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# Effect of partial root drying technique and fertigation on leaf nutrient status of pomegranate (*Punica* granatum L.) Cv. Bhagwa

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# Abstract

A field experiment was conducted to study the effect of partial root drying technique and fertigation on leaf nutrient status of pomegranate (*Punica granatum* L.) Cv. Bhagwa. The experiment comprising of 8 treatments, T<sub>1</sub>: Partial root drying technique (100% water requirement) + 100% nutrition (NPK), T<sub>2</sub>: Partial root drying technique (100% water requirement) + 75% nutrition (NPK), T<sub>3</sub>: Partial root drying technique (100% nutrition by fertigation with 100% RDF of NPK, T<sub>4</sub>: Partial root drying technique (75% water requirement) + 75% nutrition by fertigation with 75% RDF of NPK, T<sub>5</sub>: Partial root drying technique (50% water requirement) + 100% nutrition by fertigation with 75% RDF of NPK, T<sub>5</sub>: Partial root drying technique (50% water requirement) + 75% nutrition by fertigation with 100% RDF of NPK, T<sub>6</sub>: Partial root drying technique (50% water requirement) + 75% nutrition by fertigation with 100% nutrition by fertigation with 100% RDF of NPK, T<sub>6</sub>: Partial root drying technique (50% water requirement) + 75% nutrition by fertigation with 100% nutrition by fertigation with 100% RDF of NPK, T<sub>6</sub>: Control *i.e.* normal drip irrigation (100% water requirement) + 100% nutrition by fertigation with 100% RDF of NPK, T<sub>8</sub>: Control *i.e.* normal drip irrigation on both sides of the plant (100% water requirement) + 75% nutrition by fertigation with 75% RDF of NPK. During flowering stage, the maximum nitrogen (1.95%), potassium (1.95%), calcium (2.10%) and magnesium content (0.57%) was recorded in I<sub>2</sub>N<sub>1</sub> treatment. Coming to harvest stage, maximum nitrogen content (1.53%), maximum potassium of 1.40% (I4N2 treatment also). Whereas, maximum calcium (0.49%) was observed in I<sub>2</sub>N<sub>2</sub> treatment.

Keywords: Pomegranate, PRD, bhagwa, soil nutrients, drought, evapotranspiration

# Introduction

# **Material and Methods**

The present investigation on the effect of partial root drying technique and fertigation on soil nutrients of pomegranate (*Punica granatum* L.) Cv. Bhagwa was carried out at pomegranate orchard at Fruit orchard, Sector 70, UHS, Bagalkot during 2019-2020 and 2020-2021. The location comes under Northern dry zone (Zone-3) of Karnataka and situated at 16. 10° N latitude and 75.42° E longitude at an altitude of 542.00 m above Mean Sea Level (MSL). The experiment was laid out in Two Factorial RCBD, first factor with four different irrigation treatment and second factor with two different fertigation treatments replicated three times comprising of 24 plots. The treatment details include.

# Main treatments

- 1. I<sub>1</sub>: Partial root zone irrigation technique to replenish 100%  $CP_E(100\% \text{ water requirement})$
- 2. I<sub>2</sub>: Partial root zone irrigation technique to replenish 75% CP<sub>E</sub> (75% water requirement)
- 3. Is: Partial root zone irrigation technique to replenish 50%  $CP_E$  (50% water requirement)
- **4. I4:** Control *i.e.* normal drip irrigation on both sides of the plant to replenish 100% CP<sub>E</sub> (100% water requirement)

# Sub treatments

- 1. N<sub>1</sub>: 100% nutrition by fertigation with 100% RDF of NPK (400:200:200 g /plant)
- 2. N<sub>2</sub>: 75% nutrition by fertigation with 75% RDF of NPK (300: 150:150 g /plant)

# Calculation of water requirement of the crop

Based on CPE, using FAO Penman- Monteith method (http://www.fao.org or https://aggiehorticulture.tamu.edu).

The index tissue identified for pomegranate plant tissue analysis *i.e.* eighth pair leaf from non-bearing shoot (Raghupathi and Bhargawa, 1998)<sup>[7]</sup> was collected from all plants in each replication separately at three stages *viz.*, before pruning, flowering and at harvest of the fruit and subjected to acid digestion of leaf samples with di-acid (HNO3: HClO4-10:4) mixture on sand bath for the nutrient content in the plant sample except nitrogen. For nitrogen, acid digestion with concentrated sulfuric acid along with digestion mixture was carried out. For determining the nutrient content in the pomegranate leaf sample. A known weight (0.5 g) of powdered sample was digested with conc. H<sub>2</sub>SO<sub>4</sub> in presence of digestion mixture (CuSO<sub>4</sub>: K<sub>2</sub>SO<sub>4</sub>: Se 100:40:1). The digested sample was distilled in an alkaline environment (with a 40% NaOH solution) and the distilled ammonia was trapped in boric acid. The ammonia in the boric acid was titrated against 0.1 N H<sub>2</sub>SO<sub>4</sub> for nitrogen measurement (Piper, 1966) <sup>[5]</sup>. The amount of phosphorus in the leaf sample was calculated using the phospho-vanado-molybdate technique. Using a spectrophotometer, the intensity of yellow colour was measured at 430 nm. The phosphorus content was calculated using the P standard curve as a guide (Piper, 1966)<sup>[5]</sup>. The diacid digested sample was fed to flame photometer directly, with proper dilution if it is required. The reading was used along with the standard curve to estimate potassium content in the plant sample (Piper, 1966) <sup>[5]</sup>. The complexometric titration method was used to determine the Ca and Mg content of the plant sample. Calcium and magnesium (Ca +, Mg+) concentrations were assessed using an EDTA titration at pH 10.00 in the presence of an EBT indicator. Calcium was determined by EDTA titration with NaOH solution at pH 12.00 in the presence of Patton and Reader indicators (Jackson, 1973)<sup>[4]</sup>.

# **Results and Discussion**

The interpretation of pooled mean values pointed out that among the irrigation treatments, the maximum nitrogen content in leaves was recorded in I<sub>2</sub> treatment (PRD<sub>75</sub>) followed by I1 (PRD100 treatment). Whereas, minimum nitrogen content in leaves was observed in I<sub>3</sub> (PRD<sub>50</sub>) treatment during before pruning and at flowering stage (Table 1). The increased nitrogen content in PRD<sub>75</sub> and PRD<sub>100</sub> irrigation treatments could be attributed to Birch effect due to alternate wetting and drying of rootzone by improving microbial and enzymatic activity of soil enables faster conversion of complex into simpler form to be ready for uptake, similar results were revealed by earlier works of Wang et al. (2010)<sup>[9]</sup> in tomato. Among the fertigation treatments, N1 (RDF100) recorded significantly maximum nitrogen content in leaves during both the years. The relatively higher amounts of inorganic N application in this treatment might have triggered higher accumulation in succulent levels at early stages of crop growth, and its owing to higher availability of N in soil (Sheikh and Rao, 2005)<sup>[6]</sup>.

During harvest stage, significantly maximum nitrogen content in leaves was recorded in I<sub>3</sub> treatment (PRD<sub>50</sub>) followed by I<sub>4</sub> treatment (CDI<sub>100</sub>). Whereas, minimum nitrogen content in leaves was observed in I<sub>2</sub> treatment (PRD<sub>75</sub>). The decreased nitrogen content in PRD<sub>75</sub> and PRD<sub>100</sub> irrigation treatments could be attributed to Birch effect due to alternate wetting and drying of rootzone by improving microbial and enzymatic activity of soil and greater increase in volume of root system with higher activity of feeder roots, which enables faster uptake and absorption of nutrients from plants resulted in decreased quantity of nutrients. Similar results were revealed by earlier works of Dry et al. (2000)<sup>[1]</sup> in grape, Songsri et al, (2008) <sup>[8]</sup> in peanut, and Wang *et al.* (2010) <sup>[9]</sup> in tomato. In fertigation, again N<sub>2</sub> recorded higher leaves nitrogen content than that of N<sub>2</sub> This decreased nitrogen content during harvest stage than that during vegetative growth attributed to utilisation of nitrogen by fruits for its growth with the advancement of crop growth resulted in reduction in leaf N concentration.

Before pruning and at flowering stage, among four different irrigation treatments, the highest phosphorous content in leaves was recorded in I<sub>2</sub> treatment (PRD<sub>75</sub>) followed by I<sub>1</sub> treatment (PRD<sub>100</sub>). Whereas, minimum phosphorous content in leaves was observed in I<sub>3</sub> treatment (PRD<sub>50</sub>) at flowering stage (Table. 2). The increased quantity of phosphorous content in mild and even zero water stress treatment in PRD irrigation regime might be due to enlarged root system due to negative effect of PRD irrigation on excess shoot growth and diversion of carbohydrates into root growth. Further, phosphorous is having higher fixation property under drought condition resulted in lesser conversion from complex form to simpler form leads to lesser mobilization in soil and plant system. Among the fertigation treatments,  $N_1$  (RDF<sub>100</sub>) recorded significantly maximum phosphorous content during both the years, this higher amount of P in this treatment could be attributed to application of higher RDP as basal dose during bahar initiation might have resulted in higher availability in soil and there upon accumulation of P in succulent leaf (Greeshma, 2016)<sup>[2]</sup>. During harvest stage, significantly maximum phosphorous content in leaves was recorded in  $I_4$  treatment (CDI<sub>100</sub>) followed by  $I_3$  treatment (PRD<sub>50</sub>). Whereas, minimum phosphorous content in leaves was observed in I<sub>2</sub> treatment (PRD<sub>75</sub>). The decreased phosphorous content in PRD<sub>75</sub> and PRD<sub>100</sub> irrigation treatments could be attributed to Birch effect due to alternate wetting and drying of rootzone by improving microbial and enzymatic activity of soil and greater increase in volume of root system with higher activity of feeder roots. In fertigation, N<sub>2</sub> recorded higher phosphorous content in leaves. With the advancement of crop growth, the P content decreased recording lowest per cent after the harvest of crop. This may be attributed to decreased P content in soil due to precipitation of P with Ca to insoluble form (Wasterman and Leytem, 2003) [10].

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Table 1: Nitrogen content (%) of leaves at flowering and harvest stages as influenced by different irrigation and fertigation in pomegranate cv.

Bhagwa
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	Before bah	ar treatme	nt stage	At flowering stage			At harvest stage		
Irrigation (I)	Pooled			I	Pooled		Pooled		
	$N_1$	$N_2$	Mean	N <sub>1</sub>	$N_2$	Mean	N <sub>1</sub>	$N_2$	Mean
$I_1$	1.90	1.87	1.88	1.88	1.86	1.87	1.42	1.46	1.44
$I_2$	1.92	1.90	1.91	1.95	1.91	1.93	1.31	1.39	1.35
$I_3$	1.88	1.86	1.86	1.82	1.77	1.79	1.51	1.53	1.52
$\mathbf{I}_4$	1.88	1.85	1.86	1.85	1.83	1.84	1.46	1.51	1.48
Mean	1.89	1.86		1.87	1.84		1.42	1.47	
For comparing means of	S.Em. ±	C.I	C.D. at 5%		C.D. at 5%		S.Em. ±	C.D	. at 5%
Irrigation (I)	0.010	NS		0.005	0.010		0.005	0.010	
Nutrients (N)	0.006	NS		0.007	0.020		0.007	0.020	
Irrigation (I) x Nutriments (N)	0.010		0.04	0.010	0	.030	0.010	0.030	

PRD: Partial root drying, Factor-II: Two different levels of fertigation

CI- Conventional (Normal two side irrigation), N1- RDF 100

Factor-1: Four different levels of irrigation (PRD and conventional)  $N_2$ - RDF  $_{75}I_1$ -PRD  $_{100}$ ,  $I_2$ -PRD $_{75}I_3$ -PRD $_{50}I_4$ - Conventional  $_{100}$  NS- Non-Significant

 Table 2: Phosphorous content of leaves (%) at flowering and harvest stages as influenced different irrigation and fertigation in pomegranate cv.

 Bhagwa

	Before bah	ar treatme	nt stage	At flowering stage			At harvest stage		
Irrigation (I)		Pooled		Pooled			Pooled		
	N <sub>1</sub>	$N_2$	Mean	N <sub>1</sub>	$N_2$	Mean	N1	$N_2$	Mean
$I_1$	0.36	0.33	0.34	0.37	0.32	0.34	0.18	0.18	0.18
$I_2$	0.36	0.35	0.35	0.38	0.35	0.36	0.15	0.16	0.15
I <sub>3</sub>	0.31	0.31	0.31	0.28	0.27	0.27	0.20	0.21	0.21
$I_4$	0.34	0.33	0.33	0.34	0.30	0.32	0.25	0.27	0.26
Mean	0.34	0.33		0.34	0.31		0.19	0.20	
For comparing means of	S.Em. ±	C.I	D. at 5%	S.Em. ±	C.D. at 5%		S.Em. ±	C.D	. at 5%
Irrigation (I)	0.004	0.014		0.004	0.010		0.003	0.008	
Nutrients (N)	0.003	0.010		0.005	0.010		0.004	0.012	
Irrigation (I) x Nutriments (N)	0.006	(	0.020	0.008	NS		0.006	0.017	

PRD: Partial root drying, Factor-II: Two different levels of fertigation

CI- Conventional (Normal two side irrigation) N1- RDF 100

Factor-1: Four different levels of irrigation (PRD and conventional) N2- RDF 75

I1 - PRD 100 I2 - PRD75 I3 - PRD50 I4- Conventional 100

NS- Non-Significant

Before pruning and at flowering period of experiment, among four different irrigation treatments, potassium content in leaves was recorded maximum in  $I_2$  treatment (PRD<sub>75</sub>) followed by I<sub>1</sub> treatment (PRD<sub>100</sub>). Whereas, minimum potassium content in leaves was recorded in I3 treatment (PRD<sub>50</sub>) (Table. 3). The increased quantity of potassium content in mild and even zero water stress treatment in PRD irrigation regime might be due to enlarged root system due to negative effect of PRD irrigation on excess shoot growth and diversion of carbohydrates into root growth as well due to birch effect. Among the fertigation treatments, maximum potassium content in leaves was noticed in N1 (RDF100 treatment). Highest potassium content in leaf was observed in 100 fertigation treatment at flowering stage, owing to higher availability of K in soil due to high amount of basal soil application. In harvesting stage, potassium content in leaves slightly decreased than that of flowering stage. Among four different irrigation treatments, potassium content in leaves was found maximum in I<sub>3</sub> treatment (PRD<sub>50</sub>) followed by I<sub>4</sub>

treatment CDI<sub>100</sub>. Whereas, minimum leaves potassium content was observed in I2 treatment (PRD75). The decreased potassium content in PRD<sub>75</sub> and PRD<sub>100</sub> irrigation treatments could be attributed to Birch effect due to alternate wetting and drying of rootzone by improving microbial and enzymatic activity of soil and greater increase in volume of root system with higher activity of feeder roots enhances higher uptake of K from soil to plants system, and its utilization from growing fruits resulted in decreased quantity of potassium in leaves. These results are opined by earlier worker revealed by Guimera *et al.*, (1995)<sup>[3]</sup> in strawberry. Between two different fertigation treatments, leaves potassium content was recorded maximum in  $N_2$  (RDF<sub>75</sub>) treatment and it might be due to antagonistic effect of other mineral nutrients like calcium resulted in reduced uptake and translocation in plants indicate their deficiency in the crop however, the visual symptoms were not seen on the leaves as revealed by earlier workers of Raghupathi and Bhargawa (1998b)<sup>[7]</sup> in pomegranate.

Table 3: Potassium content of leaves (%) at flowering and harvest stages as influenced by different irrigation and fertigation in pomegranate cv.
Bhagwa

	Before bah	ahar treatment stage At flowering st			stage	age At harvest stage				
Irrigation (I)		Pooled		Pooled			Pooled			
_	N <sub>1</sub>	$N_2$	Mean	N <sub>1</sub>	$N_2$	Mean	N <sub>1</sub>	$N_2$	Mean	
$I_1$	1.91	1.88	1.90	1.91	1.88	1.89	1.32	1.35	1.34	
$I_2$	1.91	1.90	1.90	1.95	1.92	1.93	1.26	1.31	1.28	
I <sub>3</sub>	1.84	1.83	1.84	1.82	1.78	1.80	1.36	1.40	1.40	
$I_4$	1.86	1.86	1.86	1.86	1.84	1.85	1.33	1.40	1.38	
Mean	1.88	1.87		1.88	1.85					
For comparing means of	S.Em. ±	C.I	D. at 5%	S.Em. ±	C.D. at 5%		S.Em. ±	C.D. at 5%		
Irrigation (I)	0.004	(	0.010		0.010		0.0042	0.0122		
Nutrients (N)	0.003	NS		0.006	0.020		0.0060	0.0172		
Irrigation (I) x Nutriments (N)	0.006	0.010		0.009	0.020		0.0084	0.0243		
PRD: Partial root drying	Factor-II: Two different levels of fertigation									

PRD: Partial root drying

CI- Conventional (Normal two side irrigation)

N1- RDF 100

Factor-1: Four different levels of irrigation (PRD and conventional)  $N_2$ - RDF  $_{75}$ 

 $I_1 \text{-} PRD \ {}_{100} \ I_2 - PRD_{75} \ I_3 - PRD_{50} \ I_4 \text{-} Conventional \ {}_{100}$ 

NS- Non Significant

Before pruning and at flowering stage, among four different irrigation treatments, calcium content in the leaves was recorded maximum in I<sub>2</sub> treatment (PRD<sub>75</sub>) followed by I<sub>1</sub> treatment (PRD<sub>100</sub>). Whereas, minimum calcium content in leaves was observed in I<sub>3</sub> treatment (PRD<sub>50</sub>) (Table. 4). The increased quantity of calcium content in mild and even zero water stress treatment in PRD irrigation regime might be due to Birch effect due to alternate wetting and drying of rootzone by improving microbial and enzymatic activity of soil and greater root volume of roots with enhanced activity of feeder roots enables faster conversion of complex into simpler form to be ready for uptake. Among the fertigation treatments, maximum calcium content in leaves was noticed in N1 (RDF<sub>100</sub> treatment) than that of  $N_2$  but did not varied significantly.

In harvesting stage, among four different irrigation treatments, the maximum calcium content in leaves was recorded in I<sub>3</sub> treatment (PRD<sub>50</sub>) followed by I<sub>4</sub> treatment (CDI<sub>100</sub>). Whereas, minimum calcium content in leaves was observed in I2 treatment (PRD75). The decreased calcium content in PRD<sub>75</sub> and PRD<sub>100</sub> irrigation treatments could be attributed to Birch effect due to alternate wetting and drying of rootzone by improving microbial and enzymatic activity of soil and greater increase in volume of root system with higher activity of feeder roots enhances higher uptake and absorption of Ca from soil to plants system resulted in decreased quantity of potassium in soil. These results are in agreement with those revealed by earlier workers of Dry et al. (2000)<sup>[1]</sup> in grape with respect fertigation treatments, slightly maximum calcium content in leaves was observed in N1 treatment (RDF100).

Table 4: Calcium content (%) of leaves at flowering and harvest stages as influenced by different irrigation and fertigation in pomegranate cv. Bhagwa

	Before bah	ar treatme	nt stage	At flowering stage			At harvest stage			
Irrigation (I)		Pooled		I	Pooled		Pooled			
	$N_1$	N <sub>2</sub>	Mean	N1	$N_2$	Mean	N1	$N_2$	Mean	
$I_1$	2.02	2.01	2.02	1.29	1.26	1.27	2.02	2.00	2.01	
$I_2$	2.08	2.02	2.05	1.22	1.19	1.20	2.10	2.03	2.06	
I <sub>3</sub>	1.98	1.95	1.96	1.41	1.39	1.40	1.92	1.90	1.91	
I4	2.00	2.00	2.00	1.37	1.34	1.35	1.98	1.98	1.98	
Mean	2.00	2.00		1.32	1.29		2.01	1.97		
For comparing means of	S.Em. ±	C.I	D. at 5%	S.Em. ±	C.D. at 5%		S.Em. ± C.D. a		. at 5%	
Irrigation (I)	0.006	0.018		0.002	0.007		0.005	0.015		
Nutrients (N)	0.004	NS		0.003	0.010		0.007	0.021		
Irrigation (I) x Nutriments (N)	0.008	0.026		0.004	0.014		0.010	0.030		
PRD: Partial root drying	Factor-II: Two different levels of fertigation									

PRD: Partial root drying

CI- Conventional (Normal two side irrigation)

Factor-1: Four different levels of irrigation (PRD and conventional) N2- RDF 75

I1-PRD 100 I2-PRD75 I3-PRD50 I4- Conventional 100

NS- Non-Significant

Table 5: Magnesium content of leaves (%) at flowering and harvest stages as influenced by different irrigation and fertigation in pomegranate cv. Bhagwa

N1- RDF 100

Irrigation (I)	Before bal	nar treatme	nt stage	At flowering stage Pooled			At harvest stage Pooled		
		Pooled							
	N1	$N_2$	Mean	N1	$N_2$	Mean	N1	N <sub>2</sub>	Mean
Iı	0.51	0.50	0.50	0.55	0.53	0.54	0.48	0.47	0.48
I <sub>2</sub>	0.53	0.53	0.53	0.57	0.55	0.56	0.48	0.49	0.48
I <sub>3</sub>	0.49	0.49	0.49	0.48	0.46	0.47	0.44	0.43	0.43
$I_4$	0.50	0.48	0.49	0.52	0.50	0.51	0.47	0.45	0.46

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Mean	0.50	0.50	0.53	0.51	0.47	0.46
For comparing means of	S.Em. ±	C.D. at 5%	S.Em. ±	C.D. at 5%	S.Em. ±	C.D. at 5%
Irrigation (I)	0.005	0.016	0.021	0.003	0.003	0.009
Nutrients (N)	0.004	NS	0.015	0.004	0.004	0.013
Irrigation (I) x Nutrients (N)	0.007	0.023	NS	0.006	0.006	0.018

PRD: Partial root drying

CI- Conventional (Normal two side irrigation)

Factor-II: Two different levels of fertigation N1- RDF 100

Factor-1: Four different levels of irrigation (PRD and conventional) N2- RDF 75

 $I_2 - PRD_{75}$  $I_3 - PRD_{50}$ I4- Conventional 100 I1 - PRD 100

NS- Non Significant

Before pruning and at flowering stage, among four different irrigation treatments, the highest magnesium content in leaves was recorded in I<sub>2</sub> treatment (PRD<sub>75</sub>) followed by I<sub>1</sub> treatment (PRD<sub>100</sub>). Whereas, lower magnesium content in leaves was observed in I<sub>3</sub> treatment (PRD<sub>50</sub>) at flowering stage (Table. 5). The increased quantity of magnesium content in mild and even zero water stress treatment in PRD irrigation regime might be due to Birch effect due to alternate wetting and drying of rootzone by improving microbial and enzymatic activity of soil and greater root volume of roots with enhanced activity of feeder roots enables faster conversion of complex into simpler form to be ready for uptake. Among the fertigation treatments, N1 (RDF100) recorded significantly maximum magnesium content in leaves during both years of experimentation. The better growth of the plant with higher chlorophyll might have triggered higher uptake of nutrients especially Mg from the soil and there by their accumulation in plant. However, the leaf concentration was found to be in optimum range as revealed by earlier workers of Raghupathi and Bhargava. (1998b)<sup>[7]</sup>.

During harvest stage, similar trend has observed with significantly maximum magnesium content in leaves was recorded in both  $I_1$  treatment (PRD<sub>100</sub>) and  $I_2$  treatment (PRD<sub>75</sub>) whereas, minimum magnesium content in leaves was observed in I<sub>3</sub> treatment (PRD<sub>50</sub>). The decreased magnesium content in PRD<sub>75</sub> and PRD<sub>100</sub> irrigation treatments could be attributed to Birch effect due to alternate wetting and drying of rootzone by improving microbial and enzymatic activity of soil and greater increase in volume of root system with higher activity of feeder roots enhances higher uptake of Mg from soil to plants system resulted in decreased quantity of potassium in soil. These results are in agreement with those revealed by earlier workers of Dry et al. (2000)<sup>[1]</sup> in grape. In fertigation, again N1 recorded higher magnesium content in leaves.

# Conclusion

The maximum nitrogen content in leaves (1.93%), phosphorous (0.36%), potassium (1.93%), calcium (2.06%) and magnesium content in leaves (0.56%) was recorded in I<sub>2</sub> treatment (PRD<sub>75</sub>). During harvest stage, significantly maximum nitrogen content in leaves (1.52%), potassium (1.40%) and calcium (1.40%) was recorded in I<sub>3</sub> treatment (PRD<sub>50</sub>). While with respect to calcium content during harvest stage, significantly maximum values (0.26%) was recorded in I<sub>4</sub> treatment (CDI<sub>100</sub>) and maximum magnesium content in leaves (0.48%) was recorded in both I1 treatment (PRD100) and I<sub>2</sub> treatment (PRD<sub>75</sub>).

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