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Physiological studies and nutrient accumulation in mustard varieties in response to fertilizer doses under saline condition in semi-arid region of northwestern Haryana

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The present study was conducted to findout the salt tolrant mustard variety for semi-arid region because this region has higher amount of poor quality water for irrigation and nutrient accumulation under saline environment is the most limiting factor in the sustainable crop production. For this four mustard varieties in main plots (Kranti, Giriraj, CS-54, and CS-58) and three fertilizer doses in sub-plots (100, 125 and 150% RDF). During the experimentation, saline water irrigation (7 dS m^{-1}) was applied. The physiological parameters like RWC (75.54 to 80.34 %), total chlorophyll content (1.60 to 1.91 mg g^{-1} FW), and photosynthetic rate (10.09 to 14.79 µmol CO_2 $m^{-2}s^{-1}$) were significantly increased with increasing dose of fertilizers.Yield stability index was highest with the application of 150% RDF. HI index showed a decline trend with the increasing dose of fertilizers, whereas oil content decreased non-significantly. Irrespective of fertilizer doses, variety CS-58 recorded maximum seed yield (22.89 q ha^{-1}), biological yield (11.22 q ha^{-1}); and other physiological parameters followed by CS-54, Giriraj, and Kranti mustard varieties. Variety CS-58 had the highest nutrient content (N, P, and K) in straw, with reaching to 125% RDF being at par with 150% RDF, followed by CS-54, Giriraj, and Kranti, respectively. The salt ratio (Na^+:K^+)decreased non-significantly with fertilizer doses, its mean value was however lowest in CS-58.

Keywords: Abiotic stress, Bassica, Rapeseed, Recommended dose of fertilizer (RDF), Salinitytolerance

India rank 2nd in the rapeseed/mustard area with a share of 15.2 percent, and it is fourth in predictions with a share of 9.5 percent[1]. It has cultivated area of about 8.74 million ha and production of 10.95 MT with an average yield of 1270 kg/ha[2]. It is also an important crop of Haryana after cereals crops. Presently the area under mustard crop in Haryana state is 7.1 lakh hectares with a production of 7.9 million tonnes and an average yield of 1117 kg/ha[2].

Rapeseed mustard is generally grown under arid and semi-arid regions. The main reasons for low productivity are salinity and inadequate supply of nutrients. Small and marginal farmers generally grow rapeseed and mustard mainly without applying fertilizers and irrigation, considering this a risky crop[3]. Appropriate nutrient management, especially N fertilizers, not only improves mustard yield but also improves its quality[4]. N is the most important, limiting element for plant growth and development. Hence, optimum use of N fertilizers is one of the most crucial factors in oilseeds production[5]. Phosphorus (P) is one of the major constituents in crop nutrition and is known to play an important role in salinity-fertility interaction and increase plant vigour concerning salt-tolerance capacity. Plant growth under salinity and water stress is also influenced by the plant ability to control the K^+/Na^+ ratio in the tissues[6]. Severity of deleterious effects of Na^+ are greater under low K^+ concentrations and plants having efficient activity of potassium transporters maintains normal growth under such conditions[7]. Sulphur (S) plays an important role in photosynthesis and nitrogen fixation, particularly in the crops belonging to the family brassicacae[8].

The maximum area under mustard is centred in the northwest agro-climatic zone, where the majority of groundwater is highly saline and has medium to high sodicity problems. Use of poor quality groundwater is inevitable to grow crops under arid and semi-arid climatic regions due to non-availability or limited availability of good quality waters for irrigation[9]. Under such conditions, seed germinability, growth,

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and seed yield of mustard is adversely affected[10]. Excess amounts of salts inhibit plant growth through the more negative osmotic potential of the soil solution, specific ion toxicity, and ion imbalance[11], which further reduce nutrient uptake and affect mustard yield and quality[12]. Biosynthesis of chlorophyll and inefficiency of photosynthesis affected by salt stress[13]. However, inappropriate and the continuous use of saline water irrigation have resulted in rising groundwater levels, which can trigger salt accumulation in the soil profile[14] and negatively affects crop production[11]. Keeping in view the above facts, in the present study, we tried to assess the impact of mineral fertilizers doses on yield, quality, and physiological parameters of various mustard varieties under saline water application in the semi-arid region of Haryana.

Material and Methods

Experimental site

The study was carried out in 2018-2019 at the soil research farm of CCS Haryana Agricultural University, Hisar, India, situated in semi-arid, sub-tropics at latitude 29° 10' N, the longitude of 75° 46' E and at an altitude of 215.2 m in Haryana, India. The region has very hot summers and relatively cool winters. Climate in Hisar are dryness, extremes of temperature, and scanty rainfall. The average annual rainfall is around 429 mm, most of which occurs during monsoon season (July and August). The meteorological data related to the experimental site during *rabi* season 2017-18 is depicted in Fig. 1. The data revealed that the mean weekly maximum and minimum temperature, morning and evening relative humidity (RH), and rainfall ranged from 16.9 to 35.7°C and 3.2 to 17.5°C, 68 to 100% and 24 to 81% and 0 to 34.6 mm, respectively.The soil was sandy loam in texture and other initial physico-chemical properties of the site determined by using standard methods are given in Table 1.

Experimental design and treatments

The experiment was laid out in a split-plot design with three replications. There were four mustard varieties Kranti, Giriraj, CS-54, and CS-58, in main plots; and three fertilizer treatments *viz.* F1: 100% RDF (N, P and K) @ 60-20-20 kg ha^{-1}, F2: 125% RDF @ 75-25-25 kg ha^{-1} and F3: 150% RDF @ 90-30-30 kg ha^{-1}in sub-plots.

Cultural operations

The nutrients (NPKS) were applied through urea, di-ammonium phosphate (DAP), single superphosphate (SSP), and muriate of potash (MOP), respectively. Half of the N and full portion of P and K were applied at the sowing time, whereas the remaining amount of nitrogen was applied after first irrigation. The primary sources of irrigation in Haryana are canal and tube well water.

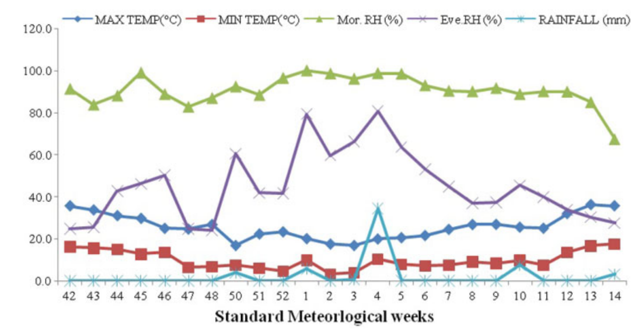

Fig. 1 — Seasonal agro-metreological data of the area during crop growth period

Table 1 — Initial physico-chemical properties of the soil of the experimental site*

Parameters	Values	Methods used
Texture	Sandy loam	International pipette method[15]
$pH_{(1:2)}$	8.10	pH meter[16]
EC_e($dS\ m^{-1}$)	3.18	Conductivity meter[16]
Organic carbon (%)	0.26	Wet digestion with $K_2Cr_2O_7$ and H_2SO_4[17]
Available nitrogen(kg ha^{-1})	102.40	Alkaline permanganate method[18]
Available phosphorus (kg ha^{-1})	16.00	Olsen's method[19]
Available potassium(kg ha^{-1})	245.50	Extraction with 1 N ammonium acetate at pH 7.0 and estimated by Flame photometer[16]
Available sulphur(ppm)	91.80	Spectrophotometrically using BaCl2 in presence of gum acacia solution[20]
CO_3^{2-}	Nil	Titration with standard H_2SO_4 using phenopthalin[16]
HCO_3^{2-}	9.35	Titration with standard H_2SO_4 using methyl red[16]
Cl^-	10.85	Titration with $AgNO_3$ using potassium chromate[16]
SO_4^{2-}	-	Spectrophotometrically[20]
Na^+	18.50	Flame photometer[16]
K^+	0.36	
$Ca^{2+} + Mg^{2+}$	8.84	Versenate titration using EBT indicator[16]

[*Standard methods adopted for analysis of irrigation water]

The desired level of EC was prepared by mixing tube-well water with canal water. Approximately three irrigations were applied in season. Irrigation water samples were taken at the time of irrigations, and standard methods (Table 1) were adopted to calculate their ionic composition. Ionic composition and quality parameters of irrigation water are given in Table 2. SAR is used as a water suitability measure for agricultural use, which the given formula calculates[21]:

$$SAR = \frac{Na^{+}}{\sqrt{\frac{Ca^{2+} + Mg^{2+}}{2}}}$$

Plant sampling and analysis

Representative grain and straw samples were collected from individual plots at the time of threshing of crop, initially dried in air, and finally in an oven at 60± 20°C. The oven-dried samples were ground in a mini grinder with stainless steel blades and stored in paper bags for analysis. Straw samples were digested in a di-acid mixture of sulphuric and perchloric acid in a 4:1 ratio. Nitrogen (N), phosphorus (P), potassium (K), and sodium (Na) were analyzed in the digested plant material by using nessler's reagent[22],vanadomolybdate yellow colourand flame photometric method, respectively. Oil content in seedwas determined by Soxhlet instrument with n-hexane (60°C) as an organic solvent.

Relative water content (RWC %) was calculated by the formula given below[24]:

$$RWC (\%) = (Fresh\ weight - Dry\ weight) / (Turgid\ weight - Dry\ weight) \times 100$$

Total chlorophyll content was measured by the dimethyl sulfoxide (DMSO) method[25]. The photosynthetic rate was estimated in fully expanded leaf with infrared gas analyzer (IRGA LCi-SD, ADC Bioscience). Harvest index was calculated by the formula given below:

$$HI = (Economic\ yield/Biological\ yield) \times 100.$$

The yield stability index (YSI) was measured by the formula given below[26]:

$$YSI = Ys/Yp$$

[where Ys and Yp are seed yield under stress and non-stress conditions, respectively]

Statistical analysis

The experimental data were analysed using the OPSTAT statistical software package developed by the Department of Statistics, CCS Haryana Agricultural University[26] to drive ANOVA (p= 0.05).

Results and Discussion

Physiological parameters

Relative water content (RWC) *and Chlorophyll content*

It was observed that RWC (Fig. 2A) and total chlorophyll content (Fig. 2B) increased with the increasing dose of fertilizers under saline water irrigation. With the consistency of other reports RWC reduced with the imposition of salinity stress but Maximum RWC was maintained by CS-58 (85.03%) and minimum in Kranti (76.59%) at 150% RDF followed by 125% RDF application. Our results are in concomitant with the findings of Alam*et al.*[28] that combination of exogenous application of ascorbic acid (AsA) and the osmotic stress restored the leaf RWC to levels at par to the control plants supporting the projected role of AsA in osmotic regulation.

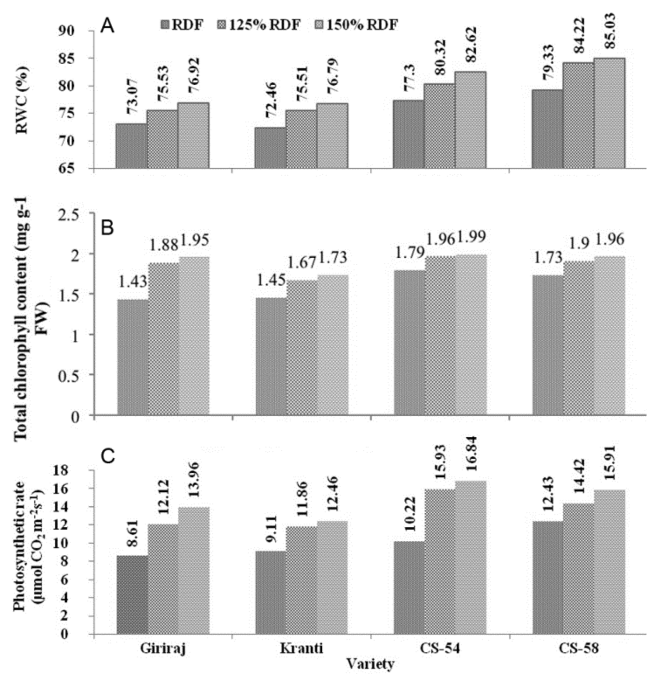

Fig. 2 —(A) Relative water content (RWC %);(B) Total chlorophyll content (mg g^{-1} FW); and (C) Efficiency of photosynthetic rate in different mustard varieties as influenced by fertilizer doses under saline water irrigation

Table 2 — Ionic composition and quality parameters of irrigation water

Treatment	pH	EC ($dS\ m^{-1}$)	Na^{+}	K^{+}	Ca^{2+}	Mg^{2+}	CO_3^{2-} ($me\ L^{-1}$)	HCO_3^{-}	Cl^{-}	SO_4^{2-}	SAR
Canal	7.50	7.09	49.39	0.21	4.40	13.20	-	4.70	42.60	20.40	16.65

Similarly, total chlorophyll content also showed an increasing trend with increasing levels of fertilizers. The highest chlorophyll content was observed in CS-54 (1.99 mg g^{-1} FW) followed by CS-58 (1.96 mg g^{-1} FW) and Giriraj (1.95 mg g^{-1} FW), respectively, at 150% RDF imposition. The values of total chlorophyll content at 125% RDF were at par with the values of 150% RDF. With the knowledge salinity stress decreased the chlorophyll content which occurs either due to inhibition of production or accelerates the degradation of active chlorophyll molecule by the enzyme chlorophyllase. This trend was reversed with the imposition of different doses of fertilizers.

Similar results were reported with individual or combined N and S application, which improved chlorophyll content in mustard cultivars Alankar and Chutki in salt-stressed plants.Application of N or S to salt-stressed plants increased salt tolerance, more conspicuously with combined application of N and S. The individual role of N in the alleviating salt stress by increasing N assimilation and osmolyte formation has been reported. However, S may also regulate the formation of osmolytes by its influence on NO_3^- reductase activity and N assimilation as the importance of S in maintaining the tertiary structure of proteins is well documented. The increased supply of sulphur (S) had improved photosynthesis and growth rate in mustard through regulating N assimilation[29]. The availability of S regulates nitrate reductase activity and the accumulation of N. An increase in RWC (%) with increased application of K_{120} in mustard and groundnut crop as compared to control (K_0) under water stress and normal conditions, is also reported[30]. Application of potassium improves RWC of plants under normal as well as water stress conditions[31]. The maintenance of plant water economy by K application in terms of a high RWC level under water stress conditions could be ascribed to the supposed role of K in stomatal resistance, water use efficiency and lowered transpiration[31]. The highest value of chlorophyll was recorded with the application of 200 kg N ha^{-1}), while no application of N fertilizer showed the lowest magnitudes of the chlorophyll content. More N consumption resulted in increasing of Chlorophyll and leaf area index (LAI) and their preservation until the end of the growth period[32]. They also reported an increase in RWC (%) with the application of N and S both. They observed 5.27% chlorophyll content enhancement in comparison to control. It has been shown that increasing N application will increase the protein synthesis, increase cell wall thickness and cause absorption of extra water by protoplasm and improve the relative water content[33].

Photosynthetic rate

Photosynthetic rate acts as a stress indicator. Present study showed clear variability in mustard genotypes in response to fertilizer doses under salinity stress. Excess salt concentration causes the adverse effects to plants but these adverse situations were overcome by the application of different fertilizer doses. The photosynthetic efficiency of different mustard varieties after applying fertilizer doses (RDF, 125% RDF and 150% RDF) under saline water irrigation (Fig. 2C). The highest photosynthetic rate was noticed in CS-54 (16.84 µmol CO_2 $m^{-2}s^{-1}$), followed by CS-58 (15.9 µmol CO_2 $m^{-2}s^{-1}1$) and Giriraj (13.96 µmol CO_2 $m^{-2}s^{-1}$) and least in Kranti (12.46 µmol CO_2 $m^{-2}s^{-1}$) after application of 150% RDF. The maintenance of photosynthetic rate at 125% RDF application was slightly lower than that 150% RDF. An increase or decrease in photosynthetic rate occurs mainly due to the changes in the activity of oxygenase enzymes and photosystem II and the stability of chlorophyll content[34]. The effect on the photosynthetic rate is due to the imposition of salt stress-causing several changes in the anatomy and physiology of the leaf-like leaf area reduction; consequently, compactness of chloroplast increases per unit leaf area and finally leads to diminished photosynthetic rate[33,35]. Inadequate N supply to the plant also causes a decline in N content of the leaf that leads to diminishing photosynthetic rate and metabolic activities[36]. It is also observed that salt stress did not adversely affect the photosynthetic rate in the existence of sufficient N[37]. Salinity reduces activity of carbonic anhydrase (CA) because of the enzyme Rubisco inactivation, consequently reducing the chlorophyll content and net photosynthetic assimilation[38]. Application of nitrogen that enhanced CA activity under salinity stress and explain why increased CA activity might have helped in the availability of CO_2 and maintain Rubisco supply[39].

Biological yield, Harvest Index and Yield Stability Index (YSI %)

Biological yield was significantly higher in variety of CS-58 (100.26 q ha^{-1}) followed by CS-54 (86.28 q ha^{-1}), Giriraj (79.82q ha^{-1}), and Kranti (74.36 q ha^{-1}), as showed in Table 3. However, under different fertilizer doses, it was recorded significantly higher under 150% RDF (96.49 q ha^{-}1) and 125% RDF (83.93q ha^{-}1) as compared to RDF (75.13 q ha^{-1}). Harvest indexwas calculated after the application of different doses of fertilizers (Fig. 2C). The HI was obtained higher in CS-54 (21.43%) followed by CS-58 (20.58%) after imposition of 150 % RDF (Fig. 3A). The yield stability index (YSI %) highest in CS-54 (1.20%) at 150% RDF application (Fig. 3B). The increase harvest index values and biological yield in mustard with increased doses of N & S was due to improved photosynthetic rate, chlorophyll content, and metabolic activities[32,35].

Nutrient content in straw

Total N, P and K in mustard straw (%)

The data on total nitrogen content in the straw of different mustard varieties as influenced by fertilizer application rates under saline water irrigation (Table 4). It was observed that variety CS-58 recorded significantly higher 0.36, 0.44, and 2.64 % mean N, P, and K content in straw whereas minimum 0.20, 0.29, and 1.38% mean N, P, and K content was observed in variety Kranti. The data also showed that different fertilizer doses influenced N, P, and K content in mustard straw significantly. Both fertilizer doses, *i.e.* 125% (0.28,0.39, and 2.09%) and 150% RDF (0.31,0.41, and 2.26%) recorded significantly at par higher mean of N, P, and K content in straw compared to RDF (0.22,0.31 and 1.74%). This might be due to mineral fertilizers had more water-soluble nutrients, which were readily available to plants. No significant interaction was noticed with respect to nutrients content in straw. Similar results were reported by many research workers[12,40].

Sodium (Na^+): *Potassium* (K^+) *in mustard straw*

The data on Na^+& K^+ in mustard straw and oil content in the mustard seed of different mustard varieties as influenced by fertilizer doses under saline water irrigation (Table 5). It was further evident from the data that significantly higher (2.85) mean Na^+: K^+ was found in the straw of variety Kranti followed by variety Giriraj (1.96), CS-54 (1.77) and CS-58 (1.57), respectively. The data showed that different fertilizer doses have non-significant influence on Na^+& K^+.However, the ratio showed a decreasing trend with increased fertilizer doses at 125% (2.00) and 150% RDF (2.21) as compared to RDF (1.91). Sodium and potassium in straw were non-significantly affected by

Table 3 — Seed and biological yield (q ha^{-1}) of different mustard varieties as influenced by fertilizer doses under saline water irrigation

Variety	Fertilizer doses (kg ha^{-1})			Mean
	RDF	125% RDF	150% RDF	
Seed yield (q/ha)				
Giriraj	16.09	17.59	17.99	17.22
Kranti	14.70	16.12	17.72	16.18
CS-54	17.41	18.13	20.94	18.
CS-58	20.92	21.72	22.89	21.84
Mean	17.28	18.39	19.89	
CD (p=0.05)	Variety (V): 0.91; Fertilizer doses (F): 0.44; V × F=0.87			
Biological yield (q/ha)				
Giriraj	70.06	79.14	90.27	79.82
Kranti	63.82	72.54	86.73	74.36
CS-54	76.01	85.10	97.73	86.28
CS-58	90.63	98.92	111.22	100.26
Mean	75.13	83.93	96.49	
CD (p=0.05)	Variety: 2.46; Fertiliser level: 1.06; V × F: NS			

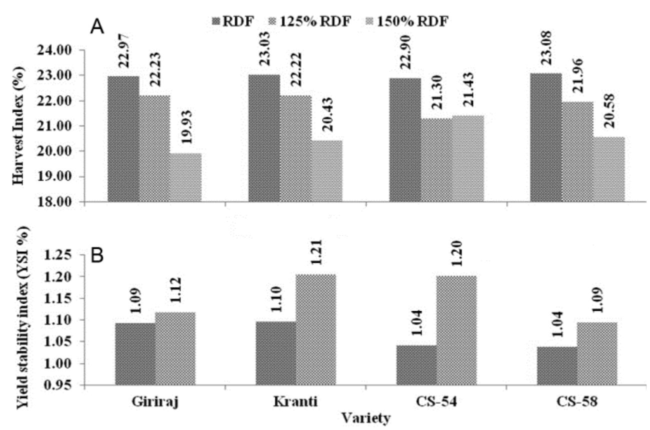

Fig. 3 —(A) Harvest index (%); and (B) Yield stability index (%) in different mustard varieties as influenced by fertilizer doses under saline water irrigation

Table 4 — Total N, P and K content (%) in straw of different mustard varieties as influenced by fertilizer doses under saline water irrigation

Variety	Fertilizer doses (kg ha^{-1}) N				P				K			
	RDF	125%RDF	150%RDF	Mean	RDF	125%RDF	150%RDF	Mean	RDF	125%RDF	150%RDF	Mean
Giriraj	0.20	0.25	0.27	0.24	0.30	0.37	0.38	0.35	1.72	1.80	1.95	1.82
Kranti	0.16	0.22	0.	0.20	0.25	0.31	0.33	0.29	1.01	1.45	1.68	1.38
CS-54	0.	0.30	0.33	0.29	0.33	0.41	0.44	0.39	1.95	2.36	2.51	2.27
CS-58	0.30	0.36	0.40	0.36	0.37	0.45	0.49	0.44	2.28	2.75	2.90	2.64
Mean	0.22	0.28	0.31		0.31	0.39	0.41		1.74	2.09	2.26	
CD (p=0.05)	Variety (V):0.05; Fertilizer doses (F):0.03; V x F:NS				Variety (V):0.06; Fertilizer doses (F):0.03 ; Variety x F=NS				Variety (V):0.34; Fertilizer doses (F):0.41 ; V x F=NS			

Table 5 — Na^+: K^+ in straw and oil content in seed of different mustard varieties as influenced by fertilizer doses under saline water irrigation

Variety	Fertilizer doses (kg ha^{-1})							
	Na^+: K^+				Oil content (%)			
	RDF	125%RDF	150%RDF	Mean	RDF	125%RDF	150%RDF	Mean
Giriraj	2.04	1.93	1.91	1.96	40.03	39.80	39.70	39.84
Kranti	3.10	2.80	2.65	2.85	39.83	39.47	39.37	39.56
CS-54	1.94	1.73	1.64	1.77	39.53	39.40	39.20	39.38
CS-58	1.76	1.52	1.44	1.57	40.17	39.83	39.73	39.91
Mean	2.21	2.00	1.91		39.89	39.62	39.50	
CD (p=0.05)	Variety (V):0.52; Fertilizer doses (F):NS; V x F:NS				Variety (V):NS; Fertilizer doses (F):NS; V x F=NS			

the interaction effect of different mustard varieties and fertilizer doses under saline water irrigation. Application of potassium not only increased significantly the concentration of K in plant parts but also significantly decreased the accumulation of Na ions as well as Na and K ratio in different plant parts under salinity[41]. Increasing potassium (K^+) concentration in growing medium in such situations may improve potassium (K^+) absorption and therefore counterbalance the adverse effect of salt stress[41,42].

Quality parameter

Oil content (%)

The data on oil content of different mustard varieties as influenced by fertilizer doses under saline water irrigation is presented in Table 4 shows that the effect of different mustard varieties on oil content was non-significant. However, maximum (39.91%) mean oil content was recorded in variety CS-58 followed by variety Giriraj (39.84%), Kranti (39.56%) and CS-54 (39.38%). The data also showed that application of different fertilizer doses did not exert their significant influence on oil content. The increased fertilizer doses up to 125 and 150% RDF showed decrease in mean oil content as compared to RDF where it was recorded maximum (39.89%). Oil content (%) was non-significantly affected by the interaction effect between different varieties and fertilizer doses. The findings showed similar results where there is non-significant decrease in oil content of Indian mustard from 100% RDF to 150% RDF, respectively[40]. This decrease might be the consequence of increased supply of nitrogen due to increased fertilizer doses. The higher rate of nitrogen leads to conversion of carbohydrates into protein. Hence, the amount of carbohydrates left to be converted into fats is too low leading to reduced oil content[40].

Conclusion

It is concluded the above study that variety CS-58 recorded maximum seed yield (22.89 q ha^{-1}), biological yield (11.22 q ha^{-1}); and other physiological parameters followed by CS-54, Giriraj, and Kranti mustard varieties. Variety CS-58 had the highest nutrient content (N, P, and K) in straw, with reaching to 125% RDF being at par with 150% RDF, followed by CS-54, Giriraj, and Kranti, respectively. The salt ratio (Na^+:K^+) decreased non-significantly with fertilizer doses, its mean value was however lowest in CS-58.

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Conflict of Interest

Authors declare no competing interests.

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