

# Effects of Bay Leaf (*Laurus nobilis* L.), Potato (*Solanum tuberosum* L.) Peel and Banana (*Musa* Species) Peel Extracts on Bio-Chemical Indicators of Some Upland Rice (*Oryza sativa* L.) Crop under Higher Iron in Acid Soil Condition

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## Abstract—

**Background:** The occurrence of high rainfall in Assam and leaching of base cations cause the soils to become acidic in reaction. The high mobility of iron in acid soil and its absorption raises the iron concentration, and it damages the physiology of crop plants. That is why excessive iron is regarded as one of the limiting factors responsible for lowering growth, development and yield of upland rice crop under irrigation through shallow tube wells. An innovative approach using extracts of potassium- and antioxidant-rich bio-inputs might be a novel approach for deterring and halting physio-biochemical aberrations due to higher iron in acid soil condition.

**Method:** A pot experiment (CRBD with three replications) was carried out to investigate the effects of bay leaf, potato peel, and banana peel extracts on biochemical indicators of some upland (Ahu) rice crop (varieties: Inglongkiri, Dehangi (Fe tolerant), Lachit (Fe susceptible), and Luit) under higher iron in acid soil conditions during the Autumn season (March-September, 2024). The five treatments were: (1) 100 ppm  $FeSO_4$  as basal at vegetative stage (control), (2) 100 ppm  $FeSO_4$  as basal at vegetative stage plus root dip treatment before transplanting and foliar spray with bay leaf extract at 20 days after transplanting, (3) 100 ppm  $FeSO_4$  as basal at vegetative stage plus root dip treatment before transplanting and foliar spray with banana peel extract at 20 days after transplanting, (4) 100 ppm  $FeSO_4$  as basal at vegetative stage plus root dip treatment before transplanting and foliar spray with potato peel extract at 20 days after transplanting; (5) Natural soil without any treatment (absolute control).

**Result:** In general, compared to the control, the treatments significantly increased biochemical indices viz., total chlorophyll (10.80-21.90%), chlorophyll 'a' (6.39-20.93%), chlorophyll 'b' contents (7.27-22.35%) at maximum tillering stage; total chlorophyll (10.96-21.38%), chlorophyll 'a' (11.88-21.89%), chlorophyll 'b' contents at heading stage (7.22-15.79%), NR activity at maximum tillering (12.43-35.52%) and heading (15-36.23%) stages, carbohydrate content (7.51-13.81%) and reduced iron content (10.57-21.34%) in grain at harvest. The bio-input extracts lessened cation leakage at maximum tillering (2.25-1.63%) and heading (3.52-2.83%) stages, and lipid peroxidation at maximum tillering (30.03-10.55%) and heading (29.64-10.96%) stages, despite the presence of high iron in the soil (initial: 200 ppm, harvest: 106 ppm). In the experiment, the intensity of blue colour following Perl's Prussian Blue staining was directly related to iron content in grain, with darker staining indicating higher iron accumulation, and this was altered by the bio-input treatments.

**Keywords—** Banana peel, Bay leaf, Chlorophyll, CMS, Cation leakage, Carbohydrate, Iron, Potato peel, Rice.

## I. INTRODUCTION

Rice is one of the staple food crops in Assam cultivated as Winter (70%), Autumn rice (23%) and Summer rice (7%) covering 2.54 million hectares. The higher iron content in the acid soil (80% of geographical area i.e. 25 m ha) of the region is one of the considerable factors for lower productivity ( $< 3 \text{ t ha}^{-1}$ ) of traditional genotypes. The high rainfall ( $>2000 \text{ mm}$ ) makes the soil acidic in nature ( $\text{pH}<5.0$ ) due to leaching of basic cations (Mandal, 1995; Mandal et al., 2019). Consequently, the ground water contains higher iron (0.25-67.0 ppm), and its absorption by plant roots enriches the concentration of iron in plants. The excess iron (150-450 ppm) in plants affects the growth and development of rice crop, which is manifested by appearance of different physiological symptoms viz., potassium deficiency, yellowing of green leaves, dark brown or bronze spots, and death of plants (Baruah et al., 1983; Bora and Borkakati, 1997). Although many efforts have been made to ameliorate the aberrations due to iron toxicity in plants (Singh and Singh, 1987; Borah and Nath, 1979; Bey, 2022; Bey et al., 2026), there is a paucity of information on how bay leaf, banana peel, and potato peel extracts halt and rectify the degradation of biochemical indices in rice crop. As the extracts of these bio-inputs contain various nutrients and bio-molecules (Burton, 1989; Nguyen et al., 2003; Buckenhüskes, 2005; Emaga et al., 2007; Cakmak et al., 2013; Sidhu and Zafar, 2018; Batool et al., 2020; Karan, 2023), it is worthwhile to investigate their effects on biochemical indices in upland (Ahu) rice crop in the presence of high iron conditions.

## II. MATERIALS AND METHODS

The laboratory pot experiment (March-September 2024) was conducted at Assam Agricultural University (Geographical position:  $26^{\circ}45' \text{ N}$  Latitude,  $94^{\circ}12' \text{ E}$  Longitude, 87 meters above mean sea level). The study comprised four upland (Ahu) cultivars: Inglongkiri, Dehangi (iron tolerant), Lachit (iron susceptible), and Luit. The 25 to 30 days old seedlings of the cultivars were transplanted in pots filled with a mixture of sand, loam soil, and farm yard manure. All management practices including application of recommended NPK fertilizers @  $60:40:40 \text{ kg ha}^{-1}$  based on the volume of soil per hectare, irrigation (2-3 cm) and prophylactic measures against insect pests were taken up when necessary.

Iron solution of 100 ppm strength was prepared using  $\text{FeSO}_4$  (MW: 151.908 g). Each pot (made leak proof by sealing bottom with mud) received a basal application of the  $\text{FeSO}_4$  solutions (10 days prior to transplanting). Locally collected matured bay leaves, ripe banana peel, and potato peel were dried at  $55^{\circ}\text{C}$  in a hot air oven. Ten grams of each dried and ground material was combined with 100 ml of distilled water separately, then the mixtures were heated to  $50^{\circ}\text{C}$  for 60 minutes in a thermostatic bath. The extracts were cooled, filtered (with a pore size of 0.45 mm), and kept in dark at  $4^{\circ}\text{C}$  (Gebre Christos et al., 2020). Before transplanting, root dip treatments (RDT) of 30-day-old seedlings were administered overnight (100 seedlings per litre solution) with the extracts of banana, potato, and bay leaf.

Soil pH was measured as suggested by Jackson (1973). Biochemical parameters viz., leaf chlorophyll contents (Hiscox and Israelstam, 1979), nitrate reductase (NR) activity (Thimmaiah, 1999), carbohydrate content in grain (Hedge et al., 1962), cation leakage (Leopold et al., 1981), and lipid peroxidation (Heath and Packer, 1968) were estimated at different growth stages. Iron content in grain was determined by colourimetric method (Sandell, 1950; Yoshida et al., 1971). The presence of iron in grains was also visualized using Perl's Prussian Blue staining technique (Velu et al., 2006) with 5% potassium ferrocyanide and 5% hydrochloric acid. Data were analyzed using the ANOVA technique suggested by Panse and Sukhatme (1967). The "F" value was calculated and critical difference between a pair of means was compared to the tabulated value at 5% significance level using appropriate statistical software.

## III. RESULTS AND DISCUSSION

### 3.1 Experimental Conditions:

The experimental environmental conditions were congenial: Temperature ranged from  $17.4$  to  $35.1^{\circ}\text{C}$ , rainfall from 14.8 to 83.6 mm, and bright sunshine hours from 2.4 to 7.2 hours. The soil was acidic with pH ranging from 4.93 to 5.60, and initially deficient in N ( $200.35 \text{ kg ha}^{-1}$ ), P ( $33.08 \text{ kg ha}^{-1}$ ), and K ( $133.03 \text{ kg ha}^{-1}$ ), which was corrected later as suggested by Baruah and Borthakur (1997). The amelioration of physiological aberrations of indigenous upland Ahu rice genotypes caused by the presence of higher available iron (at transplanting: 200 ppm; at harvest: 106 ppm) by application of bay leaf, potato peel, and banana extracts was also reported by Bey et al. (2026).

### 3.2 Chlorophyll Content:

#### 3.2.1 Total Chlorophyll Content:

The results revealed significant variations in total chlorophyll content at maximum tillering stage (Table 1a) among the treatments. Compared to the 100 ppm FeSO<sub>4</sub> (control), total chlorophyll increased by 21.90% with 100 ppm FeSO<sub>4</sub> plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous banana peel (18.61%) > 100 ppm FeSO<sub>4</sub> plus aqueous potato peel (11.13%) > natural soil (10.80%). Overall, total chlorophyll content decreased in the variety Lachit (27.05%) > Inglongkiri (14.95%) > Luit (13.91%) as compared to Dehangi (iron tolerant).

At heading stage (Table 1b), total chlorophyll content varied significantly among treatments. Compared to control, total chlorophyll increased by 21.38% with 100 ppm FeSO<sub>4</sub> plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous banana peel (17.72%) > 100 ppm FeSO<sub>4</sub> plus aqueous potato peel (15.23%) > natural soil (10.96%). Overall, total chlorophyll content decreased in the variety Lachit (23.22%) > Inglongkiri (15.14%) > Luit (12.07%) as compared to Dehangi.

**TABLE 1**  
**EFFECT OF BAY LEAF, POTATO PEEL, AND BANANA PEEL EXTRACTS ALONG WITH Fe TREATMENTS ON TOTAL CHLOROPHYLL CONTENT (mg g<sup>-1</sup> f.w.)**

Variety	Treatments					Mean
	100 ppm FeSO <sub>4</sub> (control)	100 ppm FeSO <sub>4</sub> + aqueous Bay leaf	100 ppm FeSO <sub>4</sub> + aqueous Banana peel	100 ppm FeSO <sub>4</sub> + aqueous Potato peel	Natural soil	
<b>(a) at Maximum tillering stage</b>						
Inglongkiri	1.833	2.433	2.4	2.267	2.067	2.2
Dehangi	2.367	2.767	2.667	2.533	2.6	2.587
Lachit	1.633	2.167	2.033	1.7	1.9	1.887
Luit	1.9	2.533	2.4	2.2	2.1	2.227
<b>Mean</b>	<b>1.933</b>	<b>2.475</b>	<b>2.375</b>	<b>2.175</b>	<b>2.167</b>	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.02	0.06				
Treatment (T)	0.02	0.05				
V × T	0.04	0.11				
<b>(b) at Heading stage</b>						
Inglongkiri	1.5	2	1.933	1.867	1.833	1.827
Dehangi	1.833	2.433	2.3	2.2	2	2.153
Lachit	1.5	1.8	1.7	1.667	1.6	1.653
Luit	1.667	2.033	1.967	1.933	1.867	1.893
<b>Mean</b>	<b>1.625</b>	<b>2.067</b>	<b>1.975</b>	<b>1.917</b>	<b>1.825</b>	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.02	0.06				
Treatment (T)	0.02	0.06				
V × T	0.04	0.11				

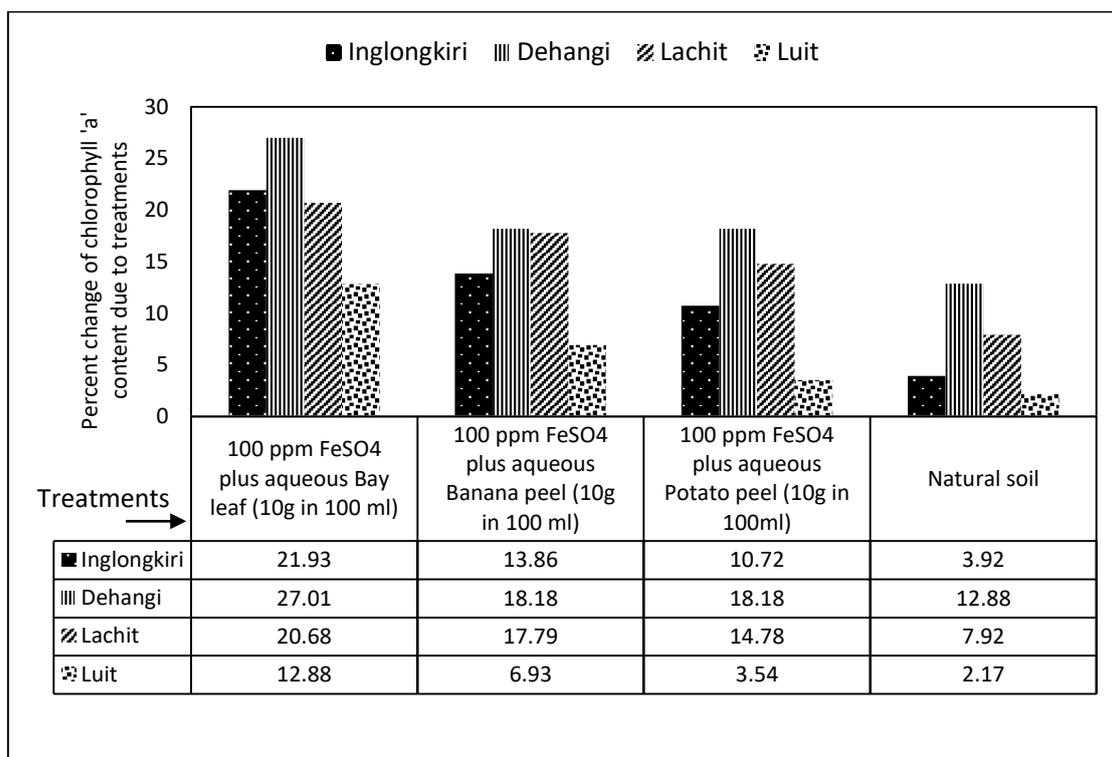
*\*Note: Values are means of three replications. Higher values indicate better chlorophyll retention. S.Ed = Standard Error of Difference; CD = Critical Difference at 5% level.\**

Bay leaf extracts are known to enhance the availability and uptake of essential nutrients like nitrogen and magnesium, which are critical for chlorophyll synthesis. Phenolics and flavonoids in bay leaf extracts mitigate oxidative stress, which often leads to chlorophyll degradation. The bioactive compounds in bay leaf extracts inhibit chlorophyll-degrading enzymes like chlorophyllase. By stabilizing chloroplast structures and reducing damage caused by reactive oxygen species (ROS), bay leaf extract enhanced chlorophyll accumulation. Furthermore, phenolic compounds caused an increase in the activity of photosynthetic pigments (chlorophyll 'a', chlorophyll 'b', total chlorophyll, and carotenoid) in plants (Aina et al., 2022). An improvement of 28% in chlorophyll content was observed by the application of Moringa leaf (Rashid et al., 2021).

#### 3.2.2 Chlorophyll 'a' Content:

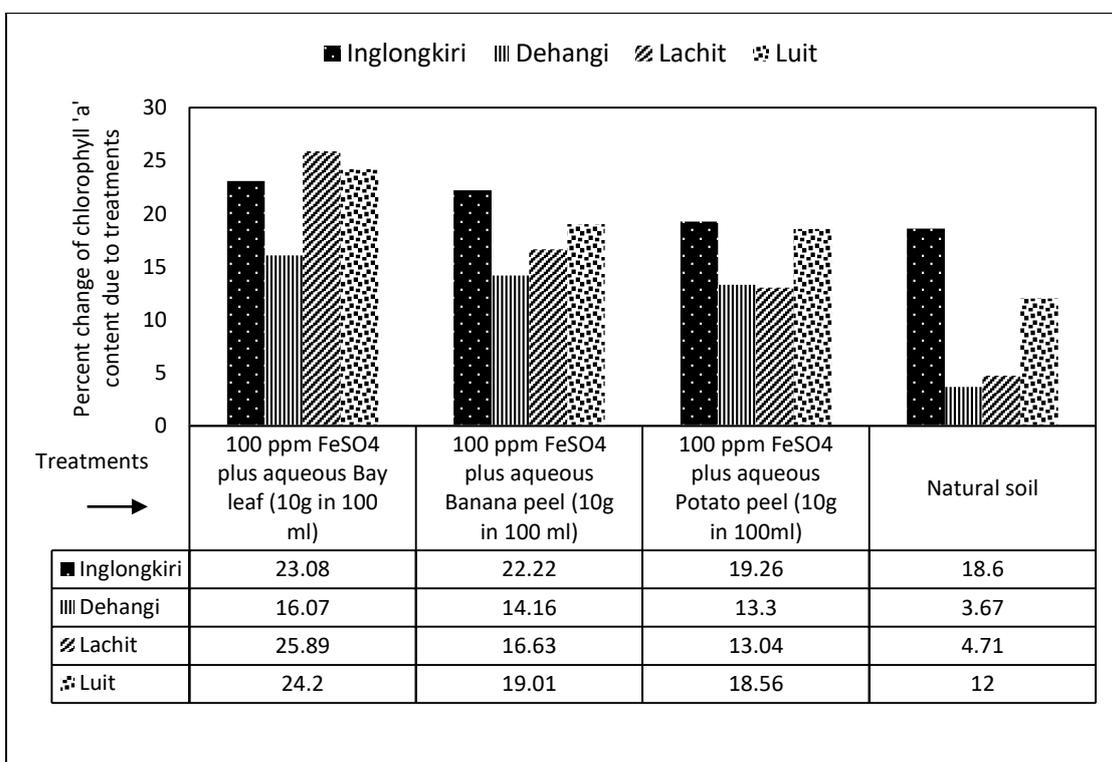
The results revealed significant variations in chlorophyll 'a' content at maximum tillering stage (Fig. 1a) among treatments. Compared to control, chlorophyll 'a' increased by 20.93% with 100 ppm FeSO<sub>4</sub> plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus

aqueous banana peel (14.31%) > 100 ppm FeSO<sub>4</sub> plus aqueous potato peel (12.10%) > natural soil (6.39%). Overall, chlorophyll 'a' decreased in the variety Lachit (17.98%) > Inglongkiri (13.04%) > Luit (11.74%) as compared to Dehangki.



**FIGURE 1(a): Chlorophyll 'a' in comparison with control at Maximum tillering stage**

At heading stage (Fig. 1b), significant variations in chlorophyll 'a' content were observed among treatments. Compared to control, chlorophyll 'a' increased by 21.89% with 100 ppm FeSO<sub>4</sub> plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous banana peel (17.56%) > 100 ppm FeSO<sub>4</sub> plus aqueous potato peel (15.20%) > natural soil (11.88%). Overall, chlorophyll 'a' decreased in the variety Lachit (20.68%) > Inglongkiri (12.40%) > Luit (10.34%) as compared to Dehangki.



**FIGURE 1(b): Chlorophyll 'a' in comparison with control at Heading stage**

### 3.2.3 Chlorophyll 'b' Content:

The results revealed significant variations in chlorophyll 'b' content at maximum tillering stage (Fig. 2a) among treatments. Chlorophyll 'b' content increased by 22.35% with 100 ppm FeSO<sub>4</sub> plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous banana peel (19.46%) > 100 ppm FeSO<sub>4</sub> plus aqueous potato peel (14.54%) > natural soil (7.27%). Overall, chlorophyll 'b' decreased in the variety Lachit (27.20%) > Inglongkiri (14.76%) > Luit (14.01%) as compared to Dehangi.

At heading stage (Fig. 2b), chlorophyll 'b' content increased significantly among treatments. Total chlorophyll content increased by 15.79% with 100 ppm FeSO<sub>4</sub> plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous banana peel (13.46%) > 100 ppm FeSO<sub>4</sub> plus aqueous potato peel (10.49%) > natural soil (7.22%). Overall, chlorophyll 'b' decreased in the variety Lachit (17.36%) > Inglongkiri (11.24%) > Luit (9.71%) as compared to Dehangi.

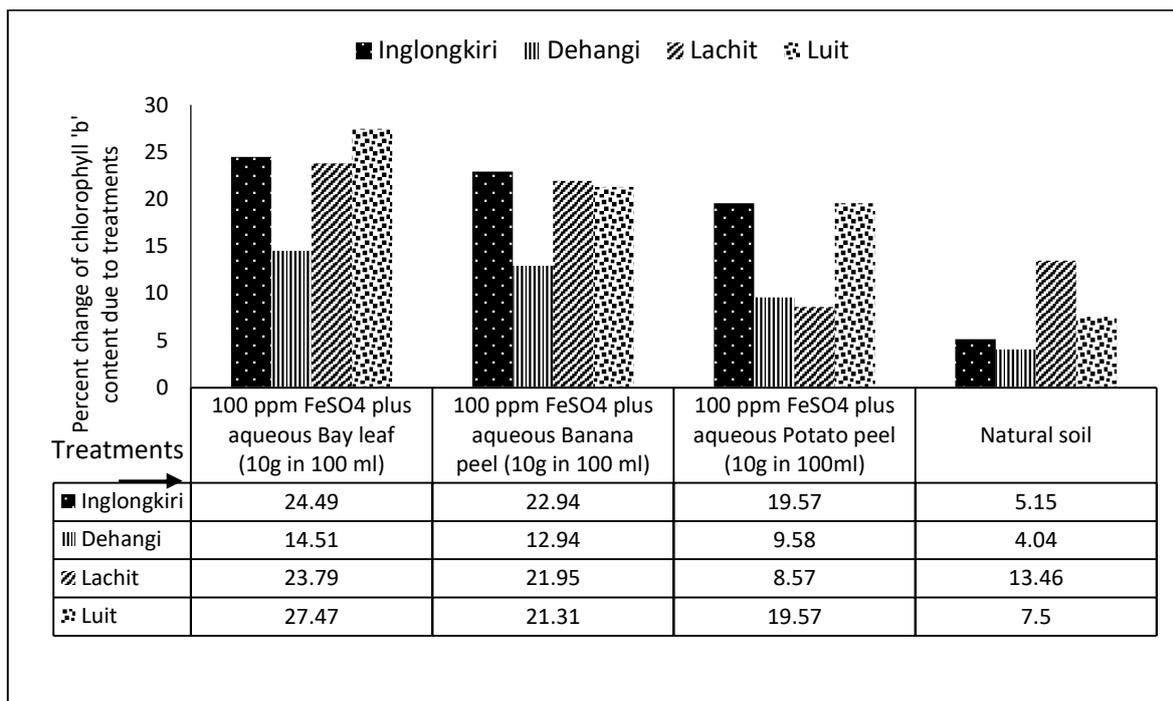


FIGURE 2 (a): Chlorophyll 'b' in comparison with control at Maximum tillering stage

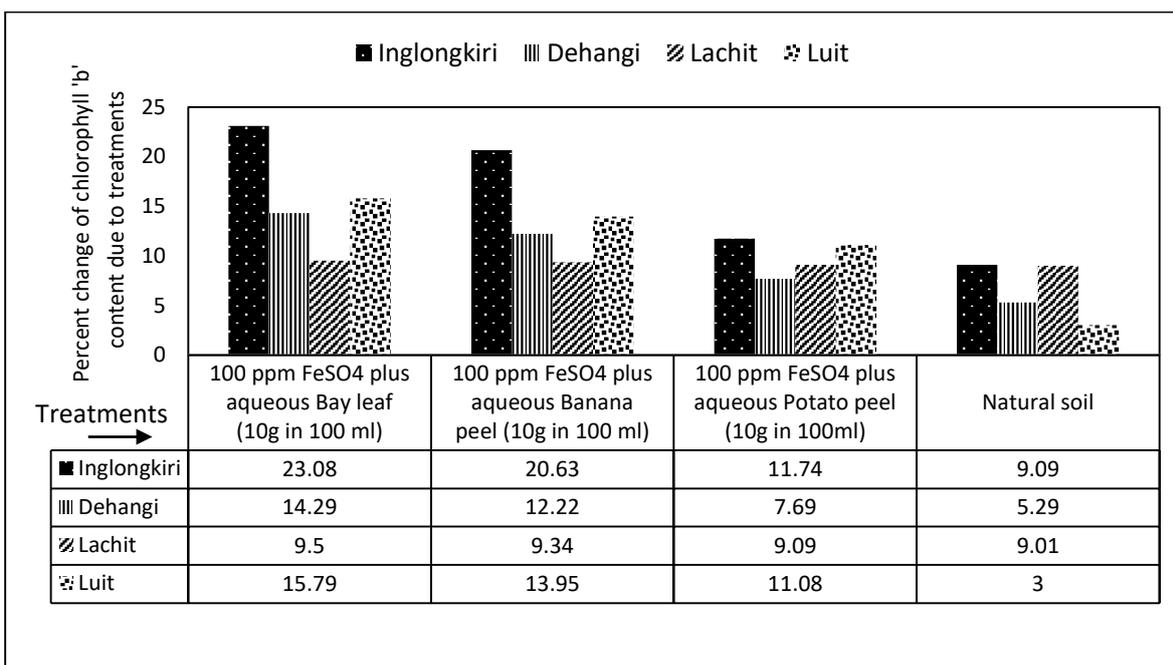


FIGURE 2 (b): Chlorophyll 'b' in comparison with control at Heading stage

### 3.3 Nitrate Reductase (NR) Activity:

The results revealed significant variations in NR activity at maximum tillering stage (Table 2a) among treatments. Compared to control, NR activity increased by 35.52% with 100 ppm FeSO<sub>4</sub> plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous banana peel (30.94%) > 100 ppm FeSO<sub>4</sub> plus aqueous potato peel (24.56%) > natural soil (12.43%). Overall, NR activity decreased in the variety Lachit (32.84%) > Inglongkiri (24.61%) > Luit (17.46%) as compared to Dehangi.

At heading stage (Table 2b), NR activity varied significantly among treatments. NR activity increased by 36.23% with 100 ppm FeSO<sub>4</sub> plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous banana peel (31.06%) > 100 ppm FeSO<sub>4</sub> plus aqueous potato peel (24.44%) > natural soil (15.00%). Overall, NR activity decreased in the variety Lachit (33.50%) > Inglongkiri (25.20%) > Luit (18.42%) as compared to Dehangi.

**TABLE 2**  
**EFFECT OF BAY LEAF, POTATO PEEL, AND BANANA PEEL EXTRACTS ALONG WITH Fe TREATMENTS ON NITRATE REDUCTASE (NR) ACTIVITY ( $\mu\text{mole NO}_3^- \text{g}^{-1} \text{f.w. hr}^{-1}$ )**

Variety	Treatments					
	100 ppm FeSO <sub>4</sub> (control)	100 ppm FeSO <sub>4</sub> + aqueous Bay leaf	100 ppm FeSO <sub>4</sub> + aqueous Banana peel	100 ppm FeSO <sub>4</sub> + aqueous Potato peel	Natural soil	Mean
<b>(a) at Maximum tillering stage</b>						
Inglongkiri	0.767	1.2	1.1	1	0.833	0.98
Dehangi	1	1.5	1.467	1.4	1.2	1.3
Lachit	0.7	1	0.967	0.9	0.8	0.873
Luit	0.8	1.367	1.267	1.033	0.9	1.073
<b>Mean</b>	<b>0.817</b>	<b>1.267</b>	<b>1.183</b>	<b>1.083</b>	<b>0.933</b>	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.01	0.02				
Treatment (T)	0.01	0.02				
V × T	0.02	0.04				
<b>(b) at Heading stage</b>						
Inglongkiri	0.8	1.267	1.167	1	0.9	1.027
Dehangi	1.067	1.6	1.433	1.467	1.3	1.373
Lachit	0.7	1.067	1	0.933	0.867	0.913
Luit	0.833	1.4	1.333	1.1	0.933	1.12
<b>Mean</b>	<b>0.85</b>	<b>1.333</b>	<b>1.233</b>	<b>1.125</b>	<b>1</b>	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.01	0.03				
Treatment (T)	0.01	0.02				
V × T	0.02	0.05				

*\*Note: Values are means of three replications. Higher values indicate better nitrogen assimilation. S.Ed = Standard Error of Difference; CD = Critical Difference at 5% level.\**

Bay leaf enhanced NR activity and nitrogen metabolism in crops. Phenolic compounds in bay leaf extracts may serve as natural activators of NR by stabilizing its structure and increasing enzymatic activity. These compounds can also reduce oxidative stress, preventing NR degradation under unfavourable conditions. Bay leaf extracts enhance root function and nutrient uptake, ensuring a higher nitrate supply for conversion by NR. Improved nitrogen metabolism leads to better protein synthesis, promoting growth and biomass accumulation. NR is sensitive to oxidative damage; antioxidants in bay leaf extracts protect NR from ROS, sustaining its activity under environmental stress (Sarkar et al., 2010; Rice-Evans et al., 1997). Bay leaf is rich in potassium, which plays a crucial role in NR activity, an essential enzyme in nitrogen metabolism. Application of foliar

mineral KNO<sub>3</sub> significantly increased nitrate content as well as NR activity in sunflower and safflower plants irrespective of their growth under non-saline or saline conditions (Jabeen and Ahmad, 2011).

### 3.4 Cation Leakage:

The results revealed significant variations in cation leakage at maximum tillering stage (Table 3a) among treatments. Compared to control, cation leakage decreased by 1.93% with 100 ppm FeSO<sub>4</sub> plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous banana peel (1.78%) > 100 ppm FeSO<sub>4</sub> plus aqueous potato peel (1.63%) > natural soil (2.25%). Overall, cation leakage increased in the variety Lachit (0.93%) > Luit (0.53%) > Inglongkiri (0.50%) as compared to Dehangi.

At heading stage (Table 3b), significant variations in cation leakage were observed among treatments. Cation leakage decreased by 3.27% with 100 ppm FeSO<sub>4</sub> plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous banana peel (3.13%) > 100 ppm FeSO<sub>4</sub> plus aqueous potato peel (2.83%) > natural soil (3.52%). Overall, cation leakage increased in the variety Lachit (1.11%) > Luit (0.48%) > Inglongkiri (0.41%) as compared to Dehangi.

**TABLE 3**  
**EFFECT OF BAY LEAF, POTATO PEEL, AND BANANA PEEL EXTRACTS ALONG WITH Fe TREATMENTS ON CATION LEAKAGE (%)**

Variety	Treatments					Mean
	100 ppm FeSO <sub>4</sub> (control)	100 ppm FeSO <sub>4</sub> + aqueous Bay leaf	100 ppm FeSO <sub>4</sub> + aqueous Banana peel	100 ppm FeSO <sub>4</sub> + aqueous Potato peel	Natural soil	
<b>(a) at Maximum tillering stage</b>						
Inglongkiri	95.167	93.633	94.433	95	93.367	94.32
Dehangi	99.733	89.167	91	93.367	88.267	92.307
Lachit	98.1	94.3	95.8	96.567	93.7	95.693
Luit	96.833	94.1	94.867	94.9	91.467	94.433
<b>Mean</b>	<b>97.458</b>	<b>92.8</b>	<b>94.025</b>	<b>94.958</b>	<b>91.7</b>	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.61	1.79				
Treatment (T)	0.54	1.55				
V × T	1.22	3.1				
<b>(b) at Heading stage</b>						
Inglongkiri	92.067	89.467	90.367	90.4	89.033	90.267
Dehangi	89.467	88.233	89.033	89.1	88.167	88.8
Lachit	98.567	90.5	90.567	93.467	89.467	92.513
Luit	91.077	89.767	89.8	91.067	89.233	90.187
<b>Mean</b>	<b>92.792</b>	<b>89.492</b>	<b>89.942</b>	<b>91.008</b>	<b>88.975</b>	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.5	1.48				
Treatment (T)	0.45	1.28				
V × T	1	2.56				

*\*Note: Values are means of three replications. Lower values indicate better membrane integrity. S.Ed = Standard Error of Difference; CD = Critical Difference at 5% level.\**

The phenolic and flavonoid compounds in bay leaf extracts strengthen cell membranes by reducing lipid peroxidation and maintaining membrane integrity, thereby preventing excessive cation loss. Reactive oxygen species cause oxidative damage to membrane lipids, leading to increased cation leakage. The antioxidant properties of bay leaf extracts neutralize ROS, protecting cell membranes from oxidative stress. Bay leaf bioactive compounds may enhance the regulation of ion transporters such as H<sup>+</sup>-ATPases and Na<sup>+</sup>/K<sup>+</sup> transporters, maintaining ionic homeostasis and preventing excessive cation efflux. Lipid peroxidation, a major cause of membrane damage, is mitigated by the bioactive compounds in bay leaf extracts, leading to

lower levels of malondialdehyde (MDA), a key indicator of membrane deterioration. Bay leaves are a good natural source of potassium. Under drought conditions, excess ROS production in plants may exaggerate cellular lipid peroxidation, leading to an increase in cellular membrane permeability, evidenced by increases in electrolyte leakage and MDA content (Fazeli et al., 2007; Degenkolbe et al., 2009). Soleimanzadeh et al. (2010) reported that an adequate supply of K significantly decreased MDA content under water shortage condition, clearly indicating the role of K in mitigating oxidative stress. The use of K against cadmium (Cd) toxicity confirmed the positive effect of this element by ameliorating Cd-induced oxidative damage in broad bean (Siddiqui et al., 2012).

### 3.5 Cell Membrane Stability (CMS):

At maximum tillering stage (Table 4a), there were significant variations in CMS among treatments. Compared to control, CMS increased by 52.02% with 100 ppm FeSO<sub>4</sub> plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous banana peel (44.76%) > 100 ppm FeSO<sub>4</sub> plus aqueous potato peel (31.40%) > natural soil (26.14%). Overall, CMS decreased in the variety Lachit (47.87%) > Inglongkiri (30.52%) > Luit (27.45%) as compared to Dehangi.

At heading stage (Table 4b), CMS varied significantly among treatments. Compared to control, CMS increased by 37.22% with 100 ppm FeSO<sub>4</sub> plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous banana peel (30.68%) > 100 ppm FeSO<sub>4</sub> plus aqueous potato peel (20.02%) > natural soil (16.22%). Overall, CMS decreased in the variety Lachit (34.30%) > Inglongkiri (21.86%) > Luit (19.66%) as compared to Dehangi.

**TABLE 4**

**EFFECT OF BAY LEAF, POTATO PEEL, AND BANANA PEEL EXTRACTS ALONG WITH Fe TREATMENTS ON CELL MEMBRANE STABILITY (CMS)**

Variety	Treatments					Mean
	100 ppm FeSO <sub>4</sub> (control)	100 ppm FeSO <sub>4</sub> + aqueous Bay leaf	100 ppm FeSO <sub>4</sub> + aqueous Banana peel	100 ppm FeSO <sub>4</sub> + aqueous Potato peel	Natural soil	
<b>(a) at Maximum tillering stage</b>						
Inglongkiri	3.06	4.12	4.02	3.22	3.127	3.509
Dehangi	3.297	6.63	5.81	4.853	4.667	5.051
Lachit	1.123	3.95	3.22	2.75	2.12	2.633
Luit	2.16	5.39	4.4	3.23	3.14	3.664
<b>Mean</b>	<b>2.41</b>	<b>5.023</b>	<b>4.363</b>	<b>3.513</b>	<b>3.263</b>	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.038	0.077				
Treatment (T)	0.034	0.068				
V × T	0.075	0.153				
<b>(b) at Heading stage</b>						
Inglongkiri	5.057	6.123	6.02	5.223	5.127	5.51
Dehangi	5.3	8.63	7.807	6.853	6.67	7.052
Lachit	3.123	5.953	5.22	4.75	4.12	4.633
Luit	4.16	7.393	6.4	5.23	5.14	5.665
<b>Mean</b>	<b>4.41</b>	<b>7.025</b>	<b>6.362</b>	<b>5.514</b>	<b>5.264</b>	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.071	0.144				
Treatment (T)	0.063	0.129				
V × T	0.142	0.288				

*\*Note: Values are means of three replications. Higher values indicate better membrane stability. S.Ed = Standard Error of Difference; CD = Critical Difference at 5% level.\**

In the study, CMS was decreased by iron and ameliorated by the bio-input extracts, especially bay leaf having higher potassium. The fall in CMS is linked to increase in lipid peroxidation regulated by ROS generation (Bharali and Bates, 2004; Bharali et al., 2015; Bharali et al., 2016) under higher iron in acid soil condition. Free iron directly or indirectly as ferritin of ferredoxin catalyzes the formation of the highly damaging hydroxyl radical (OH<sup>-</sup>) from superoxide or its dismuted product H<sub>2</sub>O<sub>2</sub>. The OH<sup>-</sup> becomes the most likely form of activated oxygen initiating peroxidative breakdown of lipids, which in turn results in disruption of the plasma membrane, reduces CMS, and increases membrane leakage (Price and Hendry, 1991).

### 3.6 Lipid Peroxidation:

The results revealed significant variations in lipid peroxidation at maximum tillering stage (Table 5a) among treatments. Compared to control, lipid peroxidation decreased by 17.49% with 100 ppm FeSO<sub>4</sub> plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous banana peel (14.29%) > 100 ppm FeSO<sub>4</sub> plus aqueous potato peel (10.55%) > natural soil (30.03%). Overall, lipid peroxidation increased in the variety Lachit (13.76%) > Inglongkiri (5.77%) > Luit (3.72%) as compared to Dehangi.

At heading stage (Table 5b), significant variations in lipid peroxidation were observed among treatments. Lipid peroxidation decreased by 17.78% with 100 ppm FeSO<sub>4</sub> plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous banana peel (13.28%) > 100 ppm FeSO<sub>4</sub> plus aqueous potato peel (10.96%) > natural soil (29.64%). Overall, lipid peroxidation increased in the variety Lachit (14%) > Inglongkiri (5.67%) > Luit (3.70%) as compared to Dehangi.

TABLE 5

EFFECT OF BAY LEAF, POTATO PEEL, AND BANANA PEEL EXTRACTS ALONG WITH Fe TREATMENTS ON LIPID PEROXIDATION (μmol MDA g<sup>-1</sup> fresh weight)

Variety	Treatments					Mean
	100 ppm FeSO <sub>4</sub> (control)	100 ppm FeSO <sub>4</sub> + aqueous Bay leaf	100 ppm FeSO <sub>4</sub> + aqueous Banana peel	100 ppm FeSO <sub>4</sub> + aqueous Potato peel	Natural soil	
<b>(a) at Maximum tillering stage</b>						
Inglongkiri	6.233	5.067	5.267	5.6	4.433	5.32
Dehangi	5.8	4.9	5.067	5.167	4.133	5.013
Lachit	6.8	5.6	5.9	6.133	4.633	5.813
Luit	6.133	5.033	5.167	5.433	4.267	5.207
<b>Mean</b>	<b>6.242</b>	<b>5.15</b>	<b>5.35</b>	<b>5.583</b>	<b>4.367</b>	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.04	0.1				
Treatment (T)	0.03	0.09				
V × T	0.07	0.18				
<b>(b) at Heading stage</b>						
Inglongkiri	6.467	5.233	5.5	5.733	4.633	5.513
Dehangi	6.033	5.067	5.267	5.333	4.3	5.2
Lachit	7	5.867	6.267	6.3	4.8	6.047
Luit	6.367	5.1	5.4	5.667	4.467	5.4
<b>Mean</b>	<b>6.467</b>	<b>5.317</b>	<b>5.608</b>	<b>5.758</b>	<b>4.55</b>	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.04	0.11				
Treatment (T)	0.03	0.09				
V × T	0.07	0.19				

\*Note: Values are means of three replications. Lower values indicate less oxidative damage. MDA = malondialdehyde.

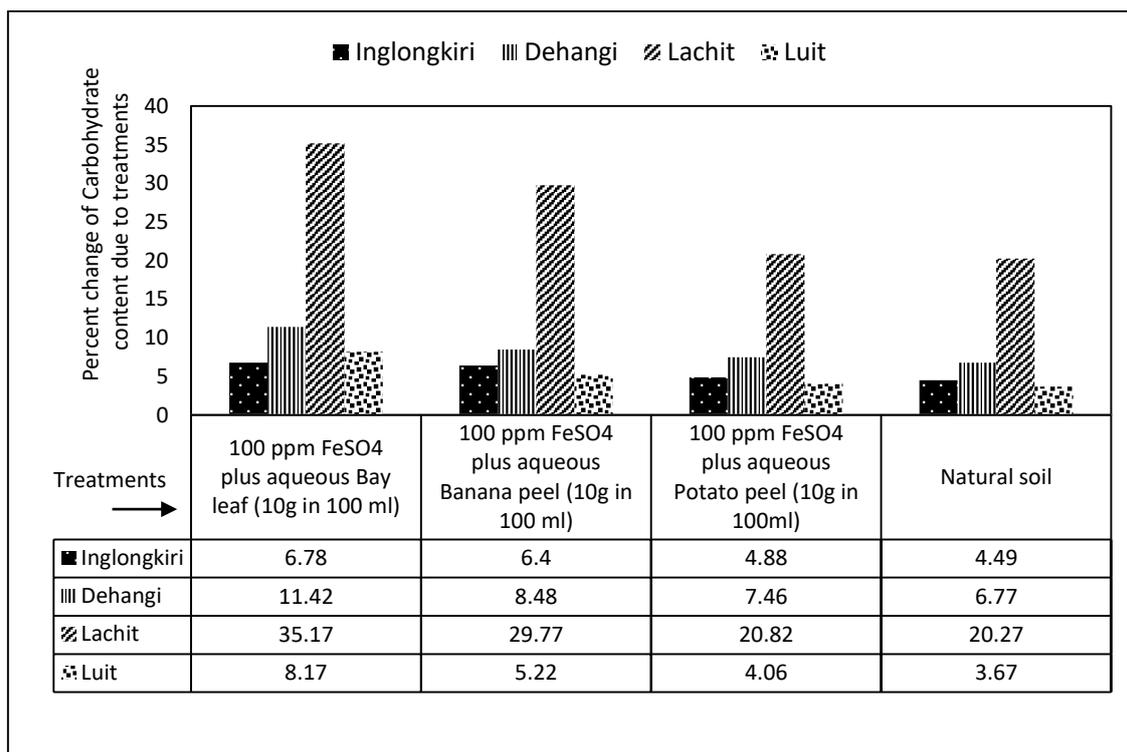
S.Ed = Standard Error of Difference; CD = Critical Difference at 5% level.\*

Bay leaf extracts contain high potassium levels and potent antioxidants such as quercetin, kaempferol, and eugenol, which scavenge ROS, preventing oxidative damage to membrane lipids. Malondialdehyde (MDA) is a byproduct of lipid peroxidation

and a key indicator of membrane damage. Studies suggest that plant-derived phenolics, like those in bay leaf extracts, significantly reduce MDA accumulation, leading to improved cell stability. Song et al. (2015) experimented with peach plants using elevated exogenous K (10 mM) against Zn toxicity (2 mM). They observed that Zn damages the plant by altering physiological processes and nutritional balance. However, K mitigated Zn toxicity by improving photosynthesis, antioxidant defence systems, and plant K nutritional status.

### 3.7 Carbohydrate Content in Grain:

The results revealed significant variations in carbohydrate content in grain at harvesting stage (Fig. 3) among treatments. Compared to control, carbohydrate content increased by 13.81% with 100 ppm FeSO<sub>4</sub> plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous banana peel (10.87%) > 100 ppm FeSO<sub>4</sub> plus aqueous potato peel (8.03%) > natural soil (7.51%). Overall, carbohydrate content decreased in the variety Lachit (42.61%) > Inglongkiri (8.03%) > Luit (7.50%) as compared to Dehangi.



**FIGURE 3: Carbohydrate content in grain compared with control at harvest**

The current studies on phenolic-rich plant extracts (bay leaf) might help in enhancing carbohydrate metabolism and grain quality. Bay leaf extracts improve chlorophyll stability, leading to increased photosynthetic activity. Higher photosynthesis rates result in greater production of photo-assimilates like sucrose, which are translocated to developing grains. Bioactive compounds in bay leaf extracts may upregulate the activity of starch-synthesizing enzymes, such as ADP-glucose pyrophosphorylase and starch synthase, leading to higher starch accumulation in grains. Bay leaf extracts may enhance phloem loading and sucrose transport from leaves to grains, ensuring a steady supply of carbohydrates for grain filling. The antioxidant properties of bay leaf extracts help mitigate stress effects, maintaining efficient carbohydrate metabolism. Improved nitrogen and potassium uptake facilitated by bay leaf extracts supports energy metabolism, promoting carbohydrate biosynthesis and storage in grains. Flavonoids constitute a wide range of substances that play an important role in protecting biological systems against the harmful effects of oxidative processes on macromolecules, such as carbohydrates, proteins, and lipids (Halliwell and Gutteridge, 1989). Potassium takes part in protein synthesis, carbohydrate metabolism, and enzyme activation (Wang et al., 2013).

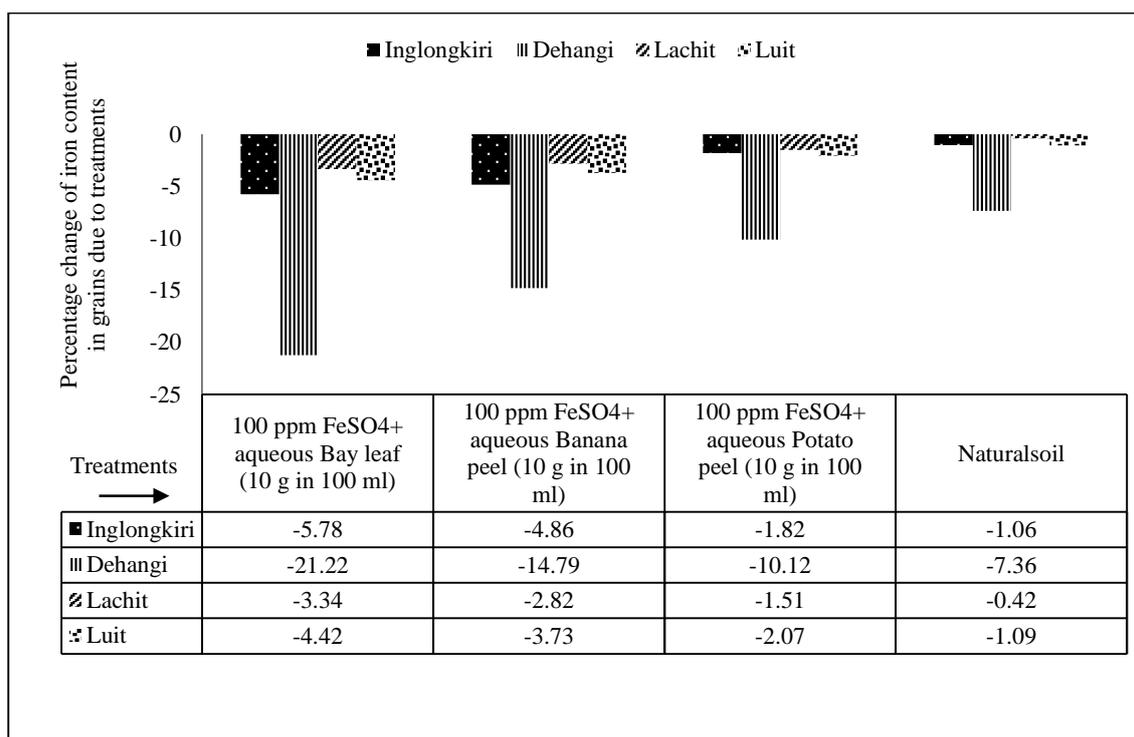
### 3.8 Iron Content in Grain:

The results revealed significant variations in iron content in grain among treatments (Table 6). Compared to control, iron content in grain decreased by 7.33% with 100 ppm FeSO<sub>4</sub> plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous banana peel (5.77%) > 100 ppm FeSO<sub>4</sub> plus aqueous potato peel (3.49%) > natural soil (2.23%). Overall, iron content in grains increased in the variety Lachit (21.34%) > Luit (15.57%) > Inglongkiri (10.57%) as compared to Dehangi (Fig. 4).

**TABLE 6**  
**EFFECT OF BAY LEAF, POTATO PEEL, AND BANANA PEEL EXTRACTS ALONG WITH FE TREATMENTS ON IRON CONTENT IN GRAINS (ppm)**

Variety	100 ppm FeSO <sub>4</sub> (control)	100 ppm FeSO <sub>4</sub> + aqueous Bay leaf	100 ppm FeSO <sub>4</sub> + aqueous Banana peel	100 ppm FeSO <sub>4</sub> + aqueous Potato peel	Natural soil	Mean
Inglongkiri	120	113.44	114.43	117.85	118.74	116.89
Dehangi	115.24	95.06	100.39	104.65	107.34	104.53
Lachit	135.01	130.65	131.3	133	134.45	132.88
Luit	126.57	121.21	122.01	124	125.21	123.8
<b>Mean</b>	<b>124.2</b>	<b>115.09</b>	<b>117.03</b>	<b>119.87</b>	<b>121.43</b>	
	S.Ed(±)	CD(0.05)				
Variety (V)	2.16	4.42				
Treatment (T)	2.49	5.1				
V × T	4.31	8.83				

*\*Note: Values are means of three replications. Lower values indicate reduced iron accumulation in grains. S.Ed = Standard Error of Difference; CD = Critical Difference at 5% level. CD values have been corrected using the formula  $CD = S.Ed \times t\text{-value (at error df)}$ .\**



**FIGURE 4: Iron content in grain as compared to the control at harvest**

### 3.9 Histochemical Localization of Iron in Grain:

There were variations in intensity of colour among the genotypes under the treatments depending upon the iron contents in grain. Higher iron content in grain resulted in greater intensity of blue colour (Table 7a). Genotypes with relatively high iron developed medium to dark blue colour intensity, and those with low iron level developed light blue or no colour. Thus, the genotypes could be sorted for iron content in grain with varying scores (Table 7b). Genotypes with more than 8 ppm iron received a score of '4', those with 4-6 ppm received a score of '3', and those with less than 4 ppm iron received a score of '2' or '1'. Using this technique, genotypes were categorized as having very low (score 1), low (score 2), medium (score 3), and high (score 4) iron contents (Purusothaman, 2010).

**TABLE 7(a)**

**CATEGORY OF GENOTYPES UNDER THE TREATMENTS BASED ON THE IRON CONTENT IN RICE GRAIN AT HARVEST AS DETERMINED BY PERL'S PRUSSIAN BLUE STAINING METHOD (Velu et al., 2006)**

Treatment	Variety	Fe Score	Colour Intensity	Fe Category
100 ppm FeSO <sub>4</sub> (Control)	Inglongkiri	4	Dark blue	High
	Dehangi	4	Dark blue	High
	Lachit	4	Dark blue	High
	Luit	4	Dark blue	High
100 ppm FeSO <sub>4</sub> + Aqueous Bay Leaf (10 g/100 ml)	Inglongkiri	3	Medium blue	Medium
	Dehangi	2	Light blue	Low
	Lachit	3	Medium blue	Medium
	Luit	2	Light blue	Low
100 ppm FeSO <sub>4</sub> + Aqueous Banana Peel (10 g/100 ml)	Inglongkiri	3	Medium blue	Medium
	Dehangi	2	Light blue	Low
	Lachit	3	Medium blue	Medium
	Luit	2	Light blue	Low
100 ppm FeSO <sub>4</sub> + Aqueous Potato Peel (10 g/100 ml)	Inglongkiri	3	Medium blue	Medium
	Dehangi	3	Medium blue	Medium
	Lachit	3	Medium blue	Medium
	Luit	3	Medium blue	Medium
Natural Soil Condition	Inglongkiri	1	No colour	Very Low
	Dehangi	1	No colour	Very Low
	Lachit	1	No colour	Very Low
	Luit	1	No colour	Very Low

**TABLE 7 (b)**

**INTERPRETATION USED FOR SORTING OUT THE GENOTYPES FOR IRON CONTENT IN GRAIN (RELATIVE SCALE FOR EXPERIMENTAL CONDITIONS)**

Score	Fe Content (ppm)	Category	Colour Intensity
4	> 120	High	Dark blue
3	100–120	Medium	Medium blue
2	80–100	Low	Light blue
1	< 80	Very Low	No colour

*\*Note: Scoring thresholds adjusted to match actual experimental values from Table 6 (range: 95-135 ppm). This relative scale allows for meaningful comparison within the experimental context. Staining intensity based on Perl's Prussian Blue reaction with 5% potassium ferrocyanide and 5% HCl (Velu et al., 2006).\**

In the treatment with 100 ppm FeSO<sub>4</sub> (control), all varieties (Inglongkiri, Dehangi, Lachit, and Luit) scored 4 with dark blue colour. In the treatment with 100 ppm FeSO<sub>4</sub> plus aqueous bay leaf, varieties Inglongkiri and Lachit scored 3 with medium blue colour, while Dehangi and Luit scored 2 with light colour. Under the treatment with 100 ppm FeSO<sub>4</sub> plus aqueous banana peel, Inglongkiri and Lachit scored 3 with medium blue colour, while Dehangi and Luit scored 2 with light colour. In the treatment with 100 ppm FeSO<sub>4</sub> plus aqueous potato peel, all varieties (Inglongkiri, Dehangi, Lachit, and Luit) scored 3 with medium blue colour. Under natural soil condition, all varieties scored 1 and exhibited no colour change. Shobhana et al. (2013) stated that the different blue colour intensities developed during the treatment provide a steadfast selection criterion for grain micronutrient (iron) contents in cereal crops. This technique could, therefore, be cost-effectively used to categorize genotypes for higher grain iron content quickly.

#### IV. CONCLUSION

Among bay leaf, banana peel, and potato extracts, bay leaf was found to be most suitable for enhancing key biochemical parameters viz., chlorophyll contents, NR activity, CMS at different growth stages, and carbohydrate content in grain. Moreover, lipid peroxidation, cation leakage, and CMS were lowered by bay leaf, particularly in the variety Dehangi, due to

iron-induced ROS activity in the plant. This was evidenced by the iron content in grain and its correspondence with the varying intensity of grain colour determined in the experiment.

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#### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in publishing the manuscript in the journal regarding the publication of this article.

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