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Humic Acid and Sulphur Modulating Phenology, Membrane Integrity, Lipid Peroxidation and Oil Yield in *Brassica juncea* under Water Regimes

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ABSTRACT

Background: Indian mustard, a key oilseed crop in India, is valued for its diverse culinary and health benefits. It faces significant challenges due to a wide range of abiotic stress wherein climate-induced water stress is one of them, which negatively impacts plant population, morpho-physiological growth and biochemical reactions. Thus not only the seed yield but also oil production is declining.

Methods: The current study aimed to evaluate the efficacy of humic acid and sulfur on phenology, membrane integrity, lipid peroxidation and oil yield in *Brassica juncea* under varying water regimes during the *Rabi* season of 2022-23 at the research farm of Lovely Professional University. The experiment was conducted in a split-plot design with a total of 16 possible combinations of water regimes and chemical treatments which include humic acid and sulfur.

Result: The results indicated that most of the traits were noticed as statistically significant for both factors *i.e.* water regimes and chemical treatments at *p*=0.05%. Among the treatments, I_3 (Three irrigation) and C_3 (Humic acid + Sulfur) were detected as the most effective treatments in terms of percent increase/decrease, resulting highest value over the respective control. The highest % increase was noticed in the initial plant population, final plant population, days taken for maturity, oil content, the moisture content in oil, chlorophyll a, b, a+b and membrane stability index while the reduction in days taken for branching, days taken for the 50% flowering, relative density and malondialdehyde were noticed in the I_3 and C_3. Thus the findings suggest that the 3 irrigations in combination with humic acid and Sulfur can effectively ameliorate the morpho-physiological growth by increasing the chlorophyll contents, integrity of the cell wall and oil yield in Indian Mustard.

Key words: Humic acid, Malondialdehyde, Membrane stability index, Sulphur, Water regimes.

INTRODUCTION

Indian mustard, botanically referred to as *Brassica juncea*, holds substantial economic importance as a vital oilseed crop, particularly in regions where it serves as a primary source of edible oil (Adhikari *et al.,* 2021 and Premi and Kumar, 2004). This crop is integral to agricultural systems, especially in India, where it considered a staple for over 4000 years (Güze, 2019 and Asha *et al.,* 2021). India produced 12 million metric tons of mustard oil in 2023-24, making it one of the largest producers globally, while out of the total production, Rajasthan alone contributed 40-50% (Chaturvedi *et al.,* 2023). However, despite this, domestic consumption due to the growing population remains dependent on the import of 60% of edible oil imports from Indonesia and Malaysia (Valiyaveettil *et al.,* 2023).

To reduce import dependency, it is crucial to enhance self-reliance in oilseed production, particularly focusing on crops like mustard (Barik, 2023). Achieving self-reliance in oilseed production is challenged by environmental stresses, particularly drought stress, which is aggravated by climate change (Pillai and Walia, 2024). India's groundwater depletion is projected to cause severe water scarcity by 2029, with rising temperatures and reduced winter precipitation further threatening mustard cultivation (Ramesh *et al.,* 2023). Consequently, significantly reduces productivity by lowering plant population, delaying vegetative growth and disrupting essential physiological processes (Batool *et al.,* 2022).

These effects are particularly pronounced during the crop establishment phase, where adequate irrigation is crucial for maintaining plant populations (Tajane *et al.,* 2023 and Murtaza *et al.,* 2016). The phenological stages are also closely tied to water availability and nutrient management, with water stress often delaying them and thus affecting yield potential (Bhattacharya and Bhattacharya, 2021). Therefore, to avoid the interventions of drought on the potential of seed yield and oil content in Indian mustard, systematic planning and formulations are

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needed to enable the plant to increase the membrane integrity, protect the degradation of chlorophyll and reduce lipid peroxidation at cellular level (Nadia and Naqvi, 2010 and Al-Gaadi *et al.,* 2024). In response to these challenges, it was noticed in literature that humic acid and sulfur application in the soil ameliorate the response of drought in mustard (Ampong *et al.,* 2022). Humic acid balances both the acidic and alkaline nature of the soil and promotes nutrient and water uptake in the plant therefore, it is known as a soil conditioner (Mosa *et al.,* 2020). Similarly, humic acid along with Sulphur act synergistically in mustard enduring the drought but also improve the yield potential of mustard (Oyege *et al.,* 2023; Akimbekov *et al.,* 2021 and Kumar and Kumar, 2007). Hence the current study was conducted to analyze the efficiency of humic acid and sulfur under variable water regimes on phenology, membrane integrity, lipid peroxidation and oil yield in mustard.

MATERIALS AND METHODS

A field-based study was conducted in the *Rabi* season of 2022 and 2023 at the research farm of Lovely Professional University Farm, Phagwara, Punjab. The experiment was comprised of a split-plot design with four levels of water regimes (I_0, I_1, I_2 and I_3) and four chemical combinations with Indian mustard (Raya RLC 3). The chemical formulations were based upon the humic acid, Sulphur and its combination. During the conduct of the experiment, standard agronomical operations were carried out as per the recommendation of the package and practices.

To analyze the impact of treatments, phenological parameters such as initial and final plant population plot-1, days to 50% flowering, days to branching and days to maturity were recorded by tracking the intervals from sowing to the maturity of crops under different water regimes. The relative density of mustard oil and its moisture content were analyzed as per the (AOAC, 2000 and 2019) which is the ratio of its density to that of water while the calculation of relative density and moisture content were calculated as per the following formula:

$$\text{Relative density of mustard oil} = \frac{A-B}{C-B}$$

Whereas

A= Weight of specific gravity bottle + oil at 30°C.

B= Weight of specific gravity bottle at 30°C.

C= Weight of specific gravity bottle + water at 30°C.

Moisture content of oil (%) =

$$\frac{\text{Wt. of fresh oil - Wt. of oven-dry oil}}{\text{Wt. of fresh oil}} \times 100$$

Chlorophyll a, chlorophyll b and Chl a + Chl b were derived as per the formula suggested (Arnon, 1949).

Chlorophyll a (mg g^{-1} fresh Wt.) = (12.7 * A 663 - 2.69 * A 645)*(V/W*1000)

Chlorophyll b (mg g^{-1} fresh Wt.) = (22.9 * A 645 - 4.68 * A 663)*(V/W*1000)

It was estimated as per (Sairam *et al.,* 2005) at regular intervals of 30 days up to 90 days after sowing (DAS) while the MSI % was computed as per the mentioned formula.

$$\text{Membrane stability index \%} = \frac{1- EC_1}{EC_2} \times 100$$

Whereas,

EC_1 and EC_2 represent the electrical conductivity of samples kept at 40°C and 100°C in water bath.

A method of Heath and Pacer (1968) was used to measure the synthesis of MDA content wherein 0.1% Trichloroacetic Acid (TCA) and 0.5% Thiobarbituric Acid (TBA) reagents were used. The MDA-TBA complex was quantified by subtracting the absorbance at 600 nm from the absorbance at 532 nm, using the extinction coefficient of εM=155 mM^{-1} cm^{-1} and the results were expressed as µmoles MDA per gram of fresh weight.

The statistical analysis of data recorded from the present piece of work was carried out by the software SPSS 22nd version. The level of significance was tested through ANOVA followed by a DMRT test at (*P*=0.05).

RESULTS AND DISCUSSION

Initial and final plant population $Plot^{-1}$

The initial and final plant population $plant^{-1}$ was evaluated along with humic acid and sulfur under variable water regimes in Indian mustard (Table 1). It was noticed that both parameters were statistically nonsignificant for the water regimes *i.e.* irrigation (I0 to I3) and interaction with chemical treatments while chemical treatment alone was detected as statistically significant (*p*=0.05). Therefore, the initial and final plant population plot-1 was relatively consistent across all irrigation and chemical treatments, ranging from 512.18 to 518.26 and 488.81 to 494.65 plants $plot^{-1}$. However, the highest initial and final plant populations were recorded at 521.94 and 498.75 in C_3 (Humic acid + Sulphur) as compared to the control (Table 1).

Days taken for branching, days to 50% flowering and maturity

Data presented (Table 1) showed the impact of treatments on days taken for branching and 50% flowering under-water regimes. As per the treatments are concerned, water regimes and chemical treatments both were recorded as statistically significant but their interaction was nonsig-nificant at (*p*=0.05). Minimum days for the branching were recorded in I_3 *i.e.* 36.12 while 33.49 was in C_3 compared to their respective control I_0 and C_0 45.06 and 45.54 days taken for the branching.

Similarly, the parameters days to 50 % flowering were also noticed statistically significant for both the kind of treatments irrigation regimes and chemical treatments while its interaction was found nonsignificant (*P*=0.05). Overall a minimum day to 50% flowering was noticed in I_3 and C_3 *i.e.* 37.45 and 39.52 days, which is a remarkable reduction in days compared to their respective control 57.88

and 52.04 days. Data of % decrease over control was 24, 35 and 54.5, 31.7% respectively were found for the days taken for the branching and days to 50% flowering (Fig 1).

In contrast, the extended crop maturation period was also noticed in I_3 and C_3 *i.e.* 131.68 and 125.51 days respectively as compared to their respective control 98.26 and 111.51 days which were statistically significant for water regimes, chemical treatments and their interaction at (p=0.05%).

Moisture content, relative density and oil content

Data depicted from Table 1 also revealed the significance of treatments on the oil quality parameters *i.e.* moisture content, relative density and oil content. A significantly gradual increase in oil content was noticed along with an increase in irrigation frequency wherein I_3 was recorded as the highest value for the parameters at 44.05% while C_3 was observed as significant among the chemical treat-ments at 45.34%. In contrast, a gradual reduction in the mois-ture content and relative density of the mustard oil was found along with the increased irrigation frequency from I_0 to I_3 and noticed a minimum in I_3 0.33 and 0.86 % while C_3 was recorded as a minimum moisture and relative density 0.44 and 0.89% among the chemical treatments (Table 1). Additionally, a negative relationship was also noticed between the relative density and oil content (Fig 2). The data depicted in Fig 1 also verify the highest reduction % of moisture content and relative density in I_3 and C_3.

Chlorophyll contents

The results depicted in (Table 2) revealed the impact of the treatments on chlorophyll content a, b and a+b wherein the entire parameters were detected as highly significant for water regimes, chemical treatments and their interaction at (p=0.05%) for both the intervals 60 and 90 DAS. A gradual increase of chlorophyll a, b and a+b was noticed along with the increased water regimes wherein the I3 was noticed as the highest value *i.e.* 1.645, 0.399 and 1.865 mg g^{-1} compared to their respective control. However, among the chemical treatments, the significantly highest amount was noticed in C_3 1.501, 0.414 and 1.915 mg g^{-1} which was followed by C_1 and C_2 at 90 DAS (Table 2). The improvement in chlorophyll a, b and a+b was also presented in Fig 3 indicating the highest in I_3 and T_3.

Membrane stability index and Malondialdehyde content

Membrane stability index (MSI%) and malondialdehyde (MDA µM g^{-1} fresh weight) were measured at 60 and 90 DAS to know the significance of the treatments. It was noticed from Fig 4 that the MSI and MDA both were statis-tically significant at (p=0.05%). Among the water regimes, a gradual increase of MSI% was noticed from I_0 to I_3 while MDA content declined and noticed maximum/minimum amount of MSI and MDA in I3 70.99 % and 12.04 µM g^{-1} fresh weight at 90 DAS. Additionally, among the chemical treatments, C_3 was noticed as a maximum MSI of 73.7% and minimum MDA content of 19.54 µM g^{-1} fresh weight at

Table 1: Impact of treatments on morpho-phenological traits and oil quality parameters under variable water regimes.

Treatment details	Initial plant population plot^{-1}	Final Plant population plot^{-1}	Days taken for branching	Days taken for 50% flowering	Days taken for maturity	Moisture content of oil (%)	Relative density (%)	Oil content (%)
Main plot								
I_o	512.18^{a}	488.81^{a}	45.06^{a}	57.88^{a}	98.26^{c}	0.79^{a}	0.94^{a}	35.78^{d}
I_1	516.41^{a}	491.78^{a}	40.46^{b}	49.29^{b}	111.76^{b}	0.63^{b}	0.92^{b}	39.05^{c}
I_2	517.15^{a}	494.65^{a}	36.41^{c}	38.71^{c}	130.09^{a}	0.40^{c}	0.89^{c}	42.51^{b}
I_3	518.26^{a}	495.76^{a}	36.12^{c}	37.45^{c}	131.68^{a}	0.36^{d}	0.86^{d}	44.05^{a}
CD (p=0.05%)	NS	NS	2.18	2.21	4.36	0.03^{1}	0.02	1.27
Sub plot								
C_0	509.76^{b}	486.35^{b}	45.54^{a}	52.04^{a}	111.51^{d}	0.68^{a}	0.92^{a}	35.26^{d}
C_1	519.31^{a}	496.03^{a}	41.19^{b}	43.83^{c}	119.79^{b}	0.57^{b}	0.88^{d}	38.98^{c}
C_2	512.97^{ab}	489.84^{ab}	37.82^{c}	47.95^{b}	114.98^{c}	0.50^{c}	0.91^{b}	41.82^{b}
C_3	521.94^{a}	498.75^{a}	33.49^{d}	39.52^{d}	125.51^{a}	0.44^{d}	0.89^{c}	45.34^{a}
CD (p=0.05%)	4.63	4.29	1.35	1.28	1.88	0.03	0.01	1.00
CD at (p=0.05%) MXS	NS	NS	NS	NS	3.76	0.06	NS	2.01

Notes:
1-I_0, I_1, I_2 and I_3 represent the number of irrigation given at critical stages.
2-C_0, C_1, C_2 and C_3 represent the control, humic acid, sulfur and humic acid + sulfur.

90 DAS. A close analysis of Fig 4 also revealed a negative relationship between the MSI and MDA content.

Limited availability of water throughout the entire phase of growth not only restricts the morpho-physiological growth especially at the critical stage by reducing the efficiency of photosynthesis due to the degradation of chlorophyll content but also poses a negative impact on the seed yield thereby limiting the oil content. In contrast, to obtain remarkable output in terms of oil content must ensure the optimum growth of morpho-phenological traits. It seems from the data presented (Table 1) that most of the phenological traits were significantly improved with the supply of three irrigations at critical growth stages along with the soil application of humic acid and sulfur because the recovery in morpho-phenological traits up to the optimum level is possible by holding the moisture in the soil and facilitating the supply of nutrient. Results are well correlated with the findings of Afu *et al.* (2024) who indicated that humic acid enhances soil structure and nutrient availability, promoting better seedling establishment while sulfur application improves morphological traits, final plant population (Kumar *et al.,* 2022 and Kodavali *et al.,* 2022).

Optimal plant density ensures effective resource use (Djalovic *et al.,* 2024). Moreover, adequate moisture and the use of humic acid and sulfur not only promote the growth of morpho-phenological traits but also reduce the flowering time and extensive branching by supporting essential metabolic processes (Paul *et al.,* 2017; Sharma *et al.,* 2023 and Maurya *et al.,* 2023 and Kumar and Dhillon, 2023). Humic acid also enhances mineral absorption and profused root development, leading to improved soil structure further promoting earlier new branching for better plant growth (Gerke, 2021 and Zanin *et al.,* 2019). Results related to chlorophyll a, b and a+b followed a similar pattern wherein the chlorophyll content was reduced due to the scarcity of water while it improved at three irrigations along

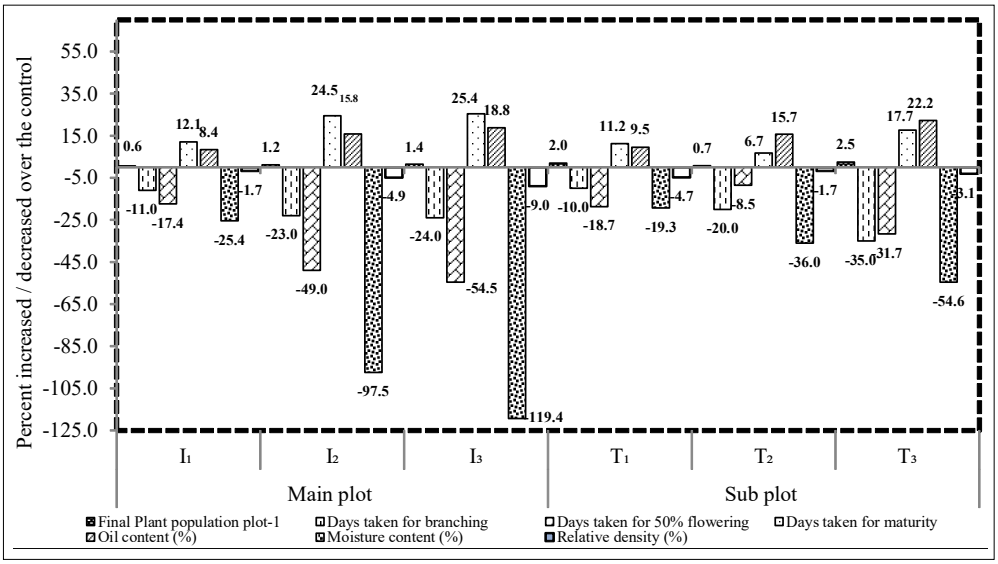


Fig 1: Impact of treatments on morpho-phenological traits and oil quality parameters under variable water regimes.

Table 2: Impact of treatments on Chlorophyll a, Chlorophyll b and a+b (mg g^{-1}) under variable water regimes in Indian mustard.

Treatments	Chlorophyll a		Chlorophyll b		Chlorophyll a + b	
	60 DAS	90 DAS	60 DAS	90 DAS	60 DAS	90 DAS
Main plot						
I_o	1.71^{d}	1.229^{d}	0.378^{d}	0.343^{d}	2.088^{d}	1.573^{d}
I_1	1.77^{c}	1.339^{c}	0.409^{c}	0.372^{c}	2.186^{c}	1.712^{c}
I_2	1.88^{b}	1.415^{b}	0.442^{a}	0.394^{b}	2.322^{b}	1.809^{b}
I_3	1.91^{a}	1.465^{a}	0.428^{b}	0.399^{a}	2.339^{a}	1.865^{a}
CD (p=0.05%)	0.025	0.03	0.007	0.007	0.031	0.033
Sub plot						
C_0	1.67^{d}	1.235^{d}	0.393^{d}	0.342^{d}	2.064^{d}	1.577^{d}
C_1	1.88^{b}	1.391^{b}	0.424^{b}	0.385^{b}	2.307^{b}	1.776^{b}
C_2	1.79^{c}	1.32^{c}	0.409^{c}	0.366^{c}	2.204$_{c}$	1.691^{c}
C_3	1.93^{a}	1.501^{a}	0.431^{a}	0.414^{a}	2.359^{a}	1.915^{a}
CD (p=0.05%)	0.009	0.03	0.005	0.009	0.012	0.039
CD at (p=0.05%) MXS	0.018	0.06	0.01	0.02	0.02	0.078

with the soil application of humic acid and sulfur (Table 2).

Similarly, the maximum MSI% and least production of MDA content were also detected in the same treatments (Fig 4). The reduction of chlorophyll content under the limited availability of water is obvious and well known (Banerjee *et al.,* 2021 and Gogoi *et al.,* 2024) consequently the potential of photosynthesis is hampered due to the collapse of cell membrane and lipid peroxidation (Fradera-Soler *et al.,* 2022). The present findings follow the findings of Moustafa-Farag *et al.* (2020) who suggested that the scarcity of water

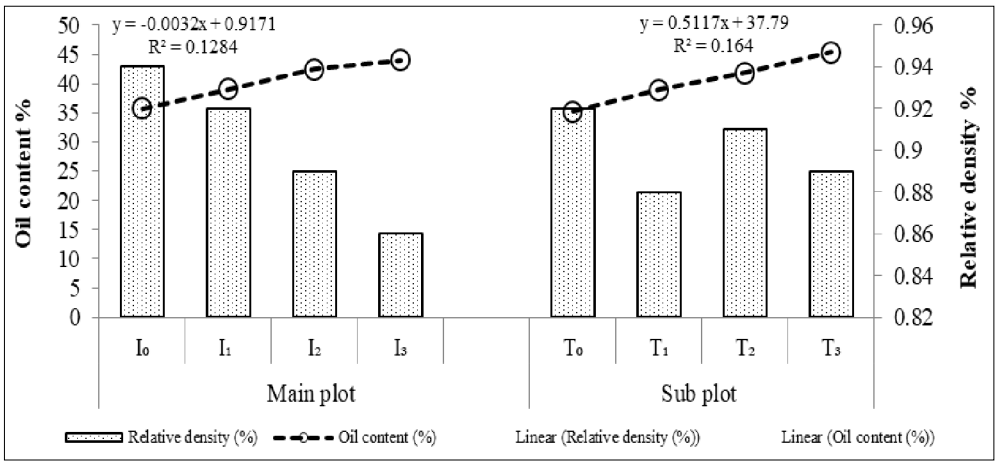


Fig 2: Impact of treatments on oil content (%) and relative density (%) under variable water regimes in Indian mustard.

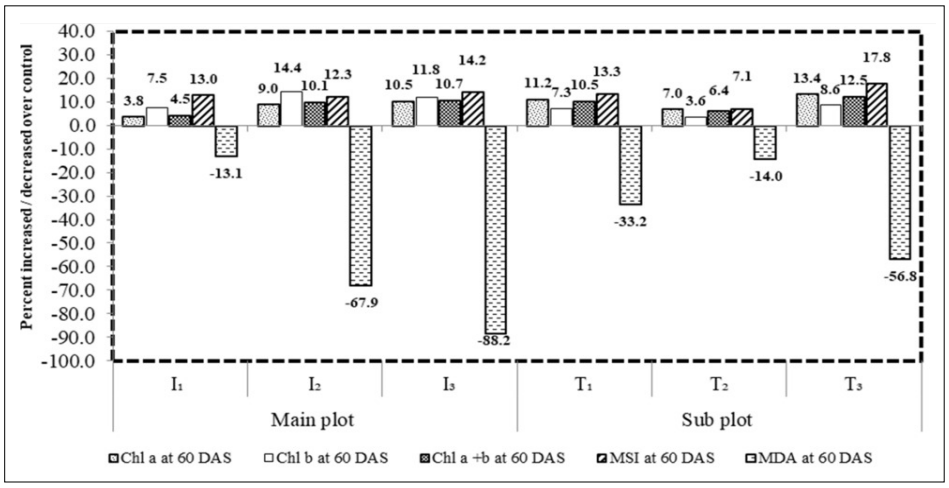


Fig 3: Impact of treatments on per cent increased/decreased over control in chlorophyll a, b and a+b content, MSI and MDA content.

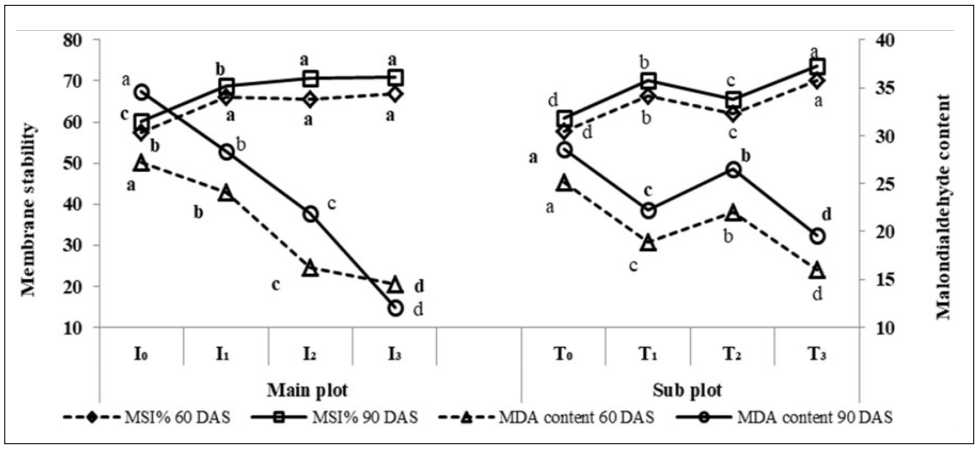


Fig 4: Impact of treatments on MSI % and MDA content (μM g^{-1} fresh weight) under variable water regimes in Indian mustard.

during the critical growth period triggers the synthesis of MDA content and consequently loss of membrane integrity. But the use of humic acid boosts MSI by improving soil structure, nutrient absorption and root growth (Nardi *et al.,* 2000) while sulfur application also strengthens the MSI by enhancing amino acid and protein mediated by the synthesis of enzymes (Ram *et al.,* 2016). Additionally, the use of humic and sulfur coordinates for the improvement of oil quality parameters like moisture content, relative density and oil yield in mustard crops. Similar findings were also noticed by Aranaz *et al.* (2023); Vikram *et al.* (2022) and Sah *et al.,* (2013) who pointed out that irrigation may dilute the oil content but the use of sulfur improves the oil content by triggering the Acetyl-CoA carboxylase activity (Maurya *et al.,* 2023; Shah *et al.,* 2022 and Singh *et al.,* 2022).

CONCLUSION

The study underscores the critical role of water regimes and nutrient management with humic acid and sulfur in Indian mustard. The optimum irrigation regimes (I_3) in combination with humic acid and sulfur (C_3) significantly influence the morpho-phenological traits like initial and final plant population, leading to earlier branching and flowe-ring and extended maturity. Unlike the morpho-phenological traits, the findings also indicated that applied treatments coordinate with the triggers of the synthesis of chlorophyll a, b, a+b which is useful for enhancing the rate of photosyn-thesis. Moreover, the study also indicates the potential of treatment in favor of membrane integrity by reducing lipid peroxidation (MDA content). Thereby improving the membrane stability and oil yield and reducing the relative density of oil. Hence, the use of irrigation at critical stages *i.e.* before flowering, after flowering and pod formation stage along with the combination of humic acid and sulfur can ameliorate the morpho-physiological growth by increasing the chlorophyll contents, the integrity of the cell wall and oil yield.

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Conflict of interest

All the authors declare that there is no conflict of interest regarding the publication of this article.

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