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EFFECT OF MICRONUTRIENTS WITH HUMIC ACID ON CARBOHYDRATE CONTENT OF SPINACH

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ABSTRACT

A study was carried out to determine the carbohydrate content of Spinach (*Spinacia oleracea* L.) which treated with only micronutrients (1ppm) stock solution and with different concentrations of humic acid. The experiment was done in four replications. Experimental treatment included seven micronutrients stock solution (1 ppm) which included $CaCl_2$, $CuSO_4$, $MgSO_4$, $MnSO_4$, $ZnSO_4$, H_3BO_3 and $FeSO_4$. Foliar application was done with micronutrients stock (1 ppm) only, micronutrients stock with different concentrations of HA (100, 1000, 10000 ppm). Soluble carbohydrate was determined by Anthrone method Spectrophotometry at wavelength of 750 nm. It was found that Spinach leaves treated with 1 ppm micronutrients stock mixed in 1000 ppm HA showed the highest percentage of carbohydrate followed by 1 ppm micronutrients stock mixed in 10000 ppm HA while control had the least. The findings of the investigation revealed that varying levels of carbohydrate content in the Spinach with different treatments analyzed which have direct comportment on their nutritional status.

KEYWORDS: Carbohydrates, Spinach, Micronutrients, Humic acid.

INTRODUCTION

As per the FAO/WHO discussion, carbohydrates recommended in the terms of soluble and insoluble fiber.[1] They have special importance as they constitute more than 50% of the dry weight of most plants.[2] Every green plant synthesizes carbohydrates by the process of photosynthesis to fulfill energy requirements. In plants, carbohydrates represent one of the most important group of organic compounds as they form a connection between photosynthesis and respiration and thus the yield of plant depends upon its carbohydrate status. As carbohydrates are the most abundant bio molecules on earth; so photosynthesis converts more than 100 billion metric tons of carbon dioxide (CO_2) and water (H_2O) into cellulose and other plant products per year.[3] Carbohydrates perform many functions ranging from stores of potential energy in animals to source of energy and as supporting tissues in plants.[4]

Soluble sugars are major constituents of osmotic adjustment maintaining turgor pressure and stabilizing cellular membranes.[5][6] Sucrose decomposes to glucose and fructose and starch break-down to glucose increases osmotic pressure of cells.[7] Sugars play an important role in disease resistance by suppressing the proteolytic and cellulotytic enzymes essential for pathogenesis.[8] The interaction by some pathogens brings about changes in the respiratory pathway and photosynthesis that leads to fluctuation in sugars in plant-pathogen interactions.[9] Sugar responsible genes give various defensive responses and cellular expansions.[10]

Recently, various evidences available indicated increasing research on micronutrient requirements in staple grains due to their critical importance in human diet.[11] There is focus on micronutrient levels research in staple grains because of their vital importance for the provision of micronutrient requirements in the human diet. Hence, future emphasis on micronutrients may expand from their role in crop production, to their importance in the main staple foods with leafy vegetables in diets for sustaining human and animal health. Experiment was carried out on Spinach, as it is a rich source of vitamin A, vitamin C, vitamin E, vitamin K, magnesium, manganese, folate, betaine, iron, vitamin B_2, calcium, potassium, vitamin B_6, folic acid, copper, protein, phosphorus, zinc, niacin, selenium and omega-3 fatty acids, opioid peptides called rubiscolins. Polyglutamyl folate (Vitamin B_9 or folic acid) is a vital constituent of cells and Spinach is a good source of folic acid.

This study was aimed at evaluating the soluble carbohydrate content in Spinach treated with only micronutrients stock and micronutrients stock with HA of various concentrations, with a view to determining whether foliar applications of these treatments enhance carbohydrate content or decrease its content. It is envisaged that the findings of the investigation would provide additional information on the improvement in nutritional status of the Spinach by application of micronutrients with humic acid.

MATERIALS AND METHODS

Sample treatment

The experiment was carried out in four replications. 1 ppm stock solution of micronutrients was prepared by using $CaCl_2$, $CuSO_4$, $MgSO_4$, $MnSO_4$, $ZnSO_4$, H_3BO_3 and $FeSO_4$. Humic acid solutions of various concentrations (100,1000,10000 ppm) were prepared and mixed in 1 ppm micronutrients stock solution separately. Distilled water treatment served as control. Morphologically healthy seeds of Spinach were first surface sterilized with 1% of $HgCl_2$ for 2 minutes and then washed with distilled water to remove toxic elements. A pot culture experiment was conducted during 2013-2015 to study the influence of foliar application of only micronutrients and micronutrients with HA on carbohydrate content of Spinach. Soil selected for this study was tested for micronutrients and macronutrients contents. Soil was low quality which contains less quantity of macronutrients as well as micronutrients. After that Spinach seedlings were treated with foliar spray of 1 ppm micronutrients stock and 1 ppm micronutrients stock with different concentrations (100,1000,10000 ppm) of humic acid and each treatment had four replicates. Plants were allowed to grow for 45 days. In these 45 days, foliar spray was given in 15 days interval. After 6 weeks, plants were harvested and carbohydrate content was determined of each treatment.

Carbohydrate analysis

5 g fresh leaf material was homogenized in mortar with pestle and extracted with 25ml distilled water. It was filtered through Buchner's funnel using Whatman No.1 filter paper. This filtrate was used for estimation of carbohydrates. From the above filtrate,1 ml of each extracted sample was taken into 25 ml volumetric flask and 2 ml of freshly prepared anthrone reagent (0.2 gm of anthrone was weighed accurately and dissolved in concentrated sulphuric acid and make up the volume up to 100 ml and finally transferred it in to a 100 ml of reagent bottle) was added in each volumetric flask.

Finally the volume was made up to the mark with distilled water. Reference was prepared by taking 2 ml of anthrone reagent in 25 ml of volumetric flask and made the volume up to the mark with distilled water. The absorbance was read at 750 nm on double beam spectrophotometer (Lab-India UV-3000^{+}). A standard curve of glucose (0.1mg.ml^{-1}) was prepared and the sugar content was calculated.

RESULTS AND DISCUSSION

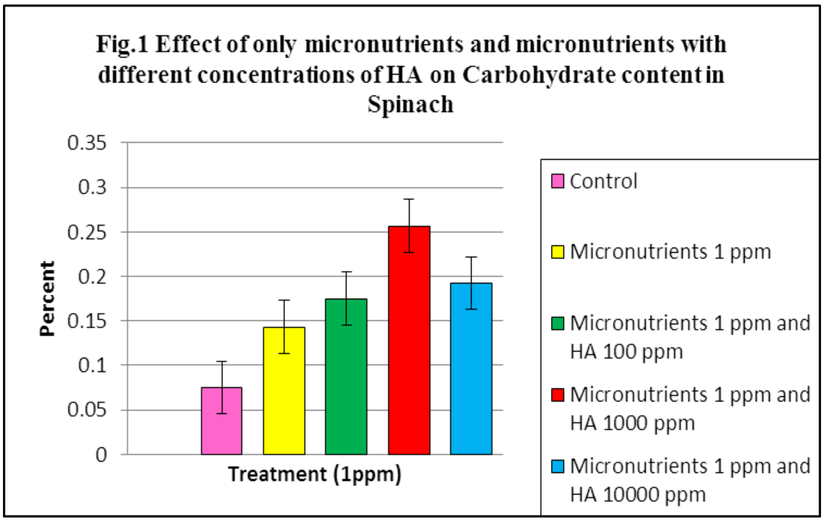

Fig.1 Effect of only micronutrients and micronutrients with different concentrations of HA on Carbohydrate content in Spinach

It is clear from figure that carbohydrate levels increased in all treated plants than control treatment. The significant accumulation of soluble sugars is observed with 1ppm micronutrients combined with1000 ppm HA and 10000 ppm HA. Similar results were observed which explained that carbohydrates content increased with humic acid and mycorrhizal inucolation.[12] Humic acid application up to 6kg/fed increased the highest N, P and K uptake and increased K/Na, Ca/ Na ratio, protein and carbohydrates contents of cowpea plants.[13] Total sugar increased by application of HA in radish plant.[14] Increased level of carbohydrate reported in *Capsicum annuum* L. because of foliar application of HA with B and Zn.[15] Other results were found that adding humic acid increased carbohydrate content of turnip roots.[16] While the increased rates of the application of humic acid caused an increment in the percentage of protein, N, P, K, carbohydrate and Fe contents, Moreover, the highest humic acid level caused the maximum significant level for total carbohydrate content.[17]

CONCLUSION

It is evident from our observations that applied combination of 1ppm micronutrient with 1000 ppm HA stimulates the accumulation of carbohydrates in leaves that may be because of higher photosynthetic activity. The higher content of carbohydrate is associated with increased disease resistance and nutrient content in Spinach.

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