



Effect of Irrigation Regimes and Mulching on the Crop Physiology and Yield of Rabi Maize (*Zea mays*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Agriculture is a vital sector of India's economy and plays a significant role in providing food security, employment, and contributing to the country's overall development. Maize is a crucial crop within the agriculture sector in India. Maize cultivation contributes to crop diversification in Indian agriculture. It serves as an alternative crop option for farmers, reducing dependence on traditional crops like rice and wheat. Diversification helps in maintaining soil health through crop rotation. A field experiment was conducted at the Farm of College of Agricultural Engineering and Technology, Godhra, Gujarat during the year 2018-19, to study the influence of drip irrigation regimes with mulching on the physiological development and yield of the rabi maize (*Zea mays* L.) in the sandy loam soil of the region of Middle Gujarat. The field experiment design was a split-split plot with 36

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treatment combinations with 3 replications including three irrigation regimes (1.0IW/CPE, 0.8IW/CPE and 0.6IW/CPE), four mulch conditions (control, paddy straw, black plastic and reflective silver plastic mulch), and three stages (tasselling, silking and dough stage). The result revealed that as the consumption of water increased, growth parameters, grain yield, and its attributes increased significantly at each higher level of irrigation regimes up to 1.0 IW/CPE with reflective silver plastic mulch under all the stages but 0.8 IW/CPE with black plastic mulch result was comparable to 1.0 IW/CPE with reflective silver plastic mulch. The reflective nature of silver mulch scatters light, ensuring better light distribution throughout the plant canopy. This can enhance photosynthesis and promote more uniform plant growth. Black plastic mulch absorbs and retains heat from sunlight, raising soil temperatures. This is advantageous in cooler climates or during the early growing season, as it promotes earlier germination, faster growth, and improved crop productivity.

Keywords: Growth parameters; growth stages; irrigation regimes; mulching and yield.

1. INTRODUCTION

Around the world, maize holds a significant position in consumer food baskets. In 165 nations and over 197 million ha of land, maize has been grown as a crop, and it has contributed about 40% (1219 million MT) of the world's cereal production (International Grains Council [1]). The United States, China, Brazil, the European Union, Argentina, India, and Mexico are the top producers of maize. The amount of rabi maize that had been planted in India as of February 4th, 2022, was about 19.31 lakh hectares (47.72 lakh acres), which is greater than the 17.51 lakh hectares (43.27 lakh acres) that had been planted during the same time period the year before (the Ministry of Agriculture and Farmers' Welfare of India [2]). Karnataka, with 3.3 million MT (14.7 percent of India's total), is the state that produces most maize, followed by Madhya Pradesh, Bihar, Tamil Nadu, Maharashtra, and Uttar Pradesh. 28.64 million tonnes of maize are anticipated to be produced in 2019–20 [3]. It was estimated that approximately 44% of the total maize consumption of the country is consumed by the poultry industry alone. For both animal feed and human nourishment, maize is a key grain crop.

Water stress affects maize quite easily [4,5]. The biggest barrier to maize production is excessive moisture stress [6]. It reduces plant density and affects young plants during the establishment. It limits the leaf's development and growth of the plant during the vegetative stage, which stunts growth [7,8]. Shaw, [9] reported that increased moisture stress on the cell development and division phase has a direct influence on the crop's ability to create dry

matter, while lower photosynthesis has an indirect impact.

The use of lack of water application lowers the number of grains per ear and kernels weight, which in turn lowers grain yield [10-13]. Intensity of the deficit and growth stage during which it occurs to influence the amount of yield losses caused by water stress. Under moderate and severe water stress throughout the most important development periods, grain yield is decreased by 70–90%. Additionally, water stress-induced delay in flowering (7 days) and ripening (5 days) can lengthen the maize growth season [10].

It's possible that an inadequate amount of water application of maize spread out across the entire growing season won't always increase crop production [13]. Using mulch in drip-irrigated fields is another crucial element for supporting soil moisture and fostering growth of plant and yield production [14,15]. Water hyacinth, straw of wheat or paddy straw, and black and white plastic film can all be used for mulching. In addition to other benefits like fewer weeds, maintaining the root zone's temperature regime, etc., it has been discovered that mulching lowers soil water losses through evaporation [16], Patel et al. [17], Paul et al. [18], and Rao et al. [19] and reported the importance of use of mulching.

The unpleasant and uncertain weather conditions cause the production and productivity of the maize crop to vary from year to year. Because there is a close interaction between plant growth and soil moisture available for plant development, the soil profile's level of moisture is a key factor in determining whether maize can be successfully produced [20]. Therefore, determining the best irrigation schedule for the

various stages of maize development will help to boost plant growth and yield without raising the cost of production.

Therefore, the present investigation was carried out with three irrigation regimes (based on irrigation depth (IW) per three days cumulative pan evaporation (CPE)) 1.0IW/CPE, 0.8IW/CPE and 0.6IW/CPE at three growth stages (tasselling, silking and dough stage) under four mulch conditions (reflective silver plastic mulch, black plastic mulch, paddy straw mulch and no mulch condition during the rabi season of years 2018-19. The objectives of this research were 1) to determine the impact of the irrigation regime on maize growth parameters, grain yield, and its attributes; 2) to assess the mulching effect on the maize growth parameters, grain yield and its attributes; 3) to find out the plant growth-stage based irrigation regime effect on the maize growth parameters, yield, and its attributes. The findings of this experiment may be used to develop a successful maize production plan that can guarantee a consistent maize yield and even boost it while lowering irrigation and successfully conserving water.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment in the year 2018-19, where data is collected, is located at the College of Agricultural Engineering and Technology (CAET), Godhra, Gujarat. CAET is situated in the north-eastern Gujarat state, west-central India. The farm was geographically situated at 22° 46' 51.1" North latitude and 73° 39' 22.9" East longitude and 132 m of altitude above mean sea level.

2.2 Weather Condition

The daily data pertaining to the various meteorological parameters recorded during the crop-growing period (from 10 December 2018 to 9 April 2019) are graphically provided in Fig. 1. It would be seen from the recorded data that the mean max and min temperatures for the crop-growing season of the year 2018-19 were 28.34°C and 11.96°C respectively. The highest maximum temperature was 34.5°C and the lowest min temperature was 7.5°C. The mean max temperature 28.34°C varied between 24 and 34.5°C. While the mean min temperature 11.96°C varied between 7.5°C and 16.5°C. The temperature was positive for development of the maize crop. Mean relative humidity for the crop-growing season was 64.38 % and its range was 77 to 45 %. Mean wind speed was 2.21m s⁻¹ and it is varied between 3.61 to 0.83 m s⁻¹. The average sunshine hour for rabi maize's growing season was 8.68 h.

2.3 Land Preparation

The experimental field had a gentle slope and moderate drainage. The groundwater table is more than 10 meters deep. Hence, there is no problem with a high water-table in that region. The experimental field was ploughed at 40 cm to completely mix the soil profile and remove any compacted layers, then chiseled with 30 cm, harrowed, and pulverized the soil. The composite soil samples were drawn at randomly from three depths of 0-15 cm, 15-30 cm, and 30-45 cm soil depth from the research fields, and were found their physical and chemical characteristics through the application of the standard procedure provided in Table 1.

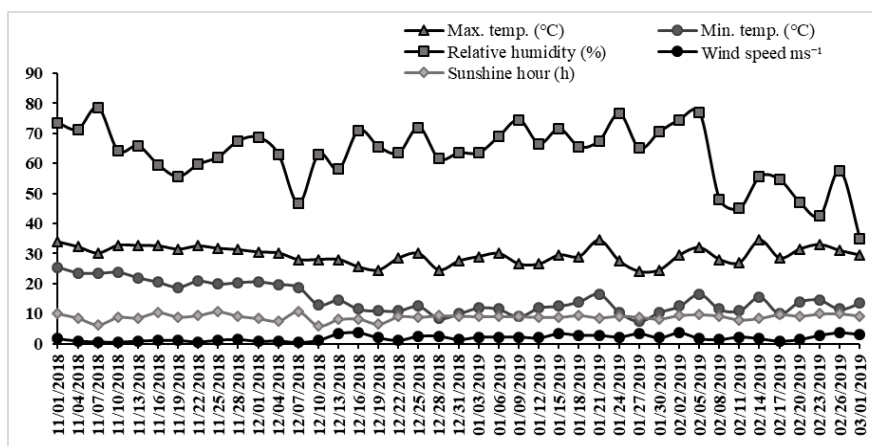


Fig. 1. Daily weather data recorded during the experimental period of Rabi Maize (2018-19)

Table 1. Physico-chemical properties of the experimental soil

Particulars	Godhra 2018-19	Method of analysis
Sand (%)	66	International Pipette Method [21]
Silt (%)	13	International Pipette Method [21]
Clay (%)	20.9	International Pipette Method [21]
Texture	Sandy Loam	
Bulk Density (g cm ⁻³)	1.41	Core Sampler method
Soil pH (1:2.5) (Soil: Water)	7.84	pH meter [22]
Electrical Conductivity dS m ⁻¹	0.18	EC meter [22]
Organic Carbon (%)	0.27	Walkley [23]
Available Nitrogen (kg ha ⁻¹)	160.2	Subbaiah and Asija, [24]
Available Phosphorous (kg ha ⁻¹)	18.85	Olsen et al. [25]
Available Potassium (kg ha ⁻¹)	128	Jackson [22]

2.4 Experimental Design and Treatment

The experimental setup was in split-split plot design under 3 replications with 3 levels of irrigation with 4 mulching conditions and 3 specific growth stages (Table 2). The total treatment combination was 108. The plot area under treatment was covered with paddy straw mulch uniformly spread at the rate of 6t/ha (i.e. 600 g/m²) just after the sowing, black plastic mulch, and reflective silver plastic mulch before sowing. Plastic mulches used for mulching had 120 cm width, and 25 μ LDPE thickness. The corners and border of plastic mulch were incorporated into the soil for trapping heat and to avoid disturbance from the wind. The round holes were made at the spacing of 60 x 20 cm with the help of galvanized iron pipe of 2-inch diameter. Paddy straw mulch with the thickness of 3cm was applied to the respective plots. The Gujarat Anand Yellow Maize Hybrid 1 variety of rabi maize was sown manually on 10 December 2018 at a spacing of 60 cm for row to row and 20 cm for plant to plant. The net plot size was 3m x1m with 30 plants. Seeds were placed at 4-5 cm depth. The recommended basal dose (120:60:40; N:P:K) of nitrogen @ 60 kg ha⁻¹ in the form of urea, phosphorus @ 60 kg ha⁻¹ in the form of single super phosphate, and potash @ 40 kg ha⁻¹ from Murata of potash were given at the time of sowing. The remaining half 60 kg nitrogen ha⁻¹ was given 30 days after sowing as fertigation practice.

The scheduling of irrigation which is based on the regimes was done using the Open-Class A pan method on three days intervals (three days cumulative evaporation amount) (Allen et al. 1998). Irrigation regimes (IW/CPE=irrigation depth/ cumulative pan evaporation; i.e. 0.6 IW/CPE, 0.8 IW/CPE, and 1.0 IW/CPE) were applied at particular growth stages under

different mulch conditions. Five tagged plants were measured at ten days intervals for each plot to find out the increment of plant height, and the number of leaves. The amount of fully opened, green leaves on a given plant was regarded as the quantity of functional leaves. The leaves that had dried over 50% of their area or more were not included in the total count of functioning leaves. The diameter was measured with the use of a Vernier Caliper in each treatment at every 30 days after the date of sowing, measured at the center of the total plant height. The leaf area of the maize plant was measured using Montgomery [26] applied an equation which was also used by McKee [27], Pearce et al. [28], and Dwyer and Stewart [29], to calculate the area of each maize leaf individually;

$$\text{Leaf area} = \text{Leaf length} \times \text{leaf maximum width} \times 0.75,$$

At harvest time, the total count of cobs per plant in each treatment was counted. The length of randomly selected six sample cobs from the treatment was measured with the centimeter scale and then calculated the average value of the length of the cob. The diameter of the above selected six samples cobs was calculated using a Vernier Caliper device, from the bottom, center, and top of the cob, and the average value of the diameter was multiplied by the pi such as π (3.14) to get the mean cob girth. The total count of grains from the sample cobs used for the measurement of cob length and girth was counted and stated as the number of grains per cob. After picking the cobs, the leftover plant residue with the husk was dried in the sun, weighed, and stated as the stover yield (q ha⁻¹). The weight of 1000 seeds were measured as a test weight in (g) after the maize was shelled. The grain yield was represented as q ha⁻¹ and adjusted to a moisture level of 15%.

Table 2. Description of experimental treatments for rabi maize at middle Gujarat

Treatments	Irrigation Regimes, (IW/CPE) (I)	Mulch Type, (M)	Crop Stages (S)	Treatments Combinations (IMS)		
T ₁	(I ₁) 0.6	No Mulch (M ₀)	S ₁ , S ₂ , S ₃	I ₁ M ₀ S ₁	I ₁ M ₀ S ₂	I ₁ M ₀ S ₃
T ₁	(I ₁) 0.6	Paddy Straw Mulch (M ₁)	S ₁ , S ₂ , S ₃	I ₁ M ₁ S ₁	I ₁ M ₁ S ₂	I ₁ M ₁ S ₃
T ₁	(I ₁) 0.6	Black Plastic Mulch (M ₂)	S ₁ , S ₂ , S ₃	I ₁ M ₂ S ₁	I ₁ M ₂ S ₂	I ₁ M ₂ S ₃
T ₁	(I ₁) 0.6	Reflective Silver Plastic Mulch (M ₃)	S ₁ , S ₂ , S ₃	I ₁ M ₃ S ₁	I ₁ M ₃ S ₂	I ₁ M ₃ S ₃
T ₂	(I ₂) 0.8	No Mulch (M ₀)	S ₁ , S ₂ , S ₃	I ₂ M ₀ S ₁	I ₂ M ₀ S ₂	I ₂ M ₀ S ₃
T ₂	(I ₂) 0.8	Paddy Straw Mulch (M ₁)	S ₁ , S ₂ , S ₃	I ₂ M ₁ S ₁	I ₂ M ₁ S ₂	I ₂ M ₁ S ₃
T ₂	(I ₂) 0.8	Black Plastic Mulch (M ₂)	S ₁ , S ₂ , S ₃	I ₂ M ₂ S ₁	I ₂ M ₂ S ₂	I ₂ M ₂ S ₃
T ₂	(I ₂) 0.8	Reflective Silver Plastic Mulch (M ₃)	S ₁ , S ₂ , S ₃	I ₂ M ₃ S ₁	I ₂ M ₃ S ₂	I ₂ M ₃ S ₃
T ₃	(I ₃) 1.0	No Mulch (M ₀)	S ₁ , S ₂ , S ₃	I ₃ M ₀ S ₁	I ₃ M ₀ S ₂	I ₃ M ₀ S ₃
T ₃	(I ₃) 1.0	Paddy Straw Mulch (M ₁)	S ₁ , S ₂ , S ₃	I ₃ M ₁ S ₁	I ₃ M ₁ S ₂	I ₃ M ₁ S ₃
T ₃	(I ₃) 1.0	Black Plastic Mulch (M ₂)	S ₁ , S ₂ , S ₃	I ₃ M ₂ S ₁	I ₃ M ₂ S ₂	I ₃ M ₂ S ₃
T ₃	(I ₃) 1.0	Reflective Silver Plastic Mulch (M ₃)	S ₁ , S ₂ , S ₃	I ₃ M ₃ S ₁	I ₃ M ₃ S ₂	I ₃ M ₃ S ₃

The shelling percentage was calculated as the ratio of weight of grains to the whole cobs' weight. The harvesting index was determined by the ratio of grain yield to total biomass production. The leaf area index is calculated as the formula i.e. Leaf area index is equal to the total of one-sided green leaves area per square meter of the ground surface. All the biometric observations were recorded and analysed statistically.

3. RESULTS AND DISCUSSION

3.1 Effect of Irrigation Regimes on Growth Characters of Maize

The height and stem diameter of the plant were not significantly varied under the irrigation regimes but the count of leaves per plant, the plant leaf area, and the leaf area index was significantly different. Plant height increased under the I_1 treatment from 3.7 cm (10 DAS) to 200.8 cm (90 DAS), in the I_2 treatment it increased up to 211.2 cm and with the application of I_3 treatment it increased up to 209.6 cm. Plant height under the irrigation regimes I_2 such as 0.8IW/CPE gives the highest plant height compared to the I_3 and I_1 but it is not significantly different from them. The mean stem diameter increased with an increase the growth of the crop, and after a certain stage, it declined. The mean stem diameter was 6.88 mm, 8.71 mm and 18.27 mm at 30, 60 and 90 DAS, under I_2 obtained maximum stem diameter but the stem diameter was obtained under I_1 and I_3 at par with I_2 . The irrigation regime I_2 produced the maximum number of leaves 15.3 as compared to other regimes and the lowest obtained under the I_1 . The data of the leaf area of the plant was 15.9 dm² at 30 DAS, 43.88 dm² at 60 DAS and 34.83 dm² at 90 DAS. The mean maximum leaf area was significantly highest under the irrigation regimes I_3 . The data of the mean leaf area index of the plant, temporally affected by the different treatments, it was 1.33, 3.66 and 2.9 at 30,60 and 90 days after sowing respectively.

The mean maximum leaf area index was significantly highest under the irrigation regimes I_3 . However, the result indicated that irrigation regime I_2 was at par with I_3 . Maize is a sensitive crop to water shortage [30,2], an adequate amount of application of water as a plant requirement promotes the plant's physiological parameters but the application of it in a deficient amount to some extent, it limits plant growth, these findings agreed with the results of Cracium

and Craclum [31] and Ashagre et al. [32] they studied the effect of different irrigation water levels on maize growth parameters and reported a similar result that an adequate amount of water promotes plant growth.

3.2 Effect of Mulching Conditions on Growth Characters of Maize

The mulches had a significant effect on the height and stem diameter of the plant, the count of leaves per plant, the leaf area, and the leaf area index. The plant's height under the control conditions (M_0 i.e. no mulch condition), straw mulch conditions (M_1), black plastic mulch conditions (M_2), and reflective silver plastic mulch conditions (M_3) increased up to 192.4 cm, 200.1 cm, 210.6 cm, and 225.3 cm respectively. The significantly highest plant height was observed under the M_3 condition and the lowest was in the M_0 condition. The mean maximum stem diameter of 20.41mm was recorded at 60 DAS and was significantly the highest in M_3 . The maximum number of leaves 16.17 was recorded at 70 DAS and was significantly the highest in reflective silver plastic mulch M_3 . The mean leaf area of the plant increased with an increase up to certain age and then decreased. It was 15.9 dm² at 30 DAS, 43.88 dm² at 60 DAS, and 34.83 dm² at 90 DAS. The mean maximum leaf area was significantly high under reflective silver plastic mulch M_3 . The data of the mean leaf area index of the plant, temporally affected by the different treatments was 1.33 at 30 DAS, 3.66 at 60 DAS, and 2.9 at 90 DAS. The mean maximum leaf area index was significantly high 3.92 under reflective silver plastic mulch (M_3). However, black plastic mulch (M_2) was at par with reflective silver plastic mulch (M_3). Masanta and Mallik [33], Zerga et al. [34], Das and Jana et al. [35], Patel et al. [17], Paul et al. [18], and Rao et al. [19] also observed similar results.

3.3 Effect of Growth Stages on Growth Characters of Maize

The crop is sown in reflective silver plastic mulch treatment with 0.8 IW/CPE at the dough stage (S_3) and showed a significantly high plant height at 60, 70, 80, and 90 days after sowing and at harvesting stage. Cakir 2004 reported that maize is susceptible to water stress in comparison to the other crops, particularly during vegetative, tasselling, and silking phases. When generating the stress condition in any one of these phases significantly affects the increment of plant height. Stages-based irrigation significantly increased

plant growth parameters such as the number of leaves per plant, the stem diameter of the plant, leaf area, and leaf area index [36], (Aulakh et al. 2013). Data showed that when irrigation regimes applied in the form of 1.0 IW/CPE at tasselling and silking stage and 0.8 IW/CPE at the dough stage under reflective silver plastic mulch provide the maximum number of leaves. The interaction effects of irrigation regimes, mulching, and growth stages had a significant effect on the number of leaves per plant, leaf area and the leaf area index [37,38].

3.4 Effect of Irrigation Regimes, Mulching Conditions and Growth Stages on Yield and Yield Attributes of Maize

3.4.1 Yield attributes

The data obtained from the result pertaining to the mean count of cobs produced per plant, the length of the cob (cm), the girth of the cob (cm), the count of grains in each cob, and the weight of the thousand-grains are showed in Table 3. The mean length of the cob (cm) per plant in 1.0 IW/CPE (I_3) was 19.7 cm, the mean girth of the cob was 17.15 cm, the mean count of grains per

cob was 429.06 and the mean thousand-grain weight was 201.56 g. The cob's length, girth, the count of grains per cob, and weight per thousand grains were all provided considerably higher values under the I_3 regime with reflective silver plastic mulch (M_3), than they were under 0.6 IW/CPE and control conditions. in the interaction effect of irrigation regimes, different mulch conditions and the plant growth stages show significant results for yield attribute.

3.4.2 Yield

The data about mean maximum grain yield (GY) (kg ha^{-1}), mean maximum stover yield (SY) (t ha^{-1}), shelling percentage, and the harvesting index (HI) are shown in Table 4. From the statistics, it was possible to conclude that the mean maximum GY, mean maximum SY, shelling percentage, and HI were 2.81 kg ha^{-1} , 5.62 t ha^{-1} , 80.71%, and 37.0% respectively under 1.0IW/CPE (I_3) and $3773.39 \text{ kg ha}^{-1}$, 5.13 t ha^{-1} , 78.15% and 35.70% respectively under 0.8IW/CPE (I_2), which was at par with I_3 . The effect of interaction among the irrigation regimes, mulching, and growth stages showed a significant result.

Table 3. Mean number of cobs per plant, length of cob (cm), girth of cob (cm), and thousand grain weight of corn as influenced by irrigation regimes, mulching and growth stages

Treatment	No. of Cobs plant ⁻¹	Length of cob (cm)	Girth of cob (cm)	No. of grains cob ⁻¹	1000 grain weight (g)
I_1	2	17.76	15.47	349.64	173.67
I_2	2	18.87	16.58	383.89	183.78
I_3	2	19.70	17.15	429.06	201.56
S.Em	-	0.29	0.62	13.82	7.62
C.D.	-	1.16	2.45	54.24	22.85
C.V.%	0	9.40	8.77	21.39	24.52
M_0	2	17.66	16.07	344.33	170.74
M_1	2	18.36	16.15	384.59	181.30
M_2	2	18.72	16.14	391.56	190.33
M_3	2	20.38	17.30	429.63	202.96
S.Em	-	0.16	0.55	7.47	2.00
C.D.	-	0.46	1.62	22.19	5.93
S_1	2	17.37	15.30	336.08	173.06
S_2	2	18.92	16.52	393.33	184.67
S_3	2	20.04	17.42	433.17	201.28
S.Em	-	0.12	0.33	3.11	2.24
C.D.	-	0.35	0.94	8.85	6.39
$I \times M$	*	*	*	*	*
$I \times S$	*	*	*	*	*
$M \times S$	*	*	*	*	*
$I \times M \times S$	*	*	*	*	*

*significant ($p < 0.05$)

Table 4. Mean grain yield, stover yield, shelling percentage and harvesting index affected by irrigation regimes, mulching and growth stages

Treatment	Yield (kg ha ⁻¹)	Stover yield (t ha ⁻¹)	Shelling percentage (%)	Harvest Index (%)
I ₁	3675.64	5.08	72.56	34.15
I ₂	3973.39	5.13	78.15	35.70
I ₃	4402.81	5.62	80.71	37.00
S.E.m	121.63	0.10	1.33	1.13
C.D.	477.50	0.39	4.15	3.39
C.V.%	28.99	18.09	7.74	8.09
M ₀	3375.33	4.71	70.43	31.60
M ₁	3596.22	4.98	71.68	33.80
M ₂	4526.93	5.50	82.40	37.60
M ₃	4570.70	5.92	82.60	37.80
S.E.m	70.57	0.05	0.26	0.44
C.D.	209.67	0.16	0.77	1.31
S ₁	3352.28	4.75	72.10	32.40
S ₂	3938.72	5.31	77.80	36.50
S ₃	4760.89	5.76	84.61	38.60
S.E.m	53.04	0.04	0.12	0.16
C.D.	151.00	0.12	0.37	0.52
I×M	*	*	*	*
I×S	*	*	*	*
M×S	*	*	*	*
I×M×S	*	*	*	*

*significant (p<0.05)

The mean maximum GY, mean maximum SY, shelling percentage, and HI was 4570.70 kg ha⁻¹, 5.92 t ha⁻¹, 82.60%, and 37.80% respectively under M₃ and 4526.93 kg ha⁻¹, 5.50 t ha⁻¹, 82.40% and 37.6% respectively under black plastic mulch (M₂), which was at par with M₃ (Table 4).

Under considering the irrigation in the dough stage (S₃) the mean maximum GY, SY, shelling percentage, and HI were 4760.89 kg ha⁻¹, 5.76 tons ha⁻¹, 84.61%, and 38.60% respectively.

4. CONCLUSION

Irrigation applied at I₃, (1.0 IW/CPE) demonstrated favourable outcomes for plant growth measures, yields, and yield traits during the S₃ (dough stage) stage of maize. Among different mulching conditions, Reflective silver plastic mulch (M₃) provides the best results for plant growth and production. Furthermore, the use of the I₂ regime (0.8 IW/CPE) under black plastic mulch provides a significantly best result which was at par with I₃ under M₃. A treatment combination of I₂M₂S₃ provide a significantly better result for rabi maize which saves water, increases the grain yield of maize, maintain soil

temperature and moisture, and create favourable condition for plant growth.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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