



Impact of Foliar Application of Sodium Nitroprusside on Certain Morpho-physiological, Biochemical and Yield Parameters of *Kharif* Maize (*Zea mays* L.) Grown under Rainfed Condition

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ABSTRACT

Background: Maize (*Zea mays* L.) belongs to the family poaceae is one of the most important cereal next to rice and wheat in the world. Maize is referred to as the “queen of cereals” and may be produced all year due to its photothermic-insensitive traits and highest genetic yield among the cereals. Sodium nitroprusside (SNP) is a chemical compound that has multiple implications and positive modulating effects on mitigating different abiotic stresses especially water deficit regimes. SNP enhances the nutrient uptake and anchoring of the plant root system and also mitigates water stress.

Methods: In this context, a field experiment was designed to study the impact of foliar application of SNP on certain morpho-physiological, biochemical and yield parameters of *kharif* maize grown under rainfed condition. A randomized complete block design (RBD) was employed, with treatments including different concentration of SNP (100, 200, 300 and 400 μ M SNP) exogenously applied on maize crop (variety- CP-858) at various critical growth stages.

Result: Significant differences were observed with reference to various morpho-physiological, biochemical and yield traits under control and treated plots. Results indicate significant improvements in these parameters following SNP application in comparison to control which significantly implies its possible role in mitigating water stress under rainfed condition.

Key words: Maize, Protein content, Rainfed condition, Sodium nitroprusside.

Abbreviation: GSH- Glutathione-S-Transferase, NO- Nitric oxide, SNP- Sodium nitroprusside, T- Treatment.

INTRODUCTION

After rice and wheat, maize (*Zea mays* L.), a member of the poaceae family, is the most significant cereal in the world. Due to its highest genetic output among the cereals and characteristics that make it resistant to photothermia, maize is known as the “queen of cereals” and can be cultivated year-round. It is a C4 plant that is highly effective at converting solar energy into dry matter. (Latif *et al.*, 2024). In addition to being utilised as food for humans and animals, maize has several industrial uses. Its common practice to roast and consume green corn. 10% protein, 4% oil, and 2% to 3% crude fibre are found in maize seed (Sain *et al.*, 2023).

Nitroprusside is a complex anion with a Fe-N-O angle of 176.2° and an idealized symmetry. It is a donor of nitric oxide (NO). Nitric oxide (NO), an endogenous signaling molecule, is believed to regulate growth and photosynthetic processes as well as reduce stress. NO crosstalk with other phytohormones is one of the primary potential factors regulating physiological processes that occur in stressed plants. NO regulates cytokinin levels, auxin transport, and signaling, which promotes root growth and development.

Due to its function as a nitric oxide (NO) donor, which aids plants in overcoming a variety of environmental stresses, applying sodium nitroprusside (SNP) to rainfed maize can be very advantageous. As rainfed maize depends

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so heavily on natural rainfall, it is susceptible to temperature changes, drought stress, and deficiencies in soil moisture. Due to water constraint, rainfed maize frequently experiences oxidative stress, decreased photosynthesis and stomatal closure. Under drought conditions, SNP reduces transpiration loss and improves water-use efficiency by releasing nitric oxide (NO), which helps control stomatal opening. Maize undergoes oxidative stress when there is insufficient rainfall because of the overproduction of reactive oxygen species (ROS).

Antioxidant enzymes that scavenge ROS and stop cellular damage, such as peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD), are activated more by SNP. This provides a plausible explanation as to why SNP is suited for kharif maize under water stress conditions to mitigate the stress.

Nitric oxide (NO) is an endogenous signaling molecule that has been shown to ameliorate stressful situations (Ahmad *et al.*, 2016; Shi *et al.*, 2016) and control development and photosynthesis (Fatma *et al.*, 2016). According to Hermes *et al.* (2013), the external injection of NO can also change gene expression and reduce oxidative stress. One of the main elements potentially involved in the physiological processes occurring in stressed plants is NO crosstalk with other phytohormones (Sanz *et al.*, 2015). NO promotes root growth and development *via* controlling auxin transport, cytokinin level, and signaling (Fernandez-Marcos *et al.*, 2011; Liu and Gou, 2013 and Mohanlal *et al.*, 2021). NO can alter the activity of the Asc-Glu cycle's enzymes and scavenge reactive oxygen species (ROS) (Begara-Morales *et al.*, 2015). In *Oryza sativa*, Singh *et al.* (2016) found that an external supply of NO increased GSH content by up-regulating gamma-glutamyl cysteine synthetase, maintaining the GSH/GSSG ratio. According to reports, NO in the form of sodium nitroprusside (SNP) has been shown to be highly successful at improving wheat's redox status, photosynthesis, relative water content, and gas exchange characteristics when it is subjected to abiotic stress (Tian and Lei, 2007). NO reduces water loss through transpiration by influencing stomatal closure. By reducing excessive water loss and upregulating genes related to

osmoprotection, antioxidant defense, and membrane stability, it works in tandem with abscisic acid (ABA) to improve drought tolerance, a common issue in rainfed environments.

Kharif maize is a crucial cereal crop in Uttar Pradesh, primarily grown during the monsoon season under rainfed conditions, (Manavalagan *et al.*, 2024). *Kharif* maize was chosen in place of *rabi* maize because erratic rainfall patterns, prolonged dry spells and increasing climate variability make *kharif* maize highly vulnerable to water stress provides an opportunity to study the effect of abiotic stress and climate change. *Kharif* maize contributes significantly to global maize production, particularly in tropical and subtropical regions. In India it is grown in Uttar Pradesh, Bihar, Madhya Pradesh, Karnataka and Andhra Pradesh.

In this context, effect of different doses of sodium nitroprusside (SNP) on key morphological, physiological, and biochemical parameters in *kharif* Maize (*Zea mays*) under rainfed condition was studied and analyzed under field conditions.

MATERIALS AND METHODS

The trial was carried out during the *kharif* season of 2022-23 at the Agricultural Research Farm of Banaras Hindu University, located approximately 10 km southwest of Varanasi railway station. The experimental field is situated at 25°18'N latitude, 83°03'E longitude, and at an elevation of 75.7 meters above mean sea level, within the North Gangetic alluvial plain. The meteorological data and

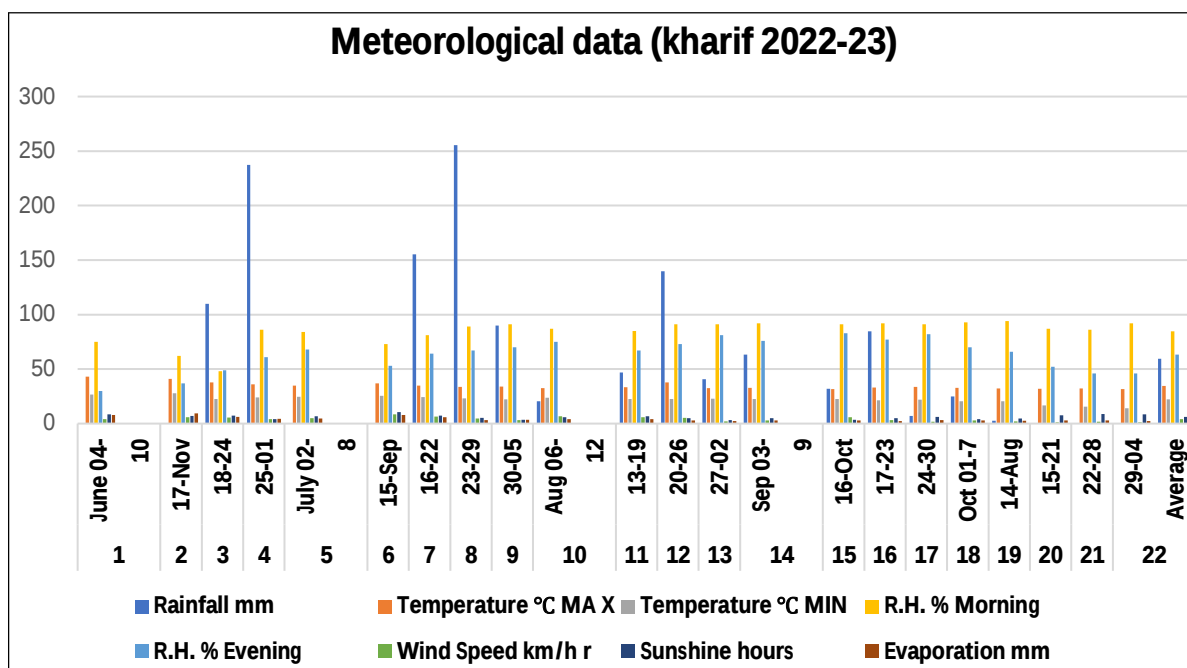


Fig 1a: Meteorological data (*kharif* 2022-23).

experimental details are given in (Fig 1a) and Table 1 respectively.

SNP is reported to have heavy metal stress mitigating effects in maize at concentrations of 100 μ M (Naseem *et al.*, 2020). Therefore, different enhanced concentrations of SNP were taken to study the rainfed cultivation of maize. The treatment details having various concentration of sodium nitroprusside are discussed in Table 2.

Plant height, leaf area index, test weight, sink size, number of leaves and yield per plot were recorded manually. Crop Growth Rate (CGR) represents the amount of dry weight grained in a unit land area per unit time and was calculated using the formula given by Watson (1952) in a non-destructive method. Chlorophyll a, Chlorophyll b and total chlorophyll content were estimated using the formula of Arnon (1949). Total protein content was estimated from leaf samples using Bradford method. Total soluble sugars were estimated using the protocol by Samogyi *et al.*, 1952. Membrane stability index was identified using formula given by Sairam *et al.*, (1997). Chlorophyll stability index was determined using the formula;

$$CSI = \frac{OD \text{ at } 652 \text{ nm of treated sample}}{OD \text{ at } 652 \text{ nm of control}} \times 100$$

Apart from morphological, physiological data, yield parameters were also recorded in terms of test weight, sink size and yield per plot (kg). The overall experimental plan has been depicted in (Fig 1). All, the statistical analysis was done using OPSTAT website (<http://14.139.232.166/opstat/>).

RESULTS AND DISCUSSION

At 30 DAE, T4 (400 μ M) had the highest mean plant height among the different concentrations of SNP, with a mean height of 143 cm, compared to the other treatment plots, which had mean plant heights of 124.33 (T0), 129.33 (T1), 134.66 (T2) and 130.33 (T3).

All SNP treatments, including T1, T2, T3 and T4, demonstrated substantial changes from the control at 60 DAE. When SNP was sprayed at 400 μ M (T4), plant height was considerably higher than in the control plot, with a mean height of 182.33 cm. With a mean height of 173 cm in comparison to the control plot, T2 (200 μ M) had the next-highest value of plant height (Table 3).

Table 1: Experimental details.

Particulars	Details
Location	Agricultural research farm
Crop	Maize
Variety	CP-858 (21°C- 27°C and 50-100cm rainfall, wideadaptability, grown in kharif season in Bihar and Uttar Pradesh)
Crop Spacing	60 cm $\times$ 20 cm
Seed rate	25 kg/ha
Fertilizer (N, P2O5, K2O)	150, 70, 60 kg/ha
Design	Randomized block design
Plot size	4 m $\times$ 3 m
Block border	1.0m
Plot border	0.5m
Irrigation	main- 1m, sub- 0.5m
Treatment	05
Replication	03
Foliar spray	Sodium nitroprusside (SNP)
Season	Kharif (2022-23)

Table 2: Treatment details.

T0	Control (water spray)
T1	SNP @100 μ M
T2	SNP @ 200 μ M
T3	SNP @ 300 μ M
T4	SNP @ 400 μ M
Stages of foliar spray	30 DAE and 60 DAE
Treatment combination	5 $\times$ 3 $\times$ 2 = 30

Table 3: Effect of exogenous application of sodium nitroprusside on plant height (cm), number of leaves per plant, leaf area index of maize grown under rainfed condition.

	Plant height (cm)		Number of leaves per plant		Leaf area index (LAI)	
	30 DAE	60 DAE	30 DAE	60 DAE	30 DAE	60 DAE
T0	124.333 ^e	160 ^e	6.333 ^d	12.333 ^e	2.31 ^e	3.153 ^e
T1	129.333 ^d	166.667 ^d	8.333 ^b	14 ^a	2.373 ^d	3.327 ^d
T2	134.667 ^b	173 ^b	7 ^c	12.667 ^d	2.5 ^c	3.573 ^c
T3	130.333 ^c	171.333 ^c	7 ^c	13.333 ^c	2.58 ^b	3.727 ^b
T4	143 ^a	182.333 ^a	9 ^a	13.667 ^b	2.64 ^a	3.79 ^a
C.D.	N/A	N/A	N/A	N/A	0.049	0.089
SE (m)	4.742	4.7	0.856	0.972	0.015	0.027
SE (d)	6.707	6.647	1.211	1.374	0.021	0.038
C.V.	6.207	4.77	19.689	12.752	1.04	1.325

Where (T0- Control, T1 - 100 μ M SNP, T2- 200 μ M SNP, T3 - 300 μ M SNP , T4 - 400 μ M SNP) .

Leaf area index of maize was recorded at 30 DAE and 60 DAE and the data is presented in Table 3. Exogenous application of SNP at 400 μM (T4) concentration at 30 DAE significantly increased LAI 2.64 compared to control plot. With a mean leaf area index of 2.58 in comparison to the control plot, exogenous application of SNP @ 300 μM (T3) produced the next higher value of the leaf area index. The remaining treatments differ compared to other treatments and from the control. Exogenous application of SNP at 400 μM (T4) at 60 DAE revealed a higher LAI value than the control plot, with a mean value of 3.79. With a mean value of 3.727, spray of SNP @ 300 μM (T3) was recorded the next higher LAI value. The remaining treatments differ compared to other treatments and from the control.

The control plot treated with water had the lowest number of leaves, with mean values of 6.33 at 30 DAE and 12.33 at 60 DAE. Nayanakantha *et al.*, (2016) sprayed varying doses of SNP on maize. SNP concentrations ranging from 200 μM to 500 μM were shown to be most effective for growth parameters such as stem diameter, shoot height, dry weight, and leaf area. The maize leaf area index was measured at 30 DAE and 60 DAE, and it considerably varied as a result of different SNP concentrations. The higher leaf area index was recorded at 400 μM SNP was 2.64 and the lowest at 30 DAE in the control plot was 2.31. The results for the leaf area index at 60 DAE vary amongst plots, SNP @ 400 μM (T4) notably displays a greater value 3.79 in comparison to other plots, while the control plot has the lowest value, with a mean value of 3.153. Barzin *et al.*, (2022) showed similar results in pot marigold, as did Chohan *et al.* (2012) in chickpea.

The data analyzed and was recorded pertaining to Crop growth rate of maize as influenced by exogenous

application of SNP is presented in (Fig 2). When compared to other treatments, the SNP @ 400 μM (T4) spray considerably was recorded the highest crop growth rate at 30 DAE with a mean value of 9.3 $\text{g/m}^2/\text{day}$. With a mean value of 8.69 $\text{g/m}^2/\text{day}$ in comparison to the control plot, treatment 300 μM had the next greater crop growth rate. In comparison to treated plots, the control plot's mean crop growth rate was the lowest, at 6.94 $\text{g/m}^2/\text{day}$. Spray of SNP, i.e., T1, T2, T3 and T4 exhibited significant differences over the control at 60 DAE among all treatments. With a mean value of 8.92 $\text{g/m}^2/\text{day}$, spraying SNP at 400 μM exhibited the highest crop growth rate, followed by spraying SNP at 300 μM with a mean value of 8.88 $\text{g/m}^2/\text{day}$ and spraying SNP at 200 μM and 100 μM with significant differences from the control.

The observations on chlorophyll 'a', 'b' and total chlorophyll content of maize were recorded at 30 DAE and 60 DAE and has been presented in Table 4. Significantly greater value for chlorophyll 'a' was obtained by SNP @ 400 μM (T4), with a mean value of 2.065 mg/g FW in comparison to the control plot. In comparison to other treatments, T3 (300 μM) had the next greater value of chlorophyll 'a'. When compared to the control plot, the spray of SNP at 400 μM considerably increased the amount of chlorophyll 'b', with a mean value of 0.901 mg/g FW. Spray of SNP @ 400 μM (T4) among the treatments showed increased total chlorophyll content with a mean value of 2.966 mg/g FW in comparison to other treatments. The next higher mean value of total chlorophyll content was found in treatment (T3) compared to other treatments, while the lowest mean value was found in the control plot, at 2.285 mg/g FW. From above data it is concluded that exogenous application of SNP at concentration @ 400 μM

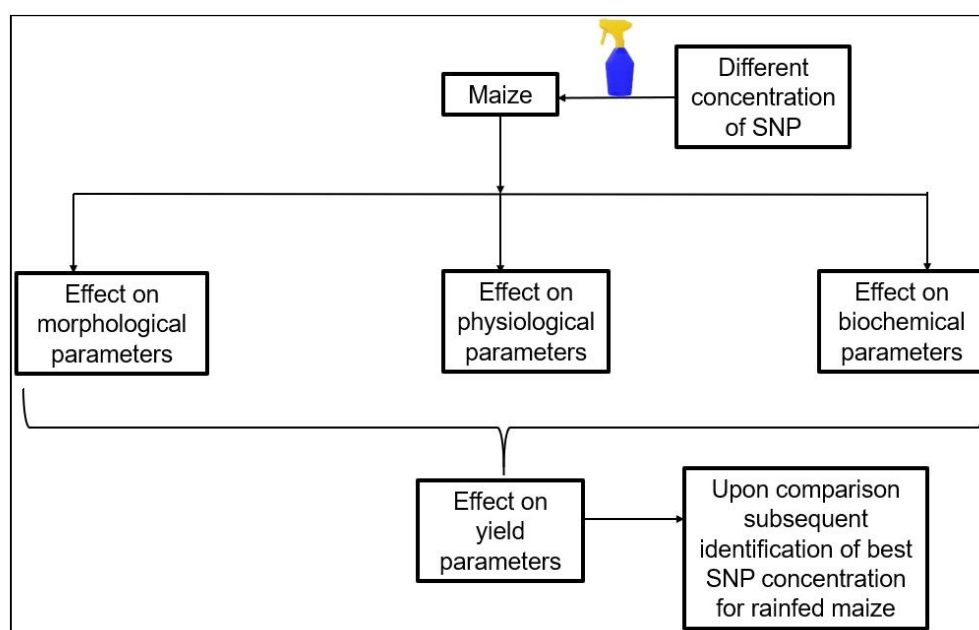


Fig 1b: Overall experimental planning.

shows higher value for chlorophyll 'a', 'b' and total chlorophyll content at 30 DAE and lowest value observed in control plot. The highest total protein content was found at T4 (400 μ M) at 30 DAE, with a mean value of 1.316 mg/g FW in comparison to other treatment plots (Fig 3). With a mean value of 1.242 mg/g FW in comparison to other

treatments, T3 (300 μ M) had the next highest value of total protein content. With a mean value of 1.19 mg/g FW and 1.113 mg/g FW when compared to the control plot, the treatments T2 (200 μ M) and T1 (100 μ M) followed by third and fourth in terms of total protein content, respectively. The control plot had the lowest mean value, 1.095 mg/g

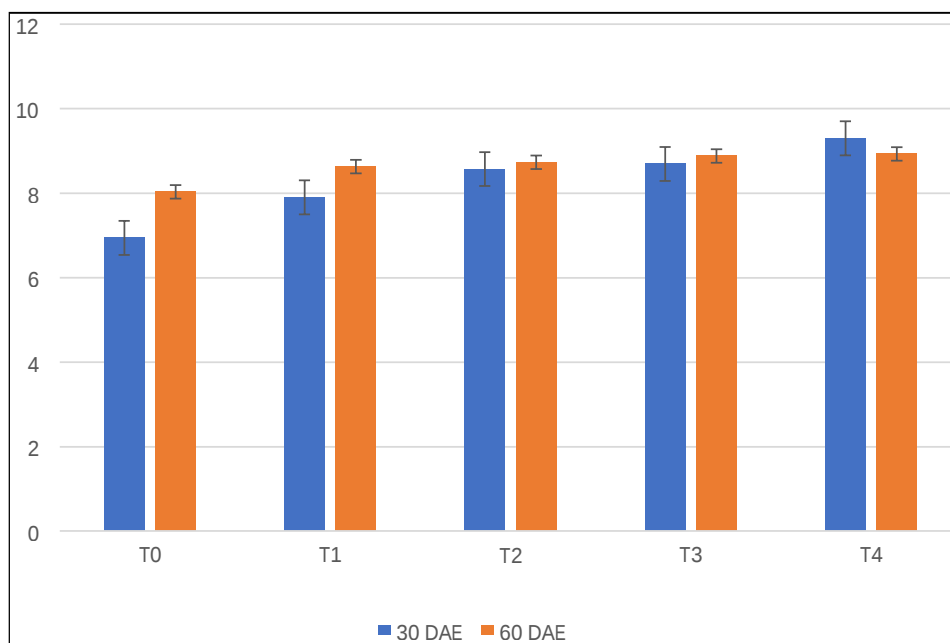


Fig 2: Effect of different Sodium Nitroprusside concentrations on the crop growth rate for 30 and 60 DAE of maize grown under rainfed condition.

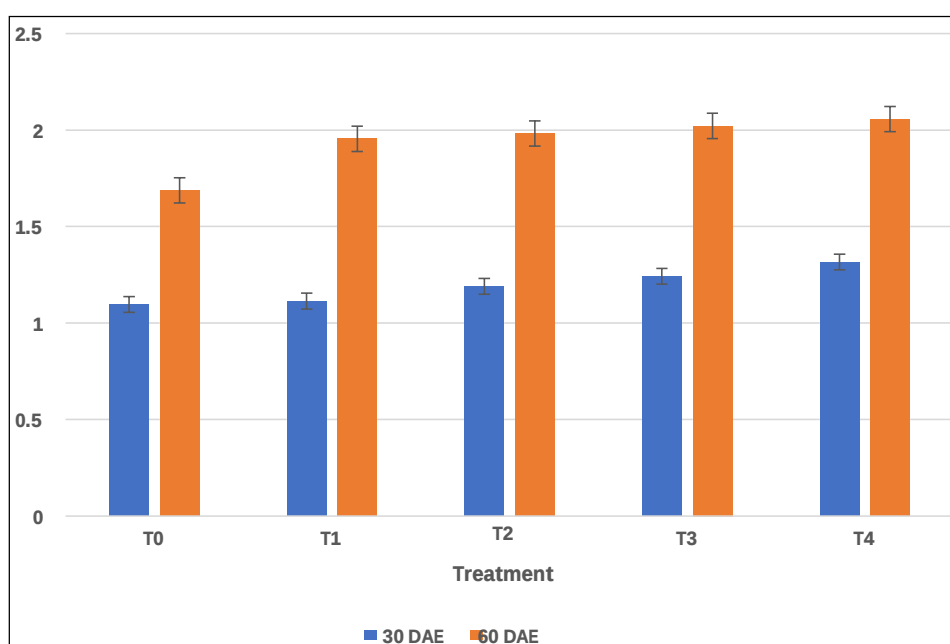


Fig 3: Effect of different Sodium Nitroprusside concentrations on the Total protein content for 30 and 60 DAE of maize leaf sample grown under rainfed condition.

Table 4: Effect of exogenous application of Sodium Nitroprusside on chlorophyll 'a', 'b' and total chlorophyll content (mg/g FW) of maize grown under rainfed conditions.

Treatment	30 DAE			60 DAE		
	Chlorophyll 'a'	Chlorophyll 'b'	Total	Chlorophyll 'a'	Chlorophyll 'b'	Total
	content (mg/g FW)	content (mg/g FW)	chlorophyll content (mg/g FW)	content (mg/g FW)	content (mg/g FW)	chlorophyll content (mg/g FW)
T0	1.767d	0.518d	2.285d	1.595c	0.59e	2.186e
T1	1.908c	0.61c	2.519c	1.64bc	0.683d	2.324d
T2	1.984b	0.806b	2.791b	1.688b	0.822c	2.511c
T3	2.029ab	0.791b	2.822b	1.751a	0.887b	2.638b
T4	2.065a	0.901a	2.966a	1.787a	1.005a	2.793a
C.D.	1.211	3.657	0.183	0.049	0.127	0.106
SE(m)	0.366	1.104	0.055	0.015	0.038	0.032
SE(d)	0.517	1.562	0.078	0.021	0.054	0.045
C.V.	1.066	3.548	3.577	1.521	8.299	2.216

Where (T0- Control, T1 - 100 μ M SNP, T2- 200 μ M SNP , T3 - 300 μ M SNP , T4 - 400 μ M SNP).

Table 5: Effect of exogenous application of sodium nitroprusside on membrane thermo-stability Index of maize grown under rainfed condition.

Treatment	30 DAE	60 DAE
T0	61.20e	60.07e
T1	64.32d	62.58d
T2	67.62c	64.61c
T3	70.32b	67.24b
T4	72.40a	70.86a
C.D.	3.657	2.887
SE(m)	1.104	0.872
SE(d)	1.562	1.233
C.V.	3.548	2.664

Where (T0- Control, T1 - 100 μ M SNP, T2 - 200 μ M SNP, T3 - 300 μ M SNP , T4 - 400 μ M SNP).

FW, when compared to the plots treated with foliar SNP treatment. From the above data it is concluded that exogenous application of SNP at different concentration (T1, T2, T3, T4) show a positive effect on total protein content at 30 DAE, whereas concentration @ 400 μ M (T4) shows higher value compared to other treatments. The observations on soluble sugar content of maize were recorded at 30 DAE and 60 DAE and has been presented in (Fig 4).

Membrane Thermo-stability index of maize as influenced by different concentration of SNP at 30 DAE and 60 DAE and the data regarding the observation has been presented in Table 5.

When compared to other treatments, exogenous application of SNP @ 400 μ M (T4) substantially was recorded the highest value chlorophyll stability index at 30 DAE with a mean value of 79.28% (Fig 5). The T3 (300 μ M) had the next-highest value of the chlorophyll stability index, with a mean value of 76.90% in comparison to the other treatments. In comparison to

the control plot, the mean values of the experimental plots treated with 200 μ M (T2) and 100 μ M (T1) concentrations of SNP are 72.43% and 68.28%, respectively. In comparison to plots treated with SNP, the control plot's mean value of 61.90% for the chlorophyll stability index was the lowest.

Over the control, the sink size was much larger after each treatment. With a mean value of 4.362 in comparison to other treatments, the exogenous application of SNP @ 400 μ M (T3) showed the highest sink size among all the treatments. Other treatments are comparable to one another but much more effective than controls. In comparison to plots treated with SNP at various concentrations, the control plot had the smallest sink size, with a mean value of 4.65. When compared to the control, all treatments had considerably higher test weight. In comparison to other treatment plots, the exogenous application of SNP @ 400 μ M (T1) was recorded the highest test weight with a primary value of 23.37 g. In comparison to the control plot, the next higher test weight value was observed at 300 μ M (T2) with a mean value of 20.805 gram. When SNP concentration was applied at 100 μ M (T1), test weight gradually decreased, with a mean value of 19.11 g. When compared to other treatment plots, the control plot had the lowest test weight value, with a mean value of 19.06 g. When compared to other treatments, exogenous application of SNP @ 400 μ M (T4) considerably increased yield per plot with a mean value of 14.06 kg. When compared to other treatments, the T3 (300 μ M) had the highest yield per plot with a mean value of 13.52 kg. The experimental plots treated with 200 μ M (T3) and 100 μ M (T1) concentrations of SNP exhibited a mean value of 12.53 kg and 11.39 kg, respectively, when compared to the control plot. In comparison to plots treated with SNP, the control plot had the lowest yield per plot, with a mean value of 9.68 kg (Fig 6).

With higher SNP doses, crop growth rate increased significantly. In comparison to other plots, SNP @ 400 μM displays the higher crop growth rate with mean values of 9.3 g/m²/day at 30 DAE and 8.92 g/m²/day at 60 DAE. The control plot had the lowest crop growth rate, with a mean value of 6.9 g/m²/day at 30 DAE and 8.03 g/m²/day at 60 DAE. Therefore, it can be summarized that morpho-physiological indices such as plant height, number of leaves, leaf area index, and crop growth rate were considerably raised with increasing Sodium Nitroprusside doses. According to current study, chlorophyll a (1.767 mg/g FW in control to 2.065 mg/g FW at 400 μM), Chlorophyll 'b' (0.518 mg/g FW in control to 0.901 mg/g FW @ 400 μM), and Total Chlorophyll Content (2.85 mg/g FW in control to 2.966 mg/g FW in 400 μM) at 30 DAE and Chlorophyll 'a' (1.595 mg/g FW in control to 1.787 mg/g FW in 400 μM), Chlorophyll 'b' (0.59 mg/g FW in control to 1.005 mg/g FW in 400 μM) and total chlorophyll content showed positive results for foliar spray of SNP at different concentrations. Chlorophyll 'a', 'b' and total chlorophyll content significantly increased in the plots treated with varying concentrations of SNP, whereas the control plot showed a decrease in all three of these components. Under rainfed conditions, sodium nitroprusside (SNP) has a favourable effect on biochemical markers in maize. The results are comparable to those obtained by Ali *et al.* (2017). Exogenous SNP treatment effectively delayed chlorophyll breakdown and yellowing of broccoli florets during storage at 20°C SNP may have accomplished this by increasing the activity of antioxidant enzymes, reducing the activity of Chl-degrading enzymes and suppressing associated gene expression (Wang *et al.*, 2015).

Of all the treatments, foliar application of SNP @ 400 μM (T4) recorded higher total protein content with a mean

value of 1.316 mg/g/FW compared to other plots, while the next higher value of total protein content was recorded in 300 μM with a mean value of 1.242 mg/g/FW compared to control plot (1.095 mg/g/FW) at 30 DAE. Total protein content gradually increased with application of SNP @ 400 μM (2.057 mg/g/FW) compare to control plot with a mean value of 1.687 mg/g/FW at 60 DAE. Foliar application of SNP @ 400 μM (T4) recorded higher soluble sugar content with a mean value of 0.933 mg/g/FW compared to other plots, followed by 300 μM with a mean value of 0.838 mg/g/FW compared to control plot (0.678 mg/g/FW) at 30 DAE and soluble sugar content gradually increased with application of SNP @ 400 μM (1.343 mg/g/FW) compared to control plot with a mean value of 0.678 mg/g/FW. T4 (400 μM) concentration of Sodium Nitroprusside has a beneficial influence on biochemical parameters such as Chlorophyll 'a', 'b', and total chlorophyll content, Total protein content, Soluble sugar content, Membrane thermo-stability index, and Chlorophyll stability index. A low concentration of NO increased soluble protein accumulation, while a high concentration of NO prevented soluble protein accumulation. NO was discovered to influence a number of protein accumulations involved in material and energy metabolism, including ATPase and LOX, according to Bai *et al.*, (2011). Foliar application of SNP considerably impacted the test weight among treatments, with the test weight achieved in T4 (23.376g) being much greater than the values recorded in rainfed maize control plots. When compared to treated plots, the higher sink size was recorded with foliar application of SNP @ 200 μM with a mean value of 5.142 and the lowest sink size was recorded in T4 with a mean value of 4.362. Among varied concentrations of SNP @ 400 μM (T4), the yield per plot increased significantly, with a mean value of 14.06 kg

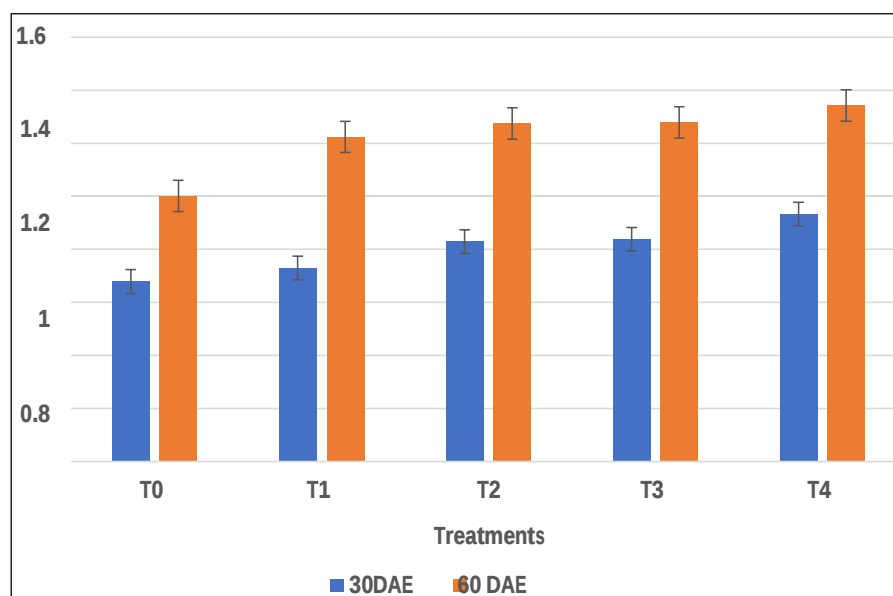


Fig 4: Effect of different Sodium Nitroprusside concentrations on the total soluble sugar for 30 and 60 DAE of maize leaf sample grown under rainfed condition.

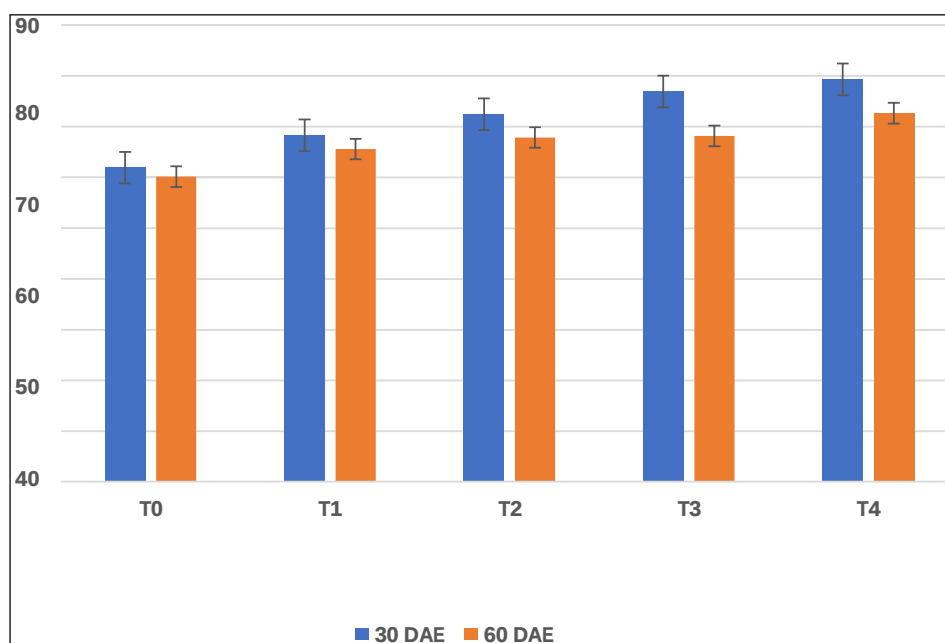


Fig 5: Effect of different Sodium Nitroprusside concentrations on the chlorophyll stability index for 30 and 60 DAE of maize grown under rainfed condition. T0, T1, T2, T3, T4 are different treatments.

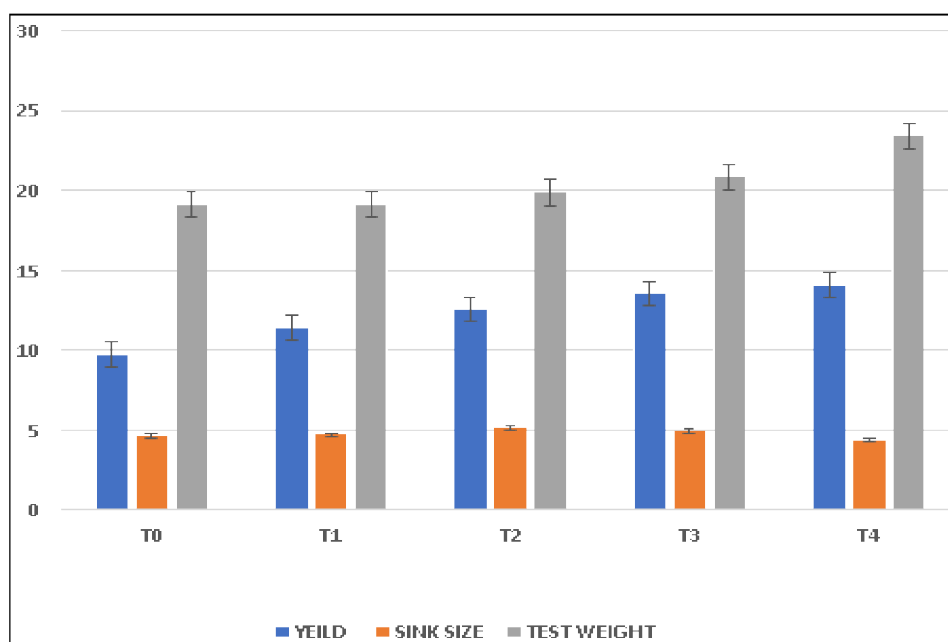


Fig 6: Impact of different Sodium Nitroprusside concentrations on the sink size, test weight and yield per plot of maize grown under rainfed condition

compared to other plots. In comparison to the treated plots, the yield per plot decreased in the control plot, with a mean value of 9.68 kg. Different Sodium Nitroprusside concentrations produced superior outcomes on yield and yield-related characteristics such as test weight, sink size,

yield per plot, however T4 and T3 were found to be the best treatments in this regard. The T4 treatment showed the highest height of the plants which can be attributed to improved root anchorage, water retention, or nutrient uptake due to SNP.

CONCLUSION

These findings suggested that foliar application of SNP at various concentrations can be employed to boost the productivity of maize variety C.P-858. On the basis of the observations and results from the current study, it can be concluded that foliar spray of Sodium Nitroprusside @ 400 μ M shows better results in maize variety C.P-858 compared to control plot grown under rainfed conditions.

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Conflict of interest

There is no conflict of interest.

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