity, number of capsules plant^{-1}, and oil yields qt ha^{-1} gradually increased along with the number of irrigations ranging from I_0 to I_2, showing a statistically significant difference at (p=0.05). The highest values were recorded under I2 with days to maturity (155.15), capsules plant^{-1} (42.43) and oil yield (4.06 qt ha^{-1}). Data presented in Table 1 also shows the significance of chemical treatments wherein the highest values were observed under the combinations of C_5 with Days to maturity (151.29), capsules plant^{-1} (48.17) and oil yield of (4.45 qt ha^{-1}). Additionally, data depicted in Fig 2 revealed the highest percent increase over the control was noticed in I2 with 7%, 23% and 22%, and in C_5 with 4%, 38%, and 32% increases for the days taken to maturity, number of capsules and oil yield.

Relative water content (RWC) and proline content

Relative water content and proline were analyzed at consecutive stages of 45 and 90 DAS under the influence of a pre-defined irrigation schedule and the combinations of chemical treatments to assess their significance in enhancing linseed oil yield. Data illustrated (Fig 1) revealed that both parameters, RWC% and proline content, were found statistically significant for irrigation and chemical treatments at both intervals, except for proline content at 45 DAS under irrigation scheduling, which was nonsignificant at (p=0.05). The amount of RWC% was gradually increased as the number of irrigations increased from I_0 to I_2 at both intervals. In contrast, the amount of proline content was reduced under the same levels of irrigation. However, among the chemical treatments, C_5 was observed as the most effective treatment to maintain the significantly highest amount of RWC, 66.91% and 59.24%, as well as produced proline content of 168.05 and 213.21 µg g^{-1} fresh weight. Data presented in Fig 2 revealed the percent increase or decrease over the control due to the irrigation and chemical treatments. The highest value of RWC was noticed under treatment I_2, showing an increase of 23% and 24% at respective intervals. In contrast, the proline content declined by 10% at both intervals under the same treatment. Similarly, C_5 resulted in an increase in

Table 1: Response of treatments on days taken for emergence, days taken for branching, days taken for 50% flowering, and days taken for maturity.

Treatment	Days taken to emergence	Days taken to branching	Days taken to 50% flowering	Days taken to maturity	Capsule plant^{-1}	Oil yield qt ha^{-1}
Irrigation schedule						
I_0	8.46^{a}	45.55^{a}	109.31^{a}	143.66^{b}	32.76^{c}	3.17^{c}
I_1	8.54^{a}	40.48^{b}	102.10^{b}	146.60^{b}	40.02^{b}	3.95^{b}
I_2	8.63^{a}	39.25^{b}	96.62^{c}	155.15^{a}	42.43^{a}	4.06^{a}
CD (p=0.05)	NS	2.403	2.278	1.55	0.698	0.068
Chemical treatments						
C_0	10.70^{a}	45.18^{a}	109.47^{a}	145.69^{c}	29.78^{f}	3.03^{g}
C_1	8.58^{b}	43.91^{b}	104.29^{b}	148.81^{ab}	36.06^{d}	3.51^{e}
C_2	9.14^{b}	44.72^{ab}	102.57^{bcd}	147.69^{bc}	32.50^{e}	3.34^{f}
C_3	8.77^{b}	40.89^{c}	103.02^{bc}	148.36^{bc}	38.78^{c}	3.75^{d}
C_4	7.68^{c}	39.06^{de}	101.22^{cd}	149.25^{ab}	40.44^{c}	4.10^{b}
C_5	7.28^{c}	38.14^{e}	97.11^{e}	151.29^{a}	48.17^{a}	4.45^{a}
C_6	7.64^{c}	40.43^{cd}	101.04^{d}	148.21^{bc}	43.11^{b}	3.90^{c}
CD (p=0.05)	0.385	1.176	1.195	1.42	1.424	0.085
CD (p=0.05) IXC	0.692	NS	2.936	2.65	2.510	NS

Note:

1-I_0= No post sowing irrigation, I_1= One post sowing irrigations, I_2= Two post sowing irrigations.

2-C_0= Control, C_1= KNO_3, C_2= Sulphur, C_3= Humic acid, C_4= KNO_3+ Sulphur, C_5= KNO_3+ Humic acid and C6= Sulphur + Humic acid.

3- As per the Duncan test, similar letters in a column indicate no significant difference among the treatments, while dissimilar letters indicate a significant difference among the treatments at p=0.05.

RWC by 7% and 9% and an increase in proline content by 21% and 18% at the respective intervals.

The phenological traits like days to emergence, days to branching, days to 50% flowering, days to maturity and the number of capsules were studied in the present piece of work to interpret the significance of irrigation schedule and combinations of chemical treatments in favor of linseed oil yield improvement under prevailing environmental conditions. The results of the study indicated that as the frequency of irrigation increased from I_0 to I_2, a gradual reduction in days to branching and days to 50% flowering. Conversely, days to maturity, number of capsules plant^{-1}, and oil yield qt ha^{-1} were increased as compared to I_0. These results are consistent with the findings of (Kariuki *et al.,* 2016), who reported that optimum irrigation can reduce the number of days to branching and days to 50% flowering, while improving days to maturity and number of capsules. Improvement in linseed is attributed to the critical role of water, facilitating physiological and biochemical processes essential for growth (Patel *et al.,* 2017). These traits influence the ability of plants to explore the available resources, escape biotic and abiotic stresses, and ultimately determine the yield potential. Moreover, analyzing phenological traits helps breeders select early or late-maturing genotypes according to the specific climatic conditions (Anastasiu *et al.,* 2016 and Hoque *et al.,* 2020). Among the chemical treatments, the combination of KNO_3 and Humic acid was noticed as one of the effective treatments that significantly reduced the days to emergence, branching and 50% flowering, while the number of capsules and oil yield were recorded comparatively better than the remaining treatments (Table 1). The use of potassium nitrate and humic acid as seed treatment and soil amendment may not directly influence the oil yield, but both treatments are efficient in supporting various physiological and biochemical processes for avoiding the consequences of drought (Abu-Ria *et al.,* 2024). Seed priming with KNO_3 acts as source of nutrient and signaling molecules effectively triggering seed germination and promoting synchronous seedling

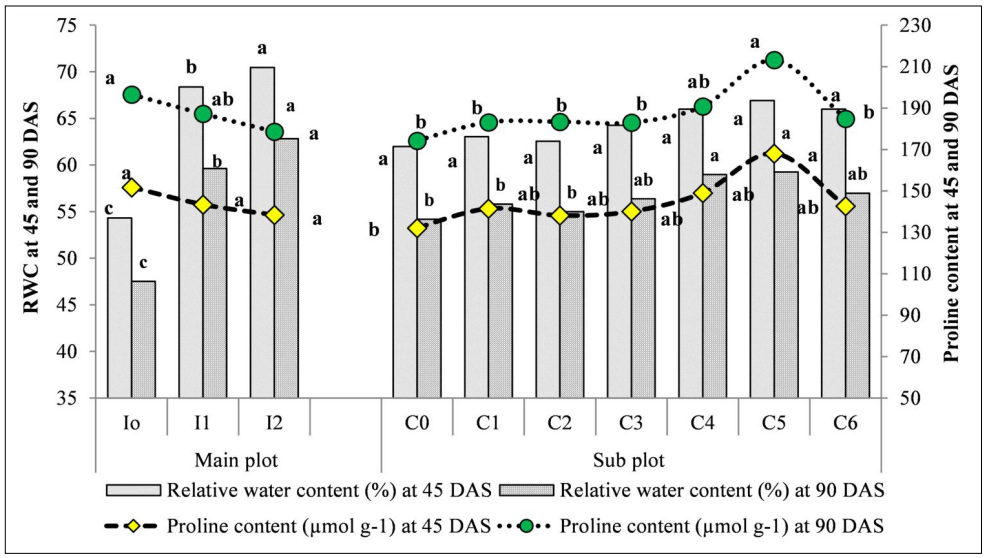


Fig 1: Response of the treatments on RWC (%) and proline content (µmol g^{-1}) in linseed.

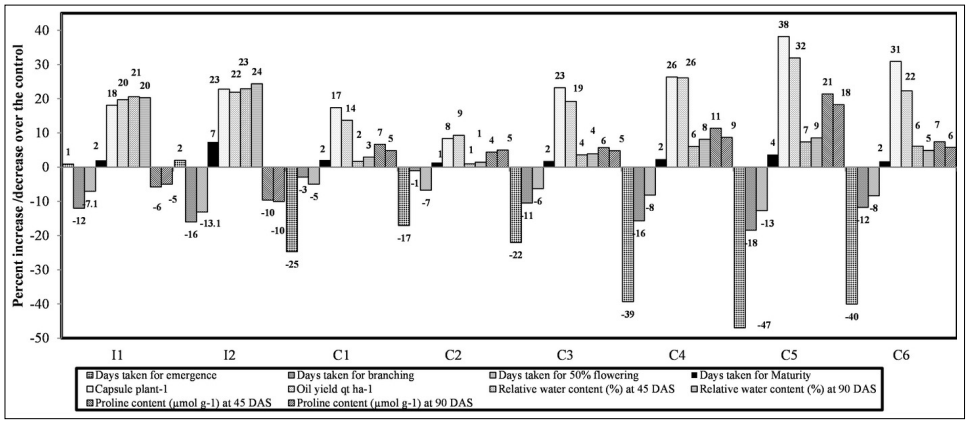


Fig 2: Response of treatments on the percent increase/decrease over the respective control in linseed.

establishment by enhancing the activity of key enzymes and supporting the hormonal balance (Zrig *et al.,* 2023 and Raj and Mallick, 2017), increasing the efficiency of water and nutrient uptake from the soil *via* triggering the root growth (Singh *et al.,* 2015 and Alizadeh *et al*., 2022). Data depicted from Table 1 reveals that the oil yield was significantly superior in C_5, which is a combination of KNO_3 and Humic acid. The result is per the findings of (Akanksha *et al.,* 2021), who elaborate significance of humic acid as a biostimulant for several physiological processes, inducing early branching and flowering. The use of humic acid alone or in combination with KNO_3 and sulphur may influence the oil yield by aligning the growth of phenological, physiological traits and yield (Bakry *et al.,* 2015; Kabak and Siddique, 2025 and Ahmad *et al.,* 2023). Data presented (Fig 1) revealed the negative relationship between the amount of proline content and RWC% due to irrigation frequency. However, the combinations of KNO_3 and Humic acid recorded the highest amount of proline and RWC%. Results are consistent with the findings (Castañares and Bouzo, 2019), potassium nitrate plays a significant role in maintaining osmotic balance through osmotic adjustment because it provides substrate K+ and NO_3 for osmotic balance and nitrogen metabolism, especially under moisture stress conditions. Proline is an amino acid; therefore, nitrate triggers the synthesis of proline through the glutamate pathway and acts as an effective osmoprotectant, contributing to cellular homeostasis and enhancing the antioxidant defence mechanism (Siddique *et al.,* 2018). Additionally, humic acid triggers root growth, water, and nutrient uptake through retention in the soil (Manna and Siddique, 2025). Together, these compounds help to mitigate the consequences of moisture stress and ultimately improve oil yield in linseed.

CONCLUSION

In the present piece of work, an integrated approach of seed priming with KNO_3, humic acid and sulphur was evaluated for the phenological growth and oil yield of *Linum usitatissimum* L. across irrigation scheduling. As per the findings are concerned, the C_5, which is a combination of potassium nitrate and humic acid, was detected as most prominent with two frequencies of irrigation. It significantly minimizes the days to branching, 50% flowering. Conversely, maximizing the days to maturity, the number of capsules plant^{-1}. Additionally, triggering the synthesis of proline while maintaining the RWC at the same level of irrigation frequency and the combinations of the treatments. Thus, it improved the oil production efficiency of linseed by aligning the phenological traits, improving the capsule plant^{-1} and improving the water use efficiency. These findings suggest that integrating seed priming with KNO*ƒ* and humic acid under appropriate irrigation regimes can serve as a cost-effective strategy to enhance oil yield and drought resilience in linseed cultivation.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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