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## Augmentation of *Trigonella foenum-graecum* L. (methi) Growth under Salinity Stress and Allelochemical stress Through Mn+B+Zn Mixture Foliar Spray

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### ABSTRACT

Salinity and allelopathy are major environmental factors that lead to deterioration of agricultural land and a reduction in crop productivity worldwide. The given investigations were undertaken to assess whether exogenous application of Mn+B+Zn mixture through foliar spray ameliorate the adverse effects of salinity and Basil leaf litter stress on growth and biochemical activities of methi plants. In this concern, three concentrations of NaCl (i.e. 0.2%, 0.4% and 0.6%) along with 5% BL were applied in soil medium and the suspension of Mn+B+Zn in ratios (1:1:1) in concentrations of 2.5ppm and 5ppm were sprayed once a week. Foliar applications of micronutrient mixture were found to improve all the studied growth parameters and biochemical activities of Methi plants irrespective to their growth under non-saline or saline conditions. The results of this study suggest that foliar spray with micronutrient may have a potential role for increasing methi tolerance to salinity stress. The application of micronutrient spray appeared to ameliorate the toxic effects of excessive Na<sup>+</sup> and allelochemical present in the growing medium which is reflected by producing economically feasible yields even beyond the threshold value of salinity for respective crop.

**Keywords:** Allelopathy; Exogenous application; Foliar spray; Mn+B+Zn.

### 1. Introduction

Agricultural productivity is severely affected by soil salinity and the damaging effect of salt accumulation in agricultural soils has become an important environmental concern<sup>[1]</sup>. Similarly, allelopathy has also a direct or indirect deleterious effect of one plant upon another through the release of chemical inhibitor (phytotoxin), it may be the main reason of failure or poor crop growth in the different environmental systems. These are the major biotic stresses that affect almost every aspect of physiology and biochemistry of a plant, resulting in a reduction in its yield<sup>[2, 3]</sup>. Salt stress limits plant growth by adversely affecting various physiological and biochemical processes<sup>[4]</sup>. The availability of most micronutrients depends on the pH of the soil solution and the nature of binding sites on organic and inorganic particle surfaces. In saline soil, the solubility of micronutrients (i.e. *Cu*, *Fe*, *Mn*, *Mo*, *Zn*) is particularly low, plants grown in these soils often experience deficiencies in these elements<sup>[4]</sup>. Salinity-induced Manganese (*Mn*) deficiency has been observed in barley (*Hordeum vulgare* L.) shoots and *Mn* additions to solution cultures increased barley's salt-tolerance<sup>[5]</sup>. Similarly, Zinc (*Zn*) concentration in shoot tissue has been found to decrease with increasing sodicity<sup>[6]</sup>. All the problems created by the presence of extra Na<sup>+</sup> and Cl<sup>-</sup> ions in the rhizosphere can be avoided by providing some sodium antagonistic minerals through foliar spray that function to minimize osmotic stress or ion disequilibrium or alleviate the consequent secondary effects caused by the salt stress<sup>[7]</sup>. Foliar application of minerals like *Fe*, *B*, *Mn* and *Cu* may be more practical than application to soil where they adsorbed to the soil particles and less available to the rooting medium<sup>[8]</sup>. Plants require specific amount of certain nutrients in specific form at appropriate time, for their growth and development<sup>[9]</sup>. It is well documented that the deficiency of micronutrients (*Zn*, *Mn*, *Fe* and *Cu*) in soils of arid and semi arid regions forms one of the major yield limiting factors and can greatly disturb plant yield and quality. Foliar feeding technique, as a particular way to supply these nutrients could avoid these factors and results in rapid absorption. Foliar feeding of micronutrients generally is more effective and less costly<sup>[7]</sup>. It is well known that soil application of NPK fertilizers may lead to some losses of these fertilizers. However, application of such micronutrients as foliar spray may decrease such losses<sup>[10]</sup>. This

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technique can also improve nutrient utilization and lower environmental pollution through reducing the amount of fertilizers added to soil <sup>[11]</sup>. Foliar feeding of a nutrient promote root absorption of the same nutrient <sup>[12]</sup> or other nutrients through improving root growth and increasing nutrient uptake <sup>[7]</sup>. Researches proved that, the foliar application of nutrients partially can overcome the negative effect of many stress conditions. <sup>[7]</sup>. They stated that foliar feeding of macro and micronutrient helps whenever; uptake of certain essential mineral through the root is restricted due to salt stress. Therefore, the aim of the present study was to assess as to whether foliar applications of micronutrients were effective in inducing salt tolerance in *Methi* plants.

## 2a. Material and method

The experiment was carried out in the department of botany, Jinnah, university for women, Nazimabad, Karachi, Pakistan under controlled greenhouse condition. Ten chemically sterilized seeds of *Methi* (*Trigonella foenum-graecum* L.) were sown in pots filled with clay loam soil. Four setups were designed such that the three groups of plants suffered various degrees of salinity i.e. 0%, 0.2%, 0.4% and 0.6% NaCl with 5% Basil leaf litter along with one set of control level, a complementary treatment of Mn+B+Zn mixture @ 2.5 ppm and 5 ppm was also applied as a foliar application on plants for the augmentation of tolerance against salinity and allelopathy. The foliar spray was imposed using water soluble Boric acid ( $H_3BO_3$ ), Zinc sulphate ( $ZnSO_4 \cdot 7H_2O$ ) and Manganese chloride ( $MnCl_2$ ). Foliar spray was applied once weekly. Set up was arranged in a randomized complete block design. Growth was recorded at harvest after a month in terms of shoot and root length and fresh and dry weight of treated plants. Soluble carbohydrates and total proteins were determined by the method of E. W. Yemm and A. J. Willis, 1956 <sup>[13]</sup> and Lawry's, 1951 <sup>[14]</sup> respectively and expressed in  $\mu$ mole/g fresh weight. Stress regulators i.e. glycine Britainwas determined Grieve and Gratan, 1983 <sup>[15]</sup> method.

## 2b. Inhibitory Percentage (I)

The percentage of inhibitory effect on shoot and root growth (length, fresh and dry weight) and biochemical contents in comparison to control was calculated by Surendra and Pota, 1978 (16)

$$\text{Formula. } I = 100 - T/C \times 100$$

Where, I is the percentage of inhibition, T is treatment reading and C is control plant reading.

## 2c. Statistical Analysis

The data were subjected to statistical analysis by the software program of SPSS version 17. Each result was the mean of at least three replicated treatments. Data are displayed as mean  $\pm$  standard deviation for three replications.

## 3. Result and Discussion

### 3a. Shoot Length (cm)

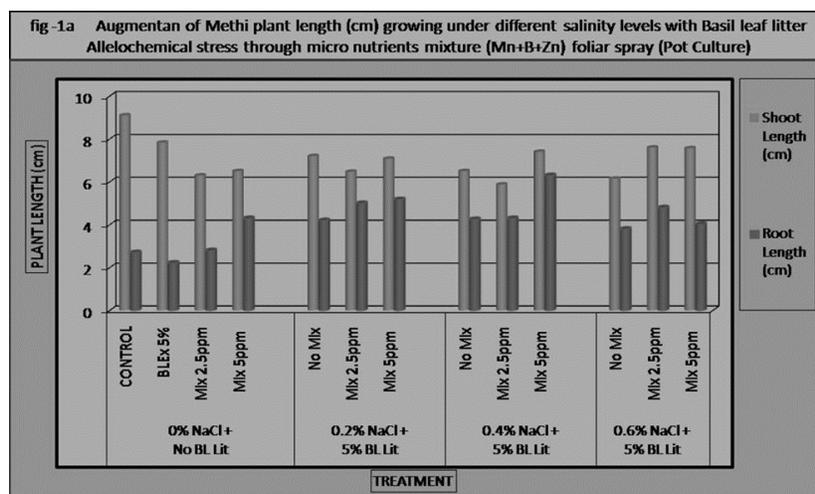
Data presented in table-1 indicated that a gradual decrease was observed in shoot length with elevated salinity in the presence of 5% BL (Fig.1a). With increasing concentration of NaCl from 0.2%-0.6%, shoot length reduced 20.87% to 32.41% respectively. These findings agree with those obtained by Morant *et al.*, 2004 <sup>[21]</sup>. Such depressive effect of salinity in *methi* growth may be attributed to the adverse effect on enzymatic processes through some interactions of salts and some organic substances of the cell <sup>[16]</sup>. Crop reduction due to salinity is generally related to the osmotic potential increase of the root zone soil solution which leads to certain physiological changes and substantial reduction in productivity <sup>[18]</sup>. The results also shown that, shoot length of *methi* increased with micronutrient i.e. Mn+B+Zn spray in both concentrations (i.e. 2.5 ppm and 5 ppm) at all salinity levels. The increment of shoot length of *methi* was found as a decrease in the reduction in inhibitory percentage appeared with stress condition. The high inhibitory percentage 32.41% reduced to 16.48% with the application of foliar Micronutrient spray @ 2.5 ppm.

TREATMENT		Shoot Length (cm)	Root Length (cm)	Plant Fr. Wt. (gm)	Plant Dry Wt. (gm)
0% NaCl	CONTROL	9.1 $\pm$ 1.193 (0)	2.706 $\pm$ 0.873 (0)	0.113 $\pm$ 0.012 (0)	0.015 $\pm$ 0.001 (0)
	Basil Leaf Extract (5%)	7.83 $\pm$ 1.159 (-13.9)	2.22 $\pm$ 0.255 (-17.9)	0.145 $\pm$ 0.087 (+24.06)	0.0155 $\pm$ 0.009 (+3.3)
	Mn+B+Zn (2.5ppm)	6.3 $\pm$ 0.411 (-30.7)	2.80 $\pm$ 0.175 (+3.7)	0.116 $\pm$ 0.006 (+2.6)	0.0144 $\pm$ 0.002 (-4)
	Mn+B+Zn (5ppm)	6.5 $\pm$ 0.933 (-28.5)	4.30 $\pm$ 1.975 (+115)	0.083 $\pm$ 0.006 (-26.5)	0.012 $\pm$ 0.001 (-20)
0.2% NaCl + Basil Leaf Litter (5%)	No Mixture	7.2 $\pm$ 0.311 (-20.87)	4.20 $\pm$ 0.857 (+55.5)	0.133 $\pm$ 0.015 (+17.69)	0.0129 $\pm$ 0.002 (-14)
	Mn+B+Zn (2.5ppm)	6.47 $\pm$ 0.095 (-28.90)	5.00 $\pm$ 1.892 (+85.18)	0.126 $\pm$ 0.059 (+11.50)	0.0146 $\pm$ 0.004 (-2.6)
	Mn+B+Zn (5ppm)	7.08 $\pm$ 1.041 (-22.19)	5.18 $\pm$ 1.030 (+91.315)	0.136 $\pm$ 0.035 (-4.14)	0.016 $\pm$ 0.0045 (+8.66)
0.4% NaCl + Basil Leaf Litter (5%)	No Mixture	6.5 $\pm$ 0.672 (-28.57)	4.25 $\pm$ 0.250 (+57.4)	0.126 $\pm$ 0.025 (+11.50)	0.0146 $\pm$ 0.001 (-2.6)
	Mn+B+Zn (2.5ppm)	5.875 $\pm$ 0.781 (-34.43)	4.30 $\pm$ 1.504 (+115)	0.196 $\pm$ 0.015 (+73.45)	0.0350 $\pm$ 0.005 (+133.3)
	Mn+B+Zn (5ppm)	7.4 $\pm$ 0.850 (-18.6)	6.30 $\pm$ 1.593 (+133.3)	0.223 $\pm$ 0.047 (+97.3)	0.0410 $\pm$ 0.017 (+173.3)
0.6% NaCl + Basil Leaf Litter (5%)	No Mixture	7.5 $\pm$ 0.271 (-17.5)	3.80 $\pm$ 0.284 (+40.74)	0.133 $\pm$ 0.025 (+17.69)	0.0155 $\pm$ 0.004 (+3.3)
	Mn+B+Zn (2.5ppm)	7.6 $\pm$ 0.439 (-16.48)	4.80 $\pm$ 1.097 (+77.77)	0.153 $\pm$ 0.012 (+35.39)	0.0166 $\pm$ 0.001 (+10.66)
	Mn+B+Zn (5ppm)	7.57 $\pm$ 0.792 (-16.81)	4.03 $\pm$ 0.872 (+49.25)	0.116 $\pm$ 0.049 (+2.6)	0.0176 $\pm$ 0.001 (+17.3)

### 3b. Root Length (cm)

The increase in NaCl level induced an increase in medium plant root length. The increment was pronounced at 0.4% salinity level which is 57.4% over control plant root length. Whereas, with 0.2% and 0.6% salinity, plant showed increased root length 55.5% and 40.74% respectively over to control untreated plant root length. Data revealed that, Root length of methi plants increased due to spraying the micronutrients in both concentrations (Fig.1a).

Micronutrient spray improved the performance of root growth and prevented the nutritional disorders and consequently caused increases the uptake of nutrients <sup>[10]</sup>. Micronutrients foliar spraying could partially counteract the negative effect of NaCl on nutrient uptake through improving root growth and preventive disorders and consequently caused an increase in the uptake of nutrients by the roots <sup>[18]</sup>.



### 3c. Plant Dry Weight (gm)

In given data (fig. 1b) dry biomass of methi plant were significantly reduced with 0.4% salinity level above of which it showed accumulation of biomass as a conservative strategy against external stress full condition. In this respect, it was reported that biomass accumulation might be considered as an indicator of crop tolerance to NaCl+allelochemical stress. Reduction in total biomass under different salinity levels was also reported by Bassil *et al.*, 2002 <sup>[19]</sup>. Data also indicate that a gradual decrease was observed in plant dry weight. The percent decrement was related to the elevation of salinity. At two different levels of NaCl (i.e. 0.2% - 0.4%) plant dry weight of methi was reduced from 14% to 2.6% respectively over control. These findings agree with those obtained by Morant *et al.*, 2004 <sup>[20]</sup>. Such depressive effect of salinity in methi dry biomass growth may be attributed to the adverse effect

on enzymatic processes through some interactions of salts and some organic substances of the cell <sup>[16]</sup>. Moreover, crop reduction due to salinity is generally related to the osmotic potential increase at the root-zone soil solution which leads to certain physiological changes and substantial reduction in productivity <sup>[17]</sup>. The results also shown that spraying micronutrients in both concentrations on plants grown under salinity conditions increased the dry weight of aerial parts and roots at all NaCl levels (Fig. 2a). The dry weight increment of methi plant was attributed to spraying micronutrients (2.5 ppm & 5 ppm). The highest increment was observed at 0.4% salinity level with 5ppm spray and gave 173.3%. Foliar mineral spray significantly affects biomass production of plants irrespective to their growth under non-saline or saline conditions. The obtained results are in agreement with the findings of Kassab, 2005 <sup>[21]</sup> and Thalooh *et al.*, 2006 <sup>[22]</sup>.

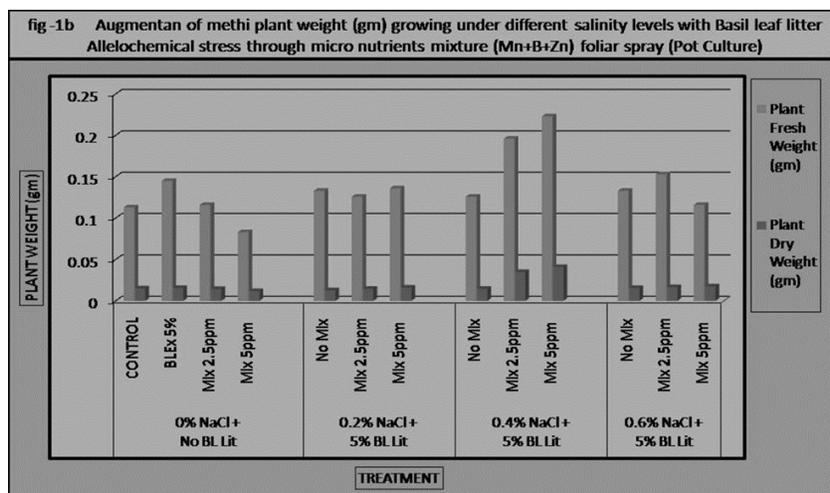


Table-1 showed that, the dry weight of micronutrient treated plants increased due to spraying the micronutrients in both concentrations (i.e. 2.5 ppm and 5 ppm). The increment of dry weights of *methi* plants was measured highest at 0.4% salinity level with the value of 0.041 gm over to 0.4% NaCl treated and untreated control plant dry weight values (i.e. 0.0146 gm and 0.015 gm respectively).

### 3d. Effect of Treatment on *Methi* Primary Metabolites

Table-2 showed the estimated amount of primary metabolites of treated plant. The data revealed the behavior of these biomolecules according to their response against salinity. At elevated salinity, production and accumulation of both biochemicals significantly affected and showed the contrasting behavior from one another.

### 3e. Total Protein Content (mg/gm fr.wt)

Table-2 indicated the response of *methi* plant's protein synthesis and accumulation when exposed to different salinity levels along with BL litter allelochemical stress. At the control level Basil leaf litter greatly reduced the biosynthesis of total protein by releasing toxic metabolites that inhibited the uptake of essential elements from the rhizosphere, 59.5% inhibition in total protein contents was estimated with the BL application of micronutrient spray also had an inhibitory effect on protein content of treated plant of about 51% and 14.8% with both doses respectively. With elevated salinity from 0.2% to 0.6%, the inhibitory percentage increased from

36.1% (at 0.2% NaCl) to 59.5% (at 0.4 % NaCl) due to the negative effect of dual stress (salinity and allelochemical) on protein biosynthesis of study crop. Under salt stress nitrogen uptake is limited by an accumulation of Na<sup>+</sup> and Cl<sup>-</sup> and its composition with NO<sup>2</sup>. A low content of N were due to decreasing synthesis of specific N compounds such as amino acids i.e. proline and aspartic acids (glutamine and asparagine) [24]. On elevating salinity to 0.6%, the estimated amount of protein content was found 31.9% inhibitory from untreated plant content this value is lower than both the above values calculated with 0.2% and 0.4% salinity. This decrease in percent inhibition showed the accumulation of protein in plant tissue which was due to the production stress protein under external stress environment. Table-2 also indicated the beneficial effect of foliar spray on both doses. The application of mixture treatment significantly reduced the inhibitory effect of salinity and allelochemical stress enable treated plant to overcome the reduction in protein synthesis at all salinity levels, the production of protein increased with micronutrient supply. The effect was dose dependent and intensified with increasing mixture concentration (i.e., 5 ppm), the value of percent inhibition reduced from 36.1% to 12.3% and 10.8%, from 59.5% to 34% and 21.2% and from 31.9% to 17% and 42% respectively at 0.2%, 0.4% and 0.6% salinity levels with 2.5 ppm and 5 ppm mixture spray.

TREATMENT		PROTEIN (mg/gm fr. Wt)	CHO (mg/gm fr. Wt)	BETAIN (mg/gm fr. Wt)
0% NaCl	CONTROL	32.66 <sup>a</sup> (0)	55.799 <sup>e</sup> (0)	17.022 <sup>c</sup> (0)
	Basil Leaf Extract (5%)	13.6 <sup>d</sup> (-59.5)	7.2919 (-86.93)	20.339 <sup>c</sup> (+19.48)
	Mn+B+Zn (2.5ppm)	16.52 <sup>d</sup> (-51)	72.2851 <sup>d</sup> (+29.54)	14.331 <sup>d</sup> (-15.80)
	Mn+B+Zn (5ppm)	28.22 <sup>a</sup> (-14.8)	80.2111 <sup>b</sup> (+43.75)	10.138 <sup>d</sup> (-40.44)
0.2% NaCl + Basil Leaf Litter (5%)	No Mixture	21.1 <sup>c</sup> (-36.1)	25.046 <sup>e</sup> (-55.11)	18.521 <sup>c</sup> (+8.806)
	Mn+B+Zn (2.5ppm)	28.6 <sup>a</sup> (-12.3)	77.04072 <sup>c</sup> (+38.06)	13.12 <sup>d</sup> (-22.92)
	Mn+B+Zn (5ppm)	29.11 <sup>a</sup> (-10.8)	90.234 <sup>a</sup> (+61.71)	12.816 <sup>d</sup> (-24.709)
0.4% NaCl + Basil Leaf Litter (5%)	No Mixture	13.85 <sup>d</sup> (-59.5)	27.582 <sup>e</sup> (-50.56)	34.482 <sup>a</sup> (+102.57)
	Mn+B+Zn (2.5ppm)	21.99 <sup>c</sup> (-34)	74.5044 <sup>c</sup> (+33.52)	26.91 <sup>b</sup> (+58.08)
	Mn+B+Zn (5ppm)	26.05 <sup>b</sup> (-21.2)	84.332 <sup>b</sup> (+51.13)	17.272 <sup>c</sup> (+1.47)
0.6% NaCl + Basil Leaf Litter (5%)	No Mixture	22.24 <sup>b</sup> (-31.9)	34.24 <sup>f</sup> (-38.63)	24.657 <sup>b</sup> (+44.85)
	Mn+B+Zn (2.5ppm)	27.33 <sup>a</sup> (-17)	91.3075 <sup>a</sup> (+63.63)	18.586 <sup>c</sup> (+9.18)
	Mn+B+Zn (5ppm)	31.52 <sup>a</sup> (-4.2)	93.8438 <sup>a</sup> (+68.18)	17.585 <sup>c</sup> (+3.311)

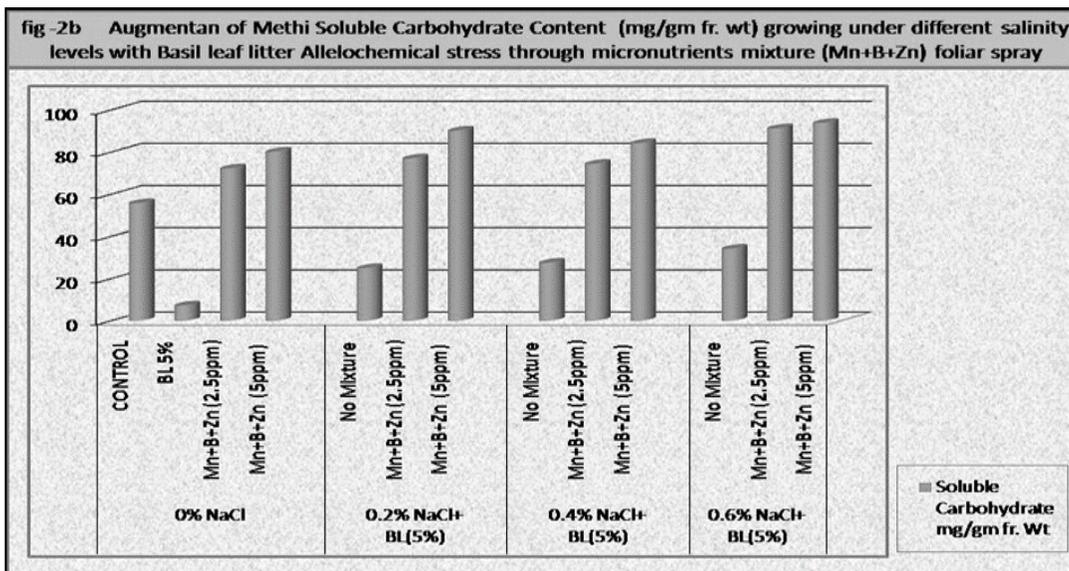
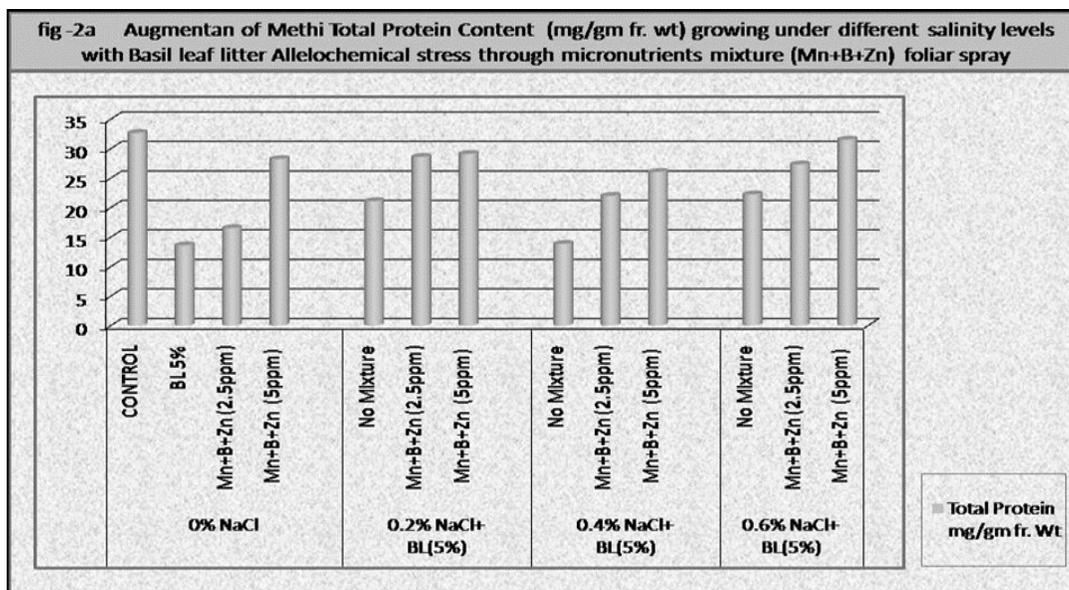
### 3f. Soluble Carbohydrate Content (mg/gm fr.wt)

Figure-2b showed that, with increasing salinity with allelochemical from 0.2% to 0.6%, the estimated amount of carbohydrates increase from 250.46 mg/g fr.wt to 342.4 mg/g fr.wt against elevated salinity stress. This may be due to the conservative strategy of treated plant to reduce the inhibitory effect of salinity and Allelochemical stress on *methi* plant's carbohydrates production and accumulation, this reduction in inhibitory percentage were calculated as 55.11% to 50.56% and then lowered

to 38.63% over control plant CHO content at 0.2, 0.4 and 0.6% salinity levels respectively. In the treated plant tissues the amount of total soluble sugar increase rapidly answering to the increasing salinity, this result agreed with the result of some researchers that indicate that salinity stress induce soluble sugar accumulation [25]. This is also the case with sorghum seeds, the stress causes a decrease in starch content and an increase in sugar content [26]. The foliar application of micronutrient (Mn+B+Zn) at both concentrations significantly helps to reduce the inhibition in

carbohydrate accumulation and promoted its production against all stress levels. The value of 55.11% inhibition at 0.2% salinity greatly overcome 238.6% and 61.71% increased over control/untreated plant CHO contents respectively with the application of 2.5 ppm and 5 ppm mixture. Similarly 0.4 and 0.6% salinity and allelopathy stress 50.56% reduction removed to

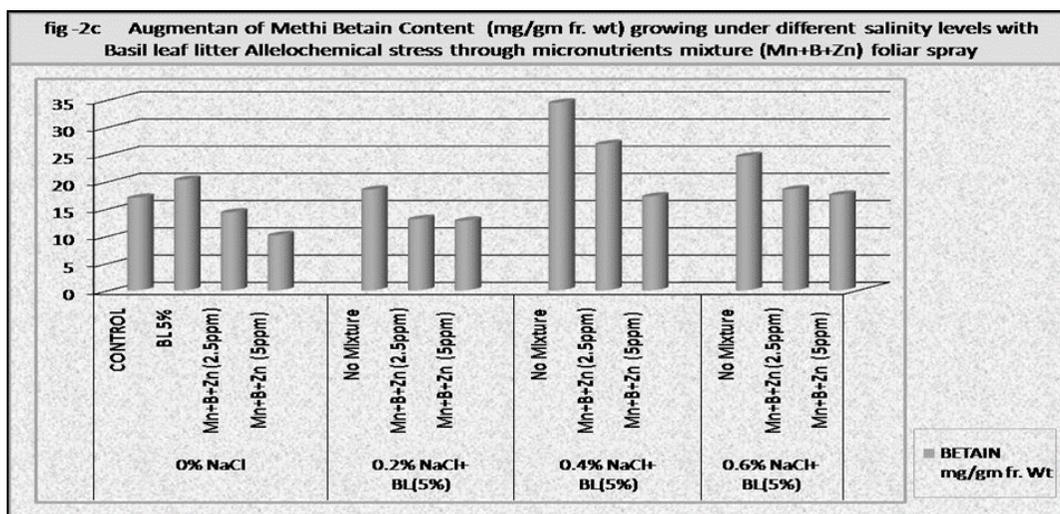
51.13% increased and 38.63% reduction converted to 68.18% increased in CHO content with 5ppm mixture spray respectively, the overall response of mixture treatment were appeared beneficent to minimized the inhibitory effect of dual stressed on carbohydrate accumulation.



**3g. Betain (mg/gm fr.wt)**

In table-2, Betain amount of methi plant increased markedly in response to increasing stress levels from 0.2% to 0.6% NaCl application along with 5% Basil leaf litter (Fig. 2c). The treated plant showed 8.8%, 102.57% and 44.85% more accumulation of Betain content at 0.2% , 0.4% and 0.6% salinity level respectively over the control untreated plant. Accumulation of betain in salinity treated plants was significantly influenced by micronutrients foliar

application at both levels. By the application of Mn+B+Zn mix @ 2.5 ppm and 5 ppm, the high Betain contents greatly inhibited to 22.92% over control plant at 0.2% salinity level whereas, the highest accumulation of batain at 0.4% salinity level i.e. 102.57% reduced to 58.08% with the application of 2.5ppm foliar mixture. Similarly, with 5ppm micronutrient spray, a remarkable inhibition in Betain contents was recorded with respect to control plant.



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