# Evaluation of a Split-Root Nutrition System to Optimize Nutrition of Basil

# EVALUATION OF A SPLIT-ROOT NUTRITION SYSTEM TO OPTIMIZE NUTRITION OF BASIL

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by

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**EVALUATION OF A SPLIT-ROOT NUTRITION SYSTEM TO OPTIMIZE NUTRITION OF BASIL**

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## DEDICATION

To my father Abbasov Djurakul Kadirovich and my mother Abbasova Mahsuma Zokirovna who made the completion of this work possible.

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## ABSTRACT

EVALUATION OF A SPLIT ROOT NUTRITION SYSTEM TO OPTIMIZE NUTRITION OF BASIL

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The plant-nutrient-water optimum interaction always has been a problematic program for plant growth and development. This work investigates this interaction using a split root nutrition system to determine possible changes in traditional hydroponics to enhance plant growth and development. While split root nutrition systems have been used experimentally to answer some specific questions, the technique has never been used in a production system for optimizing plant, nutrient, and water interaction. The introduction of hydroponics almost a hundred fifty years ago has not changed this situation fundamentally. Moreover, the norm of fertilizer application on agricultural crops has the advantage of increased productivity and reduced expenses. Results of the current research using split-root nutrition system suggest no differences between weekly application of nutrients and applying all nutrients necessary for all vegetation one time. Moreover productivity was increased significantly where the split-root, nutrition system was used to provide the experimental solution. Problems with traditional growing systems, such as optimizing pH of media, increasing productivity, improving quality of product by

increasing phytochemicals were addressed using experimental nutrient solutions specifically for basil (*Ocimum Basilicum* L). The pH of the root zone was kept at the optimum level of 6.8 during the entire vegetation period. Split root nutrition system using experimental solution significantly increased productivity due to increasing water potential in one half of the root zone, an increased quality of basil, and an increased amount of enzyme activators which would not be possible using the traditional growing system due to toxicity.

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# CHAPTER 1

# INTRODUCTION

A primary difficulty in growing plants is the problem of applying all nutrients at the same time before seeding in an attempt to optimize plant nutrient uptake to maximize plant growth and development. Applying all fertilization at one time provides economic and some other benefits, such as avoiding nutrient deficiencies, but these benefits are possible only by using slow-acting fertilizers. Slow-acting fertilizers can cause either nutrient toxicity or deficiency problems due to temperature dependence of nutrient release, if temperatures cannot be controlled (Yanishevskiy 1985; 1989; 1990; Yagodin 1983). The goal of the current research was to develop an optimum nutrition model for plants that enables quality growth in a relatively short time period and cheaply. This research, first time in the world, investigated a 'wide range of nutrient concentration' and a 'wide range of pH' (Figure 2.1.) in the root zone of the plant. This work uses a plant, split-root system to achieve better plant nutrition opportunity for the plant to satisfy nutrient needs and the use of reformulated fertilizers to make available to insure that nutrients are available as needed. Many alternative nutrient systems, especially hydroponics have failed due to relatively high expenses (Chesnokov 1983; Yagodin 1990).

The present work is based on following hypotheses: above optimum nutrient concentration is toxic for plants, but placing part of the plant roots in a high nutrient concentration and another part in a low concentration will enable the plant to optimize nutrition uptake by absorbing nutrients in the desired quantities. In addition,

important secondary metabolites can be increased by adding enzyme activators (Malusa 2006) in the low concentration part of the media, a situation that is generally impossible in traditional growing systems.

Thus, this work describes a new growing system, a "Plant Root Nutrient Selection System" that enables the plant to select mineral nutrients from an available pool of nutrients as needed. The research involved the development of new nutrient media formulas:

$$\mathbf{pA\ or\ \Delta Y_{asr}} \approx k\,\frac{\mathbf{[S]_1} * (\mathbf{V_{total}} - \mathbf{V_1})}{\mathbf{[S]_2} * \mathbf{V_1}}$$

$\mathbf{pA}$ – power of absorption or namely $\mathbf{\Delta Y_{asr}}$-deference yield of nutrient absorption due to poly-media nutrition (or wide range split-root nutrition)
$\mathbf{[S]_1}$ -nutrient concentration of high concentrated part of the root zone, % from total nutrient in overall root zone: $50 < \mathbf{[S]_1} < 100$
$\mathbf{V_{total}}$- volume of total root zone, %.
$\mathbf{[S]_2}$-nutrient concentration of low concentrated part of the root zone, % from total nutrient in overall root zone: $0 < \mathbf{[S]_2} < 50$
$\mathbf{V_1}$- volume of high nutrient concentrated part of the root zone, % from total root zone volume: $0 < \mathbf{V_1} < 50$
*k*-constant, for basil-0.01; and different for each crops

with high and low levels of various elements and the use of selected media pH to assure nutrient availability for plants as needed (Figure 5.32; 5.33).

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Using split-root system for better understanding of plant nutritional physiology

### 2.1.1 Split root system and plant growth development

The split-root system is the division of the plant root into two media with different nutrient, salinity, and pH. This system has been used by researchers (Shani and Waisel 1993; Shen and Neumann 2005; Shu. *et al*. 2005; Zhu 2000), but not for improving plant nutrition. Using localized fertilization in row crops is a similar phenomenon as the split-root system, Tworkoski and Daw (2003) report that the greatest number of roots grew at 43 to 46 cm from the root collar where localized, polypropylene, nonwoven fabric fertilizer was applied, resulting in rapid shoot growth as a response to daily fertilization. Other researchers (Rengel 2008; Shen and Neumann 2005; Ting and HengTao 2012), however, report that the split-root system is not always able to compensate for the part that has lower quantities. For example, Klein and Blum (1990) report that using the split-root system with ferulic acid in one part of the root zone suppresses root elongation.

Ma and Rengel (2008) studied phosphorus distribution in split-root systems to examine the influence of plant phosphorus status and distribution in the root zone and phosphorus acquisition on the growth of root and shoot of wheat (*Triticum aestivum* L.). The results of their research suggest that root proliferation and greater phosphorus uptake in the phosphorus-enriched zone may meet the demand for phosphorus by phosphorus - deficient plants only for a limited period of time. Accordingly, a split-root system can

solve the problem of applying all fertilization at one time instead of partial applications during the entire growing period. For this reason, a specific nutrient ratio and concentration in the rhizosphere is important but must have a positive correlation with plant nutritional physiology under the split root system (Abbasov 1991).

### 2.1.2 Plant nutritional physiology under split root system

Optimizing plant nutrition is an important aspect of plant science. Higher plants have developed a number of strategies, including morphological and physiological changes, to enhance nutrient acquisition and utilization, especially for phosphorus in phosphorus-limiting environments (Vance *et al*., 2003).

The formation of cluster roots by plants is an important phenomenon in plant development that enhances the capacity of plants to acquire sparingly soluble phosphorus from soil (Shen and Neumann 2005). Cluster roots comprise a number of tightly grouped, determinate rootlets that undergo initiation and growth in a synchronized manner (Skene, 2001). The developmental and functional synchrony within the cluster roots leads to a concentrated change in soil chemistry around the cluster roots and is thought to mobilize phosphate, iron, and other elements in the rhizosphere (Dinkelaker *et al*., 1995; Vance *et* , 2003; Watt and Evans, 1999).

Liangzuo and Shen (2007) examined cluster root formation by white lupine (*Lupinus albus* L. cv. Kiev Mutant) in response to stratified application of hydroxyapatite, demonstrating that the proportion of dry biomass of cluster roots in the whole root system was reduced significantly if phosphorus concentration was high in shoots. Such results suggest that cluster root formation is regulated by the shoot

phosphorus status. The cluster root percentage, however, increased in the soil layer supplemented with phosphorus and did not increase in other layers, especially if phosphorus was applied in a deep layer. Apparently, formation of cluster roots is regulated by internal plant phosphorus status (Ma and Zed 2008), but also is affected greatly by localized P supply (Qifu and Renge 2008). Heterogeneous phosphorus supply seems to modify the distribution of cluster roots.

Using a split-root system, Neumann and Zhang (2005) report that localized phosphorus deficiency suppressed S uptake. They subsequently suggested that cluster root formation and citrate exudation are regulated by the shoot phosphorus and all affected by localized supply of external phosphorus and that proton release is inhibited by localized phosphorus supply through alteration of the balance of anion and cation uptake. Anion active transport by unidentified guard-cell channels closes the stomata pore. (Moreover, Serna 2008)

Shu (2005) investigated growth medium and phosphorus supply on cluster root formation and citrate exudation by *Lupinus albus* L. grown in a sand/solution split medium. They concluded that phosphorus concentration and phosphorus uptake of plants in the low-phosphorus medium increased with increasing phosphorus supply to the sand compartment. Proton extrusion rate by the solution-grown roots in the phosphorus low medium was higher than that of similar roots in high-phosphorus media at the early growth stage.

Split-root experiments by Ting and HengTao (2012) demonstrated that Fe-deprivation in a portion of the root system induced a dramatic increase in Fe (III) reductase activity and proton extrusion in the Fe-supplied portion.

Based on the current available information and observation, mineral nutrient compartmentalization is a way to improve plant nutrition, because as described above, different concentrations of nutrients in different parts of the medium have an impact on plant physiology either positively or negatively. Accordingly, finding the right location and right concentration of nutrients in the medium may positively impact plant physiology.

### 2.1.3 Plant stress compensation under split root system

Research (Flores and Botella 2002; Kirkham 1983) has clarified plant mechanisms for avoiding stress by compensation. In investigating the response of tomato seedlings to salinity with a split-root system, they observed that plants could tolerate high salinity in part of the root system if the remaining roots were exposed to low salinity. The results indicate that under non-uniform salt distribution, plants can compensate for the restricted water uptake from the more saline zone by increasing water uptake from the low salinity zone so that the overall water uptake by the entire root may remain relatively unchanged. The salt stress in one half of the root system of tomato seedlings did have a slight effect on overall $NO_3^-$ uptake, although $NO_3^-$ uptake in the stressed root part was strongly reduced relative to the unstressed zone (Flores and Botella 2002).

Kirkham (1983) previously reported that increased mineral nutrition increases water movement into the tomato plant and, perhaps more rapid xylem flow that can transport mineral elements quickly throughout the plant, leading to more foliar growth. Moreover, use of the split-root system demonstrated that water could move from one side of a root system to the other side of root for compensation of the side with less water.

### 2.1.4 Relationships between pH, nutrient transport and mineral nutrition

Experiments studying the transport of copper from an aqueous solution of, cadmium, cobalt, nickel, and zinc through liquid membrane demonstrated that $Cd^{2+}$, $Co^{2+}$, $Ni^{2+,}$ $Zn^{2+}$ were not transported at pH 1–5 (Osman, 2005). Similar phenomena have been demonstrated by other researchers (Yanishevskiy 1983; Muravin 1990; Ataullaev 1973).

The nutrition system investigated in the current research has nutrient source based on relationships between pH and nutrient uptake (Figure 1.2). Apparently compartmentalizing nutrient source by dividing in different pH zones is a way of optimizing plant nutritional physiology due to keeping pH range at 6 to 7 during entire vegetation period.

## 2.2 Plant nutrition mechanisms, osmosis and rhizosphere pH under split root system

### 2.2.1 Plant nutrition mechanisms-active transport

Plant nutrient uptake occurs by two mechanisms-active and passive transport. Active transport, the movement of a substance against a concentration gradient (from low to high concentration), requires metabolic energy. Passive transport is driven by kinetic energy. Active transport can be two types - primary and secondary. (Marschner *et al*. 1995; Yanishevskiy 1990; Epstein and Bloom 2005). If the transport uses metabolic energy, such as adenosine triphosphate (ATP), the transport is termed primary active transport.

Primary active transport, also called direct active transport, uses energy to transport molecules across membranes. Most enzymes responsible for this type of transport are transmembrane ATPases (Bronwyn and Pantoja 1996). A primary ATPase universal to all life is the sodium-potassium pump that maintain cell potential. Bronwyn and Pantoja (1996) report that the vacuole of plant cells is involved in the regulation of cytoplasmic pH, sequestration of toxic ions and xenobiotics, regulation of cell turgor, storage of amino acids, sugars and $C0_2$ in the form of malate, and possibly as a source for elevating cytoplasmic calcium, activities that are driven by primary active transport mechanisms present in the vacuolar membrane.

Other sources of energy for primary active transport are oxidative phosphorylation and photosynthetic phosphorylation. For example redox energy is the mitochondrial electron transport chain that uses the reduction energy of NADH to move protons across the inner mitochondrial membrane against their concentration gradient.

Secondary active transport involves the use of an electrochemical gradient in which energy is used to transport molecules across a membrane. This process is commonly referred to as passive absorption. In contrast to primary active transport, secondary transport has no direct coupling of ATP. Instead, the electrochemical potential difference created by pumping ions from the cell is used. The two main forms of secondary active transport are antiport and symport. In antiport, two species of ion or other solutes are pumped in opposite directions across a membrane. One of these species flows from high to low concentration which yields the entropic energy to drive the transport of other solute from a low concentration to a high. An example is the sodium-

calcium exchanger (antiporter) that allows three sodium ions into a cell to transport one calcium out from the cell (Schumake and Sze 1985). Symport uses the movement of one solute species from high to low concentration to move another molecule from low concentration to high concentration (against an electrochemical gradient). In symport, two species move in the same direction across the membrane. According to Lu and Briskin (1993), maize plasma membrane $H^+/NO_3$- symport activity can be modulated in accordance with the $NO_3$- status of root cells.

According to Rea and Poole (1993) the enzyme may function as an energy conservation system through the establishment of a pH gradient across the tonoplast that is utilized to energize secondary active transport. The enzyme also may function as a mechanism for the regulation of cytosolic pH.

Water does not require active transport to cross a membrane. For what, increasing water potential in part of the rhizosphere by specific constructing split root system is important and is one of the interests of our research (Abbasov and Craker 2009).

Metal ions, such as $Na^+$, $K^+$, $Mg^{2+}$, or $Ca^{2+}$, require ion pumps or ion channels to cross membranes. The pump for sodium and potassium is called sodium-potassium pump or $Na^+/K^+$-ATPase. Kuiper (1979) demonstrated that the addition of the metabolic inhibitor 2,4-dinitrophenol to the root medium, increased passive ion transport and decreased active ion transport.

Being able to absorb nutrients in over a wide concentration range would be important for plants, and the possibility of this phenomenon has been confirmed. For example, AMTs (ammonium transporter genes) in roots exhibit different characteristics

in transport and absorption of ammonium ions through transcriptional regulation, enabling the plant root to absorb ammonium ions from a wide concentration range of ammonium and provides a theoretical basis for intracellular homeostasis of an ammonium ion pool. In crops, AMTs can contribute to absorption of nitrogen effectively, providing a favorable opportunity for improving of agricultural production (Yuan and ShenKui 2009).

The importance of ion transport related to energy has been described. For example, Felle (2004) noted cells under anoxia conditions produced an energy crisis for the plant. The pH remained relatively stable for some time, but then dropped due to an energy shortage, leading a general breakdown of transmembrane gradients and, finally, to cell death unless the plant is able to gains access to another energy source.

### 2.2.2 Passive transport

Unlike active transport, passive transport the transport across a membrane is coupled with an increase the in the entropy of the system. (Pryanishnikov 1948; Marschner *et al.*1964;). Thus passive transport is dependent on the permeability of the cell membrane, which is related to the organization and characteristics of the membrane lipids and proteins. Integral currents of passive ion transport through the membrane of isolated vacuoles investigated by Velikanov and Parfenova (1992) indicate that, abscisic acid could switch two-directional conductivity to unidirectional.

The four main kinds of passive transport are diffusion, facilitated diffusion, filtration, and osmosis (Pryanishnikov 1948; Peterburgskiy 1949 Marschner *et al.*1964; Yanishevskiy 1965; Epstein 2005).

Diffusion is the net movement of material from an area of high concentration to an area with a lower concentration. The difference in concentration between the two areas is often termed as the concentration gradient, and diffusion will continue until this gradient has been eliminated. Preventing high concentration with specific barrier will delay continuation time of diffusion, and in the future, using such a barrier can be used for nutrient construction in the root zone (Moshkov 1953). Since diffusion moves materials from an area of higher concentration to the lower the process can be described as moving solutes "down the concentration gradient". In contrast active transport, often moves material from area of low concentration to area of higher concentration and is referred to as moving the material "against the concentration gradient". Diffusion and osmosis are similar with diffusion being the passive movement of solute from a high concentration to a lower concentration until the concentration of the solute is uniform throughout the solution. Osmosis is diffusion but specifically describes the movement of water (not the solute) across a membrane until an equal concentration of water on both sides of the membrane is reached. Diffusion and osmosis are forms of passive transport and do not require any ATP energy. Active transport requires ATP.

Facilitated diffusion, also called carrier-mediated diffusion, is the movement of molecules across the cell membrane via special transport proteins that are embedded within the cellular membrane. Many large molecules, such as glucose, are insoluble in lipids and too large to fit through the membrane pores. Thus, the sugar binds with a specific carrier protein, and the sugar-protein complex will then be bound to a receptor site and moved through the cellular membrane. The process, however, facilitated diffusion, a passive process, and the solutes still move “down” the concentration gradient.

According to Macklon and Sim (1983), reductions in $K^+$ absorption can be attributed to promotion by the ionophores of facilitated diffusion “down” the electrochemical diffusion gradient, countering the efficiency of the $K^+$ influx pump. An important consideration relates boron (B) uptake occurs via passive diffusion across the lipid bilayer by facilitated transport through major intrinsic proteins (MIPs) and energy-dependent transport through a high affinity uptake system. No indications suggest soluble B complexes play a major role in either uptake or primary translocation of B (Dannel *et al.* 2001). These, B complexes do not necessarily need to be in soluble complexes, suggesting that a colloidal nutrient solution could be used to improve plant nutritional physiology.

### 2.2.3 Osmosis, water potential in the root zone

Osmosis a Greek word meaning 'to collide' or 'to hit', is the flow of a solvent (usually water) through a semi-permeable membrane in the direction of the concentrated solution. The osmotic flow usually is attributed to the natural tendency to balance water potential on both sides of the membrane. The osmotic flow stops when the concentrations is balanced.

The first description of an experiment involving osmosis was given in 1748 by Jean-Antoine Nollet (1700-1770), a French priest and scientist. But a more accurate description of this phenomenon was given by Jacobus Henricus van't Hoff in 1885 when he proposed that dissolved particles in the solvent behave like ideal gas particles. According to this theory, the partial pressure (p) of these particles is given by the following equation $p = (n/v)RT$, earning van't Hoff the Nobel prize in chemistry in 1901.

The quotient (n/v) is the molar concentration (c), R is the gas constant (0.08205 L.atm $K^{-1}$ $mol^{-1}$ ) and T the absolute temperature (a constant in the process), enabling, simplification to the partial pressure being directly proportional to the concentration, making clear that osmosis is a phenomenon that takes place whenever there is a semi-permeable membrane. The cell membrane being a semi-permeable membrane being one making osmosis an important phenomenon that must be taken into account by all living organisms. Plants use osmosis to increase turgor. The cell walls surrounding plant cells are made of cellulose, a sugar polymer. This wall is quite rigid and prevents the cell from bursting due to osmosis. The solution in the inner part of a plant cell is normally more concentrated than the outside, and for that reason, if a plant cell has good access to water the cells will be stiffened and filled with water (due to osmosis), making the whole plant rigid. If the water is more concentrated (salty water) then the plant will lose rigidity and wilt (Epstein and Bloom 2005, Barker 2005, Craker 2007).

A semi-permeable membrane is one that allows the solvent (water) to pass through but not the solute (such as dissolved sugar or sodium cations). Many cells have developed cytoskeletons made of proteins, or sugar-chains that prevent them from bursting or over-shrinking due to osmosis. Accordingly, the nutrient source of plant nutrition system is built on the described phenomenon. Relationships exist between the water potential of media and plant shoot, which can be controlled by an osmotic adjustment mechanism (Acevedo 1979, Drake and Gallagher 1984, Flores 2000, Gadallah 2000, Garicia 1978). Relationships between osmosis and specific factors such as heat (Katja 2006), light (Buligar 2006, Causin and Wulff 2003) and oxygen

concentration (Boyd and Acker 2004) are known as a function of the plant growth and development.

Plants have mechanisms to survive under salinity and drought by using osmotic regulation, and this action is the interest of this research. For example by using a split-root system Kusvuran (2012) demonstrated that melon genotypes have efficient stress-protection mechanisms to survive under salinity and drought conditions.
Relationships between water stress and nutrient supply have been investigated by many researchers. For example, Waraich and Ahmad (2011) demonstrated that exposure of plants to water and nitrogen stress will lead to noticeable decrease in leaf water potential, osmotic potential, and relative water content, confirming that, relative water content (RWC) of stressed plants dropped from 98 to 75% with the decrease in number of irrigation and nitrogen nutrition. The higher leaf water potential, and relative water contents were associated with higher photosynthetic rate. Water use efficiency (WUE) is reduced with increasing number of irrigations and increased with increasing applied nitrogen at all irrigation levels.

Osmotic regulation is the one of the important mechanisms of plants and has been confirmed by other researchers (Lei and Yunzhou 2009 ; HaiJun and Yong, 2010). Experiments with rice have demonstrated that under water stress, supplied ammonium could sufficiently accumulate and transfer amino acids and potassium, leading to relatively higher amino acids and potassium contents in xylem and phloem sap. When leaf water potential decreased under water stress (here referring to too much water), ammonium-supplied rice plants could enhance or maintain relative higher leaf water content through osmotic regulation. When leaf water potential was decreased under water

stress, the maintenance of leaf water content in ammonium-supplied rice plants ensured relative high photosynthesis, which subsequently enhanced the tolerance of rice plants to water stress (HaiJun and Yong, 2010).

Increased osmotic regulation has been confirmed by experiments using split root system. Researchers Lei and Yunzhou (2009) reported that, moderate water stress induced osmotic regulation under PRD (partial root drying) conditions, leading to normal water status, higher antioxidant enzymes activities, the same level of biomass and lower water use, thus providing some part of mechanism to higher WUE (water-use efficiency) under PRD condition. Accordingly the nutrition system investigated in the current research has partial root drying media, which improve plant nutrition due to increasing antioxidant enzyme activity.

### 2.2.4 Relationships between metabolic activity, selective ion transport and transpiration.

Schubert and Yan (1999) demonstrated that higher metabolic activity in roots supplied with nutrients will increase ATP concentrations and effects on net proton release. Moreover exposure of roots to complete nutrient solution, using split root system techniques, will increase net proton release relative to control. They concluded that the reason for more proton release is 'depolarization of the electrical membrane potential by cation uptake' and doubling ATP concentration due to nutrient supply.

Active and passive ion transports are related to transpiration. Factors which are impacted by transpiration will make changes in ion transport. Results of experiments done by Bowling (1968) show that, in the standard solution, the uptake of $K^+$, $NO_3^-$ and $SO_4^{--}$ were sensitive to changes in water flux across the root. $Ca^{++}$ uptake was

independent of water uptake. Transpiration affects only the non-metabolic transport of ions across the root.

According to Marschner (1964), in glasshouse experiments with barley and bean grown under different conditions of relative atmospheric humidity, low transpiration (1/3-1/4 of the normal) greatly decreased the uptake of $Na^{+}$ and $Mg^{++}$ from the nutrient solution by the shoots especially of bean plants, whereas $K^{+}$ uptake was affected by low transpiration in bean but not in barley. It is concluded that, increased ion uptake at high rates of transpiration is associated with high concentrations of ions of low affinity to specific locations of bonding in the apparent free space, rather than with a passive transport by water in the vessels.

Soil and also in some cases nutrient solutions may contain high concentration of mineral elements not needed for plant growth. The mechanisms by which plants take up nutrients are selective. This selectivity was demonstrated by many scientists: Hoagland (1948), Arnon (1939), Epstein and Bloom (2005), Pryanishnikov (1947) Smirnov (1957), Muravin (1995). Experiments with algal cell by Hoagland (1948) is good example of selectivity.

Selective transport is active transport and requires energy. Strong support for the involvement of ATP in carrier-mediated ion transport was first presented by Fisher (1970). One of the main interests of our research is, by increasing passive transport decrease active transport which requires energy consequently more energy can be used for other physiological processes such as photosynthesis.

### 2.3 Cation anion in rhizosphere and enzyme activators in split root system

#### 2.3.1 Influence of rhizosphere cation anion physic-chemical phenomenon on plant nutrition.

For better understanding of plant nutrition, it is important to use split-root techniques. Los (1993) did investigate $H^+/OH^-$ excretion and nutrient uptake in upper and lower parts of lupin (*Lupinus angustifolius* L.) by using vertical split-root experiments. It is reported that, the cultivation of narrow-leafed lupins (*Lupinus angustifolius* L.) increased of subsoil acidification, and this action is thought to be partly related to their pattern of nutrient uptake and $H^+/OH^-$ excretion. The main hypothesis of this study was that H+ and OH- excretion is not distributed evenly over the entire length of the root system but is limited to zones where cation or anion uptake occur in more than the amount needed.

The excess of cation over anion uptake was correlated positively with $H^+$ excretion in each rooting zone. In zones where $K^+$ was supplied at 1200 µM, cation uptake was dominated by $K^+$ and up to twice as much $H^+$ was excreted than in zones where $K^+$ was absent. In zones where $NO^{-3}$ was supplied at 750 µM, the anion/cation uptake was balanced: however $H^+$, excretion continued to occur in the zone. When $NO_3$ was supplied at 5000 µM, anion uptake exceeded cation uptake but there was no $OH^-$ excretion.

#### 2.3.2. Cations classified as enzyme activators and plant nutritional physiology.

Certain cations are considered as enzyme activating elements $Cu^{++}$, $Fe^{++}$, $K^+$, $Mg^{++}$, $Mn^{++}$ and $Zn^{++}$. Magnesium activates more enzymes than does any other mineral nutrient. (Epstein and Bloom 2005).There is a large number of enzymes in which zinc is

an integral component of the enzyme structure (zinc-enzymes). In these enzymes zinc has three functions: catalytic, cocatalytic (coactive), and structural (Vallee and Auld, 1990). During growth, plants need more nutrients than might be available (Abbasov 1991), therefore, supplying of optimum nutrients always been one of the problematic questions of plant science. A large copper supply usually inhibits root growth before shoot growth (Trehan and Sechon 1977, Mattoo 1986).

Enzyme activating elements need to be in optimum quantity and optimum ratio in the rhizosphere. Importance of these elements and their physiological roles were reported by other researchers. For example the activity and stability of mushroom tyrosinase were studied in ionic liquid (IL)-containing enzyme activators by Zhen and YaJun (2009). They report that, ILs and their inorganic salts were able to trigger enzyme activation. The effect of ILs on enzyme performance largely can be attributed to their ionic nature via interaction with the enzyme structure, the substrate, and the water molecules associated with the enzyme.

Magnesium and zinc were very low in activation efficiency in all cases, while manganese was optimally efficient. Cobalt was essentially equal to manganese for activation of the enzyme phosphoribosyltransferase from L. mexicana and L. braziliensis (Kidder and Nolan, 1982).

Potassium is highly mobile in plants at all levels, that is, from individual cell to xylem and phloem transport. This cation plays a major role in: enzyme activation. High levels of $K^+$ increase fruit size with thick and coarse peel (Alva 2006). In contrast, $K^+$ deficiency produces smaller fruits with thin peel. High K availability in the soil can

reduce the uptake of other cations, primarily magnesium, calcium, and ammonium N (Alva 2006).

Chakraborti and Banerjee (1979) report that, malathion (an organophosphorus) compound activated cation-activated enzyme-ATPase at 400 p.p.m. This increase in activity might be associated with some alteration in the membrane structure, and stimulation by malathion was non-competitive nature when the divalent and monovalent ions were included in the system. Plasma membrane bound ATPase of cowpea was activated by $Mg^{2+}$ and was further stimulated by monovalent cations like $Na^{+}/K^{+}$ at a definite pH and substrate concentration. The true substrate for the enzyme was $Mg^{2+}$ ATP. $Ca^{2+}$ could not replace $Mg^{2+}$ so far as the activation of this enzyme was concerned.

Importance of $Ca^{++}$ was described by Matsumoto and Kawasaki (1981). They report that, Activation of membrane-associated ATPase by various cations was decreased or lost during $Ca^{++}$ starvation. The basal ATPase activity of $Ca^{++}$-deficient enzyme increased for various substrates including pyrophosphate, p-nitrophenol phosphate, glucose-6 phosphate, β-glycerophosphate, AMP, ADP and ATP. $Mg^{++}$ activation was found only for ADP and ATP in both the complete and $Ca^{++}$ deficient enzymes, but the activation for ATP was greatly reduced by $Ca^{++}$ starvation. The heat inactivation curves for basal and $Mg^{++}$ activated ATPase did not differ much between the complete and $Ca^{++}$ deficient enzyme. The delipidation of membrane-associated enzyme by acetone affected the protein content and the basal activity slightly, but inhibited the $Mg^{++}$ activated ATPase activity clearly with somewhat different behavior between the complete and $Ca^{++}$ deficient enzyme.

Importance of monovalent cations was described by Hall, J. (1971). The author reported that the ATP-ase activity of cell-wall preparations from barley roots was stimulated by monovalent cations at alkaline pH values to levels higher than those obtained with $Ca^{++}$ or $Mg^{++}$ ions. $Na^{+}$ was the most effective cation, followed by $K^{+}$, $Li^{+}$ and $Rb^{+}$. Similar activation was obtained with a soluble enzyme fraction and with excised root tips. beta -glycerophosphatase activity was not stimulated by $Ca^{++}$ and only slightly by $Na^{+}$ and $K^{+}$.

It is important to note that, single type of ion can be transported by several enzymes, which need not be active all the time (constitutively), but may exist to meet specific, intermittent needs. This is one of the interests of this research and for what enzyme activators used with specific compartmentalization.

Based on the current knowledge in the literature, it can be concluded that other metabolic processes require increasing ATP concentration to achieve an increase in activity by enzyme activators by decreasing active transport due to increasing passive transport, the ATP concentration can reach high levels. By increasing passive transport can be decreased active transport which requires energy consequently more energy can be used for other physiological processes such as photosynthesis.

Split-root system can solve the problem of applying all fertilization at one time instead of partial applications during the entire growing period. For this reason, a specific nutrient ratio and concentration in the rhizosphere is important but must have a positive correlation with plant nutritional physiology under the split root system.

Mineral nutrient compartmentalization is a way to improve plant nutrition, because as described above, different concentrations of nutrients in different parts of the medium have an impact on plant physiology- either positively or negatively. Accordingly, finding the right location and right concentration of nutrients in the medium may positively impact plant physiology.

Moreover, by use of the split-root system water could move from one side of a root system to the other side of root for compensation of the side with less water.

Compartmentalizing nutrient source by dividing in different pH zones is a way of optimizing plant nutritional physiology due to keeping pH range at 6 to 7 during entire vegetation period.

The pH remained relatively stable for some time, can be dropped due to an energy shortage, leading a general breakdown of transmembrane gradients and, finally, to cell death unless the plant is able to gains access to another energy source.

Besides, literature review suggests that, colloidal nutrient solution could be used to improve plant nutritional physiology.

The nutrition system investigated in the current research has partial root drying media, which improve plant nutrition due to increasing antioxidant enzymes activity.

# CHAPTER 3

# SPLIT ROOT NUTRITION SYSTEM MECHANISMS

## 3.1 Introduction

The split-root nutrition system investigated in this research is the same as common-traditional split root nutrition, but it differs with including an advanced design of root zone media based on high, low concentration of nutrients. Namely this system can be called root nutrient selection system due to promoting passive transport. Below we will discuss about mechanisms related to basil root phenomenon in different media using initial trials with Petri plates and applying it to container experiment.

## 3.2 Materials and methods

### 3.2.1. Plant material and preparing nutrient solutions

Sweet basil (*Ocimum basilicum* L) was used in these studies because basil has a short growing period of fifty days (Craker 1998). Locally purchased seeds were germinated, because these seeds are best in the climate condition where current experiments were conducted. Germination was in sand that had been washed with distilled water to remove any water-soluble nutrients was washed with a weak sulfuric acid solution to remove organic nitrogen, and subsequently uses washed several times

with distilled water to remove any acid residue. At 12 days after seeding, individual seedlings were removed gently from the medium and tested for growth in the experimental split-root system.

The split-root system involved a division of the plant roots into two parts of the medium with each part having a different level of nutrition, different salinity, and different pH (Shani and Waisel 1993; Shen and Neumann 2005; Shu L. *at all*. 2005; Zhu, Y. 2000). The medium was prepared from premade stock solutions as described in Figure 3.1. Preparation of each of the SSH (stock solution Hoagland) and SSE (stock solution experimental) are described in Figure 3.2.

SSH-1. Hoagland modified solution (Epstein and Bloom 2005) was prepared as a modified Hoagland solution which included nitrogen as $NH_4^+$ and $NO_3^-$. The Hoagland nutrients solution was prepared as described in Figure 3.1., and Table 3.1.; 3.2.; 3.4., by following steps described below:

1) Made up stock solutions and stored in separate bottles with appropriate label.

2) Added each component in the amount described in Figure 3.1 to 800 mL deionized water then filled to 1L.

3) After the solution is mixed, it called SSH-1 (Stock solution Hoagland), and it considered as one dose or 1X.

The total volume of this stock solution is 1L. From this stock solution, a volume of 725 mL was used for all solutions on the low concentrated side of the HS (Hoagland solution) treatments.

The procedure for preparing SSH-2 solution was the same as that for the SSH-1, except that the stock solutions were made 30 times more concentrated than in the normal preparation of stock solutions for Hoagland's solution (Figure 3.1.). The total amount of

this stock solution prepared was 3.5L, with 3.339L being used. SSE (stock solutions experimental) also prepared same as SSH and differences are shown in Figures 3.1., and 3.2.

1) SSH-1: (SSH-Stock Solution-Hoagland). Prepared for low concentrated side of Hoagland treatments.
2) SSH-2: (SSH-Stock Solution-Hoagland) prepared for high concentrated side of Hoagland treatments.
3) SSE-1: (SSE-Stock Solution-Experimental) prepared for low concentrated side of the experimental solution treatments.
4) SSE-2: (SSE-Solution-Experimental) prepared for high concentrated side of the experimental treatments.

In the next step prepared following diluted solutions as described in Figure 3.1:

1) DSH-1 (Diluted Solution-Hoagland) which was prepared by diluting SSH-1 for use of low concentrated side of the HS treatments
2) DSH-2 (Diluted Solution–Hoagland) which was prepared by diluting SSH-2 for use on high concentrated side of the HS treatments
3) DSE-1 (Diluted Solution-Experimental) which was prepared by diluting SSE-1 for use of low concentrated side of the ES treatments
4) DSE-2 (Diluted Solution-Experimental) which was prepared by diluting SSE-2 for use of high concentrated side of the ES treatments

The diluted Hoagland's and Experimental solutions for the low level side were prepared in the following concentrations by addition of distilled water to make test samples that were 100, 20, 15, 10, 5, or 2.5 percent of the diluted nutrient solutions, because these levels were best results of the initial mathematic probability calculations (Tables 3.1.; 3.2; 3.4.;). The 100 percent solution was used on the high concentration side and dilutions, in separate trials, were used on the low concentration side (Table 3.3.)These levels, arrangements used because of preliminary calculations results and according to results of previous experiments. The Hoagland solution is well known popular in the world solution for traditional growing plants, for what as a control were chosen Hoagland solution.

The diluted Hoagland's and Experimental solutions for the high level side were prepared in the following concentrations by the addition of distilled water to make test samples that were 3.33, 33.33, 49.9, 66.66, 83.25, and 100 percent of the stock solution. The 3.33% was used as the control, given that this is the typical percentage for growing plants (Smirnov 1957; Peterburgskiy 1949; Chesnokov 1983 and Duke 1990).

SSE-1. This is the base experimental solution prepared in the same way as the Hoagland Solution (Tables 3.1.; 3.2., and 3.4). Differences between experimental solution and Hoagland solution are described in Tables 3.1; 3.2.; 3.4., and Figures 3.1; 3.2. Chemical compounds for preparation of experimental solution are described in Table 3.1. Amounts necessary for stock solutions are measured from these, and then experimental macro and micro nutrient stock solutions are prepared. From the stock solutions, the macronutrient final solution is first prepared, followed by the micronutrient final solution. The micronutrient final solution is then poured gently into the

macronutrient final solution and mixed well. This mixture is called 1 dose. The total volume of this stock solution is 1L and from this 725 mL were used for all solutions in the low concentrated side of the ES treatments.

SSE-2 Procedure of preparing this solution is same as SSE-1 and only difference is from the Stock solutions taken 30 time more than Experimental base solution preparation procedure. It called 30 doses. Total amount of this stock solution prepared is 3.5L and used 3.339L.

DSE-1. From SSE-1 prepared 6 solutions by diluting with distilled water: 100%; 20%; 15%; 10%; 5% and 2.5%. It called 1d (d-dose), 0.2d; 0.15d; 0.1d and 0.025d. 1d used for Control treatment of the ES treatments and rest of them used for Low concentrated side of the ES treatments (Figure 3.2.).

DSE-2. From SSE-2 prepared 6 solutions by diluting with distilled water: 3.33%; 33.33%; 49.95%; 66.66%; 83.25% and 100%. It called 1d (d-dose), 10d; 15d; 20d; 25d and 30d. 1d used for Control treatment of the ES treatments and rest of them used for High concentrated side of the ES treatments (Figure 3.2.).

Experimental media preparation procedure of the Experiment with containers are same as described above, only differences is using better treatments from the Initial experiment and skipping not important treatments.

### 3.2.2. Experimental methodology

#### 3.2.2.1 Initial trials using Petri plates

Initial trial

was done in controlled environment facility (25 ± 3$^{o}$ C, 16 h light-8 h dark cycle, RH 65-75%), using a low-concentrated nutrient solution and a high-concentrated nutrient solution contained in separate Petri plates. The dishes were subsequently placed next to each other, and a cotton ball (CVS store brand) was placed on the edges of where the two plates touched each other. A basil seedling was placed on the cotton ball, and the seedling roots were split (divided) into two, approximately equal portions. One portion of the roots was placed in a dish containing the low-concentrated media and the other portion of the roots was placed in a dish containing the high-concentrated media (Figure 3.3).

The seedlings were allowed to stay in Petri dishes for 30 days. During this 30 day period there was no significant increase in the sizes of the basil seedlings. However, between treatments, there were significant differences in leaf color changes and seedling survival over the 30-day optimum period for visual observation. Observations on the growth and development (leaf color, leaf size, and plant mortality) were made weekly and scored using a 1 (worst) to 30 (best) scale for overall plant appearance.

#### 3.2.2.2. Container trial.

Using containers is one of the best traditional experiment methods after initial trial with Petri dishes and before starting to test in big boxes. For what in the next step we did use container experiment. The container trial for testing the split root system was done using four replications, two paired media containers (2 L each). The paired containers (5 cm wide x 20 cm length x 20 cm deep) were fit side by side inside a larger

plastic box to hold the containers upright (Figure 3.4; 3.5.). Each of the paired media containers had a Tygon tube attached to an opening at the bottom so that additional media could be added. The chambers were filled with the washed sand described above to a height of 4.8 cm, the top of the containers was covered with cotton balls (CVS Brand), and a plastic mesh to support the plant material.

To initiate the experiment, the sand in each container was moistened with distilled water, and six sweet basil (cv. German) were placed on the cotton below the mesh for seed germination. The containers were placed in the previously described controlled environmental chamber (Figure 3.6.; 3.7.). After the seeds had germinated, the seedlings were thinned to three per container, and except for controls-traditional growing systems, each container was filled with the treatment media (high concentration in one container and a low concentration in the paired container). A control container pair was filled with Hoagland's solution and a control container pair was filled with the experimental solution. The root systems of the plants in the treatment chambers were divided with approximately one-half placed in the high concentrated media and one-half in the low concentrated media. Roots of the plants in the comparing-treatment were not split, but remained in the same media. The nutrient solution in the treated containers was never changed, but the plants were watered from the bottom as needed to maintain the plants. The nutrient solution in the none-split chambers was changed weekly by flushing the containers with water and adding fresh nutrient solution.

After 45 days growth, the foliage of the plants was harvested and re-harvested two weeks later by cutting the stems 5 cm above the plastic mesh. The plant fresh weight and dry weight (air-dried to a constant weight) of the harvested plant tissue were

measured. The dry matter was analyzed subsequently for macro and micro element content and for essential oil yield. Total N analyzed using Kheldal method, and other elements analyzed using spectrophotometer in UMass Amherst plant and soil test laboratory.

The above container trial was repeated, but in trials the basil seedlings (3 to 4 cm high) were used in place of the seeds. The experimental solution was based on Hoagland solution.

### 3.3 Results and Discussion

#### 3.3.1. Basil response to high-low nutrient concentration of the root zone.

The basil plants growing in split root, high-low nutrition system demonstrated usual growth and development patterns. The highest concentration of nutrient solution for the Hoagland or Experimental nutrient solution to produce good growth of the basil was limited to 25 times normal nutrient concentration (Table3.6).

During preparation of high concentrated solution, such as 10, 20, 25 times more concentrated than traditional normal, $CaSO_4$ may occur as sediment. But, results of our experiment showed that, plant did not have Ca or S deficiency due to $CaSO_4$ sediment, because of two reason: 1) amount of sediment is more significant only after 25 time concentrated solution and 2) plant can receive Ca and S from $CaSO_4$, because during growing period $CaSO_4$ may dissolve, which makes Ca and $SO_4$ available for plant uptake. The hydrated $CaSO_4$ formed has a solubility of 2.49g/L in saturation (American Chemical

Society, 2000) at $20^{o}$ C. All reaction was performed at or above $25^{o}$ C, the actual solubility was greater than 2.4 g/L. High concentrated part of experimental containers had -6025 mg/L $Ca^{++}$ and 4000 mg/L $SO^{-}$ and 50% uptake from total is equal to 2000 g/fresh weight of basil in each container, so this solubility is more than enough $Ca^{++}$ and $SO_4^{-}$ for the basil plant growth and developments.

The most growth of the basil plants occurred in the experimental solution in which the solution ratio of high to low ranged from 25 to 0.1 percent of normal solution levels. The plants in the experimental solution produced growth judged to be two times better than growth in Hoagland's solution. Plants grown in nutrient solutions without using a split root system exhibited better growth in the Hoagland's solution than with the Experimental solution. This experiment determined approximate what highest doses can be tested for future professional test. 20 and 25 dose used both with Hoagland solution and experimental solution show good results (Table 3.7.; 3.8.). Especially with experimental solution which has 20 doses combined with 0.1 low doses show best result which had 30 visual value score. Gaining nutrients from isolated compartments reported by researcher Ivanov (2009) confirm phenomenon of our experiment. Experimental solution comparing with Hoagland solution had two time better result. It is known that Hoagland solution (Hoagland 1950) is general universal solution which used for many different plants (Takano 1993; Marschner 1995 and Kane 2006).

Our experimental solution was prepared based on Hoagland solution and according to chemical constituents of basil as well, so experimental solution is specifically for basil. With no split, one dose of Hoagland solution had much better result than one dose of no-split experimental solution, but by increasing concentration with

split-root Experimental solution showed much better result than Hoagland solution. Other researchers reported strong relationships between nutrient solution, turgor and osmotic potential in individual epidermal cells (Kenneth 1987) and stomatal conductance (Kirkham 1972). Accordingly from Hoagland solution and experimental solution 20 and 25 time more concentrated than base solution were chosen for future container experiments.

### 3.3.2. Effect of mineral nutrients on productivity of basil using split root nutrition.

Productivity of the basil was increased through the use of the split-root system (Table 3.7.). With the experimental solution, plant productivity was increased over 120% compared to none split-root system. Use of the split root system with a Hoagland's solution increased yields 13% compared to none split-root system. Nutrient treatments in the split-root system producing higher foliar yields growth had increased uptake of macro-elements as compared with plants not grown in a split root system (Figure 3.9.; 3.10.; 3.11.; 3.12; 3.13.)

Split root nutrition system increased basil productivity (Table 3.14). Especially with experimental solution productivity increased more than 120 % than none-split Hoagland solution treatment, when split root nutrition with Hoagland solution increased productivity only 13 % more than its none-split treatment. It shows that split root nutrition is effective way for growing plant but needs to have a specific solution for each plant according to chemical constituents of plant. Increasing productivity had relationships with macroelement uptake. (Jackson 2000; Kane et al. 2006, and Karioti 2003) Treatments which had more productivity (Table 3.7.), had more accumulation of

macroelements (Figure 3.9.), especially treatments with split root nutrition in 20 dose combined with 0.1 dose low concentrated media. All macro-elements significantly increased in split-root treatments, comparing with no split root. However, treatments with experimental solution had much higher macro-element accumulation than treatments with Hoagland solutions.

Analysis of essential oil did show same as productivity increased in treatments with split root and best results was with Experimental solution 20 doses with split root-20 doses high concentrated and 0.1 dose low concentrated media. Difference between split-root with experimental solution and none split-root using Hoagland solution was more than two time (Table 3.7.). Productivity and essential oil depends on plant nutrition (Epstein 2005; Gan 2008) and same time plant nutrition depends on pH of the media (Kane 2006; Hitsuda 2005). According to results of analysis dynamic of the pH showed that with up to 20 doses of high concentrated media with one time application, it is possible keep optimum pH for plant. Researchers Lykas et al. (2006), Laulhere et al. (1993) confirm that it is possible predict pH in a nutrient solution during growing period and needs to be adjusted time to time. So, with treatments 20 times more concentrated than base nutrient solution combined with 0.1 time diluted than base nutrient solution, media pH was same all vegetation period as treatment which had change of nutrient solution weekly. Increasing nutrient solution concentration up to 25 times more than base nutrient solution in split-root nutrition acidified top media. Usually when solution is highly concentrated, it will be more acidic, but in split-root nutrition case, it was true only in treatment with 25 times more concentrated solution than base solution. In treatment with 20 times more concentrated solution had optimum pH conditions for plant same as

the treatment with none concentrated base nutrient solution (Table 3.8.). Changing pH also very much depends on dynamics of nutrient elements. Dynamics of $NO_3^-$ (Table 3.12), and $NH_4^+$ (Table 3.13.) prove that ,in split-root nutrition even high dose of nutrient such as 20 dose, $NO_3^-$ and $NH_4^+$ level in top media were in optimum amount, which allowed plant grow and develop.

### 3.3.3 Basil root development and productivity depending on seeding and transplanting under split root nutrition system

Main mechanism of split-root nutrition system is a phenomenon of osmotic regulation which allows normal amount of nutrients in the top of the media (Table 3.12.and Table 3.13.). Other phenomenon is: 'plant regulates its uptake of nutrients' (Figure 3.9.) according to its root development (Table 3.16. and Figure 3.17). For what, we did check experiment in split-root nutrition system with seeding and transplanting.

Treatments that were seeded comparing transplanted treatments had best result with increased productivity (Table 3.14) and increased root mass (Table 3.15) growth and development was active (Figure 3.11; 3.12.), and in 5 days height increased up to 19 cm. It is the results of seeded plants root locating in optimized nutrients. Effect of the optimized nutrients to better root formation confirmed with researchers Liangzuo *at al*. (2007) and Loughrin (2001).

Where was transplanted, productivity and root mass of split-root nutrition treatments was 74 to 88% less than treatment with no split-root, weekly changed solution. With treatments with increased doses, even basil plant could not survive (Figure 3.10.; 2.13; 2.14.). Negative effect of increased doses can be explained by effect of low pH and toxicity; however, it is known that continual pH lowering will have negative affect on

growth and development (Mizuno *et al*. 2006). The results of the present experiment showed that if some part of root is inverted manually in high concentrated nutrient solution and other part is in the low concentrated solution, plant shoot will have toxicity problem. With seeding instead of transplanting, plant roots will develop everywhere, but root development in high-concentrated area will slow down, resulting in less root volume in high concentrated media and more root volume in low concentrated media (Figure 3.3.; 3.4.; 3.16.;3.17.) will regulate nutrition due to signal when root reach in high concentrated media.

### 1.4. Conclusions

Using split-root nutrition compared to no split-root nutrition can significantly increase productivity of sweet basil due to balanced plant nutrition. It is known that pH of media is important for plant nutrition; however, split nutrition allows optimum pH to be in media and improves nutrient uptake. Results of experiments with split-root nutrition did show that it is possible to apply 100% nutrients necessary for potential production of basil at one time before seeding and avoiding many other expenses that are used in traditional growing systems.

According to experiment which we called “Seeding and transplanting in split-root nutrition system” we found that plant can avoid nutrient toxicity by different way of developing its root due to high water potential of low-concentrated media of the split-root nutrition system.

Table 3.1 Compounds used in Hoagland solution (HS) and compounds used in experimental solution (ES)

| # | HS | # | ES |
|---|---|---|---|
| 1 | $KNO_3$ | 1 | $KNO_3$ |
| 2 | $Ca(NO_3)_2.4H_2O$ | 2 | $Ca(NO_3)_2.4H_2O$ |
| 3 | $NH_4H_2PO_4$ | 3 | $NH_4H_2PO_4$ |
| 4 | $MgSO_4.7H_2O$ | 4 | $MgSO_4.7H_2O$ |
| 5 | KCl | 5 | $(NH_4)_2SO_4$ |
| 6 | $H_3BO_3$ | 6 | $KH_2PO_4$ |
| 8 | $MnSO_4.H_20$ | 7 | KCl |
| 9 | $ZnSO_4.7H_2O$ | 8 | $H_3BO_3$ |
| 10 | $CuSO_4.5H_2O$ | 9 | $MnSO_4.H_20$ |
| 11 | $H_2MoO_4$ | 10 | $ZnSO_4.7H_2O$ |
| 12 | Na Fe-EDTA(10% Fe) | 11 | $CuSO_4.5H_2O$ |
| 13 | $NiSO_4.6H_2O$ | 12 | $H_2MoO_4$ |
| 13 | $Na_2SiO_3.9H_2O$ | 13 | Na Fe-EDTA(10% Fe) |

Table 3.2 Base modified Hoagland solution (HS) compared with experimental solution (ES)

| Element | Final concentration of element mg/L | Final concentration of element mg/L |
|---|---|---|
| | HS | ES |
| Macroelements | | |
| N | 224 | 308 |
| P | 62 | 93 |
| K | 235 | 469 |
| Ca | 160 | 241 |
| Mg | 24 | 73 |
| S | 32 | 160 |
| Microelements | | |
| Cl | 1.77 | 21.3 |
| B | 0.27 | 1.89 |
| Mn | 0.11 | 1.37 |
| Zn | 0.13 | 0.85 |
| Cu | 0.03 | 0.27 |
| Mo | 0.05 | 0.10 |
| Fe | 3 | 6 |
| Ni | 0.03 | 0 |
| Si | 28 | 0 |

Table 3.3 Nutrient ratio in Hoagland solution (HS) and experimental solution (ES)

| Element | Ratio of elements based on N equal to 1; | | Element | Ratio of elements based on N equal to 1; | |
|---|---|---|---|---|---|
| | HS | ES | | HS | ES |
| N | 1 | 1 | B | 0.0012 | 0.0061 |
| P | 0.28 | 0.3 | Mn | 0.0005 | 0.0045 |
| K | 1.05 | 1.52 | Zn | 0.0006 | 0.0028 |
| Ca | 0.72 | 0.78 | Cu | 0.0001 | 0.0009 |
| Mg | 0.11 | 0.24 | Mo | 0.0002 | 0.0003 |
| S | 0.14 | 0.52 | Fe | 0.0134 | 0.0194 |
| Cl | 0.0079 | 0.069 | Ni | 0.0001 | 0.0000 |

Table 3.4 Treatments of Laboratory experiment with Petri dishes.

| Treatments with Hoagland solution or with experimental solution |
|---|
| (1X),no Split, control |
| (1X); (1X);* |
| (10X); (0.025X); |
| (10X); (0.05 X); |
| (10X); (0.10X); |
| (10X); (0.15X); |
| (10X); (0.20X); |
| (15X); (0.025X); |
| (15X); (0.05 X); |
| (15X); (0.10X); |
| (15X); (0.15X); |
| (15X); (0.20X); |
| (20X); (0.025X); |
| (20X); (0.05 X); |
| (20X); (0.10X); |
| (20X); (0.15X); |
| (20X); (0.20X); |
| (25X); (0.025X); |
| (25X); (0.05 X); |

| |
|---|
| (25X); (0.10X); |
| (25X); (0.15X); |
| (25X); (0.20X); |
| (30X); (0.025X); |
| (30X); (0.05 X); |
| (30X); (0.10X); |
| (30X); (0.15X); |
| (30X); (0.20X); |

*First parenthesis represents one side and second one represents other side of the basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

Table 3.5 Response of Basil seedlings under Split its root into high and low concentrated media (Visual valuation based on survived day, color and size of leafs, 1 is worst and 30 is best).

| Treatments (1st split side) | Treatments (2nd split side) | | | | | | |
|---|---|---|---|---|---|---|---|
| | None | 1X | 0.025X | 0.05X | 0.1X | 0.15X | 0.2X |
| | Hoagland | | | | | | |
| 1X | 7c | 5d | | | | | |
| 10X | | | 3e | 3e | 3e | 6d | 6d |
| 15X | | | 3e | 3e | 6de | 9c | 9c |
| 20X | | | 9b | 9c | 14c | 12bc | 9c |
| 25X | | | 3e | 8d | 15c | 7d | 3e |
| 30X | | | 4d | 3e | 3e | 2e | 3e |
| | Experimental | | | | | | |
| 1X | 5d | 8b | | | | | |
| 10X | | | 6c | 9c | 9d | 15b | 9c |
| 15X | | | 15a | 15a | 21b | 22a | 15bc |
| 20X | | | 15a | 17a | 30a | 23a | 21b |
| 25X | | | 13ab | 12b | 27a | 20ab | 25a |
| 30X | | | 3e | 3e | 2e | 3e | 2e |

Mean separation in columns by Duncan’s multiple range test at P=0.05.

Note:

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

Table 3.6 Effect of split-root nutrition on basil productivity

| Treatments | | g/pot, f.w. | g/pot, d.w. | Differences from control, % |
|---|---|---|---|---|
| With Hoagland solution | | | | |
| 1 | (1X), no split, control | 361bc | 44.8bc | 0 |
| 2 | (1X); (1X);* | 353c | 41.8c | -6.7 |
| 3 | (20X); (0.1X); | 426b | 50.7b | 13.2 |
| 4 | (25X); (0.1X); | 281d | 33.8d | -24.5 |
| Mean HS | | 355 | 42.8 | |
| With experimental solution | | | | |
| 5 | (1X), no split, control | 112e | 13.1e | -70.8 |
| 6 | (1X); (1X); | 116e | 14.2e | -68.3 |
| 7 | (20X); (0.1X); | 771a | 100.2a | 113.7 |
| 8 | (25X); (0.1X); | 278d | 32.4d | -27.7 |
| Mean ES | | 319 | 40 | |

Mean separation in columns by Duncan's multiple range test at P=0.05
Note:
*First bracket represents one side and second one represents other side of the basil roots for all treatments.
X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

Table 3.7 Effect of split root nutrition on basil essential oil

| # | Treatments | % d.w. | Differences from control, % | mg/pot | Differences from control, % |
|---|---|---|---|---|---|
| With Hoagland solution | | | | | |
| 1 | (1X), no split, control | 0.23bc | 0 | 103c | 0 |
| 2 | (1X); (1X);* | 0.21cd | -8.7 | 88d | -15 |
| 3 | (20X); (0.1X); | 0.25b | 8.7 | 127b | 23 |
| 4 | (25X); (0.1X); | 0.21cd | -8.7 | 71de | -31 |
| | Mean HS | 0.23 | | 97 | |
| With experimental solution | | | | | |
| 5 | (1X), no split | 0.18e | -21.7 | 25e | -77 |
| 6 | (1X); (1X); | 0.22cd | -4.3 | 31e | -70 |
| 7 | (20X); (0.1X); | 0.32a | 39.1 | 311a | 211 |
| 8 | (25X); (0.1X); | 0.32a | 39.1 | 103c | 0 |
| | Mean ES | 0.26 | | 118 | |

Mean separation in columns by Duncan's multiple range test at P=0.05.
*First bracket represents one side and second one represents other side of the Basil roots for all treatments.
X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

Table 3.8 Effect of split root nutrition on dynamics of pH

| # | Treatments | Day | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 15 | 30 | 45 | 60 |
| With Hoagland solution | | | | | | |
| 1 | (1X), no split, control | 6.5 | 6.3 | 6.3 | 6.5 | 6.8 |
| 2 | (1X); (1X);* | 6.6 | 6.1 | 6.1 | 6.6 | 6.7 |
| 3 | (20X); (0.1X); | 6.5 | 6.2 | 5.8 | 5.5 | 5.3 |
| 4 | (25X); (0.1X); | 6.5 | 5.1 | 4.5 | 4.5 | 4.3 |
| With experimental solution | | | | | | |
| 5 | (1X), no split | 6.7 | 6.8 | 6.5 | 6.6 | 6.9 |
| 6 | (1X); (1X); | 6.5 | 6.2 | 6.6 | 6.5 | 7.0 |
| 7 | (20X); (0.1X); | 6.5 | 6.3 | 6.5 | 6.3 | 6.2 |
| 8 | (25X); (0.1X); | 6.3 | 5.1 | 4.1 | 4.3 | 4.1 |

Note:
*First bracket represents one side and second one represents other side of the Basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

Table 3.9 Initial amount of cations and anions in both media, mg/L

| # | Treatments | Medium | Cations | | | | |
|---|---|---|---|---|---|---|---|
| | | | K+ | Ca+2 | NH4+ | Mg+2 | H+ |
| With Hoagland solution | | | | | | | |
| 1. | (1X), no split, control | Mb | 236 | 160 | 36 | 24 | 2.03 |
| 2. | (1X); (1X);* | M1 | 236 | 160 | 36 | 24 | 2.03 |
| | | M2 | 236 | 160 | 36 | 24 | 2.03 |
| 3. | (20X); (0.1X); | M1 | 4731 | 3206 | 720 | 480 | 40.6 |
| | | M2 | 23 | 16 | 3.6 | 2.4 | 0.2 |
| 4. | (25X); (0.1X); | M1 | 5914 | 4008 | 900 | 600 | 50 |
| | | M2 | 23 | 16 | 3.6 | 2.4 | 0.2 |
| With experimental solution | | | | | | | |
| 5. | (1X), no split | Mb | 469 | 220 | 72 | 72 | 2.67 |
| 6. | (1X); (1X); | M1 | 469 | 220 | 72 | 72 | 2.67 |
| | | M2 | 469 | 220 | 72 | 72 | 2.67 |
| 7. | (20X); (0.1X); | M1 | 9384 | 4408 | 1440 | 1440 | 53.4 |
| | | M2 | 47 | 22 | 7.2 | 7.2 | 0.27 |
| 8. | (25X); (0.1X); | M1 | 11730 | 5511 | 1801 | 1800 | 66.75 |
| | | M2 | 46 | 22 | 7.2 | 7.2 | 0.27 |

Note:
*First bracket represents one side and second one represents other side of the Basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

Mb-medium in the bottom (lower side, after 5 cm of top medium);

M1-medium in one side of the root

M2- Medium in second side of the root

Table 3.10 Initial amount of cations and anions in both media, mg/L

| # | Treatments | Medium | Cations and anion | | | | |
|---|---|---|---|---|---|---|---|
| | | | $Mn+^{2}$ | $Zn+^{2}$ | $Cu+^{2}$ | $Fe+^{3}$ | $NO^{-3}$ |
| With Hoagland solution | | | | | | | |
| 1. | (1X), no split, control | Mb | 0.11 | 0.13 | 0.03 | 3 | 620 |
| 2. | (1X); (1X);* | M1 | 0.11 | 0.13 | 0.03 | 3 | 620 |
| | | M2 | 0.11 | 0.13 | 0.03 | 3 | 620 |
| 3. | (20X); (0.1X); | M1 | 2.2 | 2.6 | 0.6 | 60 | 12402 |
| | | M2 | 0.01 | 0.01 | 0.003 | 0.3 | 62 |
| 4. | (25X); (0.1X); | M1 | 2.75 | 3.25 | 0.75 | 75 | 15502 |
| | | M2 | 0.01 | 0.01 | 0.003 | 0.3 | 62 |
| With experimental solution | | | | | | | |
| 5. | (1X), no split | Mb | 1.37 | 0.85 | 0.27 | 6 | 682 |
| 6. | (1X); (1X); | M1 | 1.37 | 0.85 | 0.27 | 6 | 682 |
| | | M2 | 1.37 | 0.85 | 0.27 | 6 | 682 |
| 7. | (20X); (0.1X); | M1 | 27.4 | 17 | 5.4 | 120 | 13642 |
| | | M2 | 0.14 | 0.09 | 0.03 | 0.6 | 68 |
| 8. | (25X); (0.1X); | M1 | 34 | 21 | 6.75 | 150 | 17052 |
| | | M2 | 0.14 | 0.09 | 0.03 | 0.6 | 68 |

Note:
*First bracket represents one side and second one represents other side of the Basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

Mb-media in the bottom;

M1-Media in one side of the root

M2- Media in second side of the root

Table 3.11 Initial amount of anions in both media, mg/L

| Treatments | Medium | Anions | | | | |
|---|---|---|---|---|---|---|
| | | $H_2PO_4^-$ | $SO_4^{-2}$ | $Cl^{-1}$ | $BO_3^{-3}$ | $MoO_4^{-3}$ |
| With Hoagland solution | | | | | | |
| (1X), no split, control | Mb | 189 | 101 | 1.77 | 1.47 | 0.08 |
| (1X); (1X);* | M1 | 189 | 101 | 1.77 | 1.47 | 0.08 |
| | M2 | 189 | 101 | 1.77 | 1.47 | 0.08 |
| (20X); (0.1X); | M1 | 3798 | 2033 | 35 | 29.4 | 1.6 |
| | M2 | 18 | 10 | 0.18 | 0.15 | 0.008 |
| (25X); (0.1X); | M1 | 4748 | 1541 | 44 | 36 | 2 |
| | M2 | 19 | 10 | 0.18 | 0.15 | 0.008 |
| With experimental solution | | | | | | |
| (1X), no split | Mb | 237 | 494 | 121.7 | 10 | 0.16 |
| (1X); (1X); | M1 | 237 | 494 | 121.7 | 10 | 0.16 |
| | M2 | 237 | 494 | 121.7 | 10 | 0.16 |
| (20X); (0.1X); | M1 | 4748 | 9894 | 2434 | 205 | 3.2 |
| | M2 | 23 | 49 | 12.17 | 1.03 | 0.02 |
| (25X); (0.1X); | M1 | 5935 | 12368 | 3042 | 257 | 4 |
| | M2 | 24 | 49 | 12.17 | 1.03 | 0.02 |

*First bracket represents one side and second one represents other side of the Basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

Mb-media in the bottom;

M1-Media in one side of the root

M2- Media in second side of the root

Table 3.12 Effect of Split root nutrition on dynamics of $NO_3$ in all media

| Treatments | Medium | $NO_3^-$ in pot | | | | |
|---|---|---|---|---|---|---|
| | | Day 1 | | Day 20 | Day 40 | Day 60 |
| | | mg/L | mg/pot | mg/pot | mg/pot | mg/pot |
| With Hoagland solution | | | | | | |
| 1. (1X), no split, control | Mt | 0 | 0 | 75 | 42 | 32 |
| | Mb | 620 | 1860 | 940 | 350 | 90 |
| 2. (1X); (1X); | Mt | 0 | 0 | 70 | 38 | 30 |
| | Mb1 | 620 | 930 | 520 | 210 | 50 |
| | Mb2 | 620 | 930 | 470 | 220 | 52 |
| 3. (20X); (0.1X); | Mt | 0 | 0 | 90 | 75 | 62 |
| | Mb1 | 12400 | 18600 | 9700 | 4550 | 1870 |
| | Mb2 | 62 | 93 | 55 | 78 | 65 |
| 4. (25X); (0.1X); | Mt | 0 | 0 | 150 | 120 | 95 |
| | Mb1 | 15500 | 23250 | 18070 | 7890 | 3700 |
| | Mb2 | 62 | 93 | 76 | 56 | 80 |
| With experimental solution | | | | | | |
| 5. (1X), no split | Mt | 0 | 0 | 80 | 75 | 55 |
| | Mb | 682 | 2046 | 1050 | 780 | 105 |
| 6. (1X); (1X); | Mt | 0 | 0 | 40 | 35 | 30 |
| | Mb1 | 680 | 1020 | 480 | 195 | 95 |
| | Mb2 | 680 | 1020 | 520 | 180 | 25 |
| 7. (20X); (0.1X); | Mt | 0 | 0 | 30 | 28 | 25 |
| | Mb1 | 13640 | 20460 | 9860 | 3720 | 325 |
| | Mb2 | 68 | 102 | 85 | 45 | 120 |
| 8. (25X); (0.1X); | Mt | 0 | 0 | 60 | 54 | 34 |
| | Mb1 | 17050 | 25580 | 12880 | 6800 | 3600 |
| | Mb2 | 68 | 100 | 45 | 30 | 35 |

*First bracket represents one side and second one represents other side of the Basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

Mt-media in the top; Mb-media in the bottom; M1-Media in one side of the root;M2- Media in second side of the root;

Table 3.13 Effect of Split root nutrition on dynamics of $NH_4^+$ in all media, mg/pot in high and low concentrated media and mg/kg in top media

| Treatments | Medium | $NH_4^+$ in pot | | | | |
|---|---|---|---|---|---|---|
| | | 1st day | | 20 day mg/pot | 40 day mg/pot | 60 day mg/pot |
| | | mg/L | mg/pot | | | |
| With Hoagland solution | | | | | | |
| 1. (1X), no split, control | Mt | 0 | 0 | 15 | 7 | 3 |
| | Mb | 36 | 108 | 40 | 13 | 4 |
| 2. (1X); (1X);* | Mt | 0 | 0 | 13 | 6 | 3 |
| | Mb1 | 36 | 54 | 19 | 10 | 3 |
| | Mb2 | 36 | 54 | 20 | 11 | 2 |
| 3. (20X); (0.1X); | Mt | 0 | 0 | 20 | 15 | 11 |
| | Mb1 | 720 | 1080 | 490 | 245 | 103 |
| | Mb2 | 3.6 | 5.4 | 5 | 4 | 2 |
| 4. (25X); (0.1X); | Mt | 0 | 0 | 14 | 11 | 8 |
| | Mb1 | 900 | 1350 | 687 | 338 | 227 |
| | Mb2 | 3.6 | 6 | 5 | 3 | 3 |
| With experimental solution | | | | | | |
| 5. (1X), no split | Mt | 0 | 0 | 30 | 14 | 7 |
| | Mb | 72 | 216 | 123 | 59 | 11 |
| 6. (1X); (1X); | Mt | 0 | 0 | 10 | 7 | 4 |
| | Mb1 | 72 | 108 | 49 | 19 | 7 |
| | Mb2 | 72 | 108 | 51 | 24 | 6 |
| 7. (20X); (0.1X); | Mt | 0 | 0 | 27 | 16 | 5 |
| | Mb1 | 1440 | 2160 | 943 | 109 | 41 |
| | Mb2 | 7.2 | 11 | 8 | 7 | 3 |
| 8. (25X); (0.1X); | Mt | 0 | 0 | 37 | 30 | 8 |
| | Mb1 | 1800 | 2702 | 1413 | 970 | 562 |
| | Mb2 | 7.2 | 11 | 11 | 10 | 9 |

*First bracket represents one side and second one represents other side of the Basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

Mt-media in the top; Mb-media in the bottom; M1-Media in one side of the root; M2- Media in second side of the root;

Table 3.14 Effect of seeding and transplanting on productivity of basil under split root nutrition system

| # | Treatments | Transplanting | | Seeding | |
|---|---|---|---|---|---|
| | | g/pot, f.w. | Differences from control, % | g/pot, f.w. | Differences from control, % |
| With Hoagland solution | | | | | |
| 1 | (1X), no split, control | 315a | 0 | 334c | 0 |
| 2 | (1X); (1X);* | 298b | -5 | 323c | -3 |
| 3 | (20X); (0.1X); | 57de | -82 | 395b | 18 |
| 4 | (25X); (0.1X); | 35e | -89 | 256d | -23 |
| | Mean HS | 176 | | 327 | |
| With experimental solution | | | | | |
| 5 | (1X), no split | 265c | -16 | 84e | -75 |
| 6 | (1X); (1X); | 278bc | -12 | 89e | -73 |
| 7 | (20X); (0.1X); | 73d | -77 | 836a | 150 |
| 8 | (25X); (0.1X); | 38e | -88 | 248d | -26 |
| | Mean ES | 163 | | 314 | |

Mean separation in columns by Duncan's multiple range test at P=0.05.

Note:
*First bracket represents one side and second one represents other side of the Basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

Table 3.15 Effect of seeding and transplanting on root mass of basil under split root nutrition system

| # | Treatments | Transplanting | | Seeding | |
|---|---|---|---|---|---|
| | | g/pot, f.w. | Differences from control, % | g/pot, f.w. | Differences from control, % |
| | | With Hoagland solution | | | |
| 1 | (1X), no split, control | 95a | 0 | 103c | 0 |
| 2 | (1X); (1X);* | 90ab | -5 | 97d | -6 |
| 3 | (20X); (0.1X); | 20d | -79 | 135b | 31 |
| 4 | (25X); (0.1X); | 14e | -85 | 89d | -14 |
| | Mean HS | 55 | | 106 | |
| | | With experimental solution | | | |
| 5 | (1X), no split, control | 81c | -15 | 23e | -78 |
| 6 | (1X); (1X); | 90ab | -5 | 21e | -80 |
| 7 | (20X); (0.1X); | 25d | -74 | 235e | 128 |
| 8 | (25X); (0.1X); | 11e | -88 | 75de | -27 |
| | Mean ES | 52 | | 89 | |

Mean separation in columns by Duncan's multiple range test at P=0.05.

Note:
*First bracket represents one side and second one represents other side of the Basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

| Volume of SSH taken for SSH-1, mL/L | SSH (Stock solutions-Hoagland), mM | Volume of SSH taken for SSH-2, mL/L |
|---|---|---|
| Macronutrients | | |
| 6 | $KNO_3$ ----------1000 | 180 |
| 4 | $Ca(NO_3)_2.4H_2O$---1000 | 120 |
| 2 | $NH_4H_2PO_4$ --------1000 | 60 |
| 2 | $MgSO_4.7H_2O$----- -500 | 60 |
| Micronutrients | | |
| 2 | KCl ----------25 | 60 |
| 2 | $H_3BO_3$ ----------12.5 | 60 |
| 2 | $MnSO_4.H_20$ ----------1 | 60 |
| 2 | $ZnSO_4.7H_2O$ ----------1 | 60 |
| 2 | $CuSO_4.5H_2O$----- -0.25 | 60 |
| 2 | $H_2MoO_4$ (85% $MoO_3$) ----------0.25 | 60 |
| 1 | NaFeDTPA(10% Fe) ----------53.7 | 30 |
| 27 mL/L | Total | 810mL/L |

For SSH-1

For SSH-2

SSH (stock solution Hoagland)-1.

SSH (stock solution Hoagland)-2.

For low side

For high side

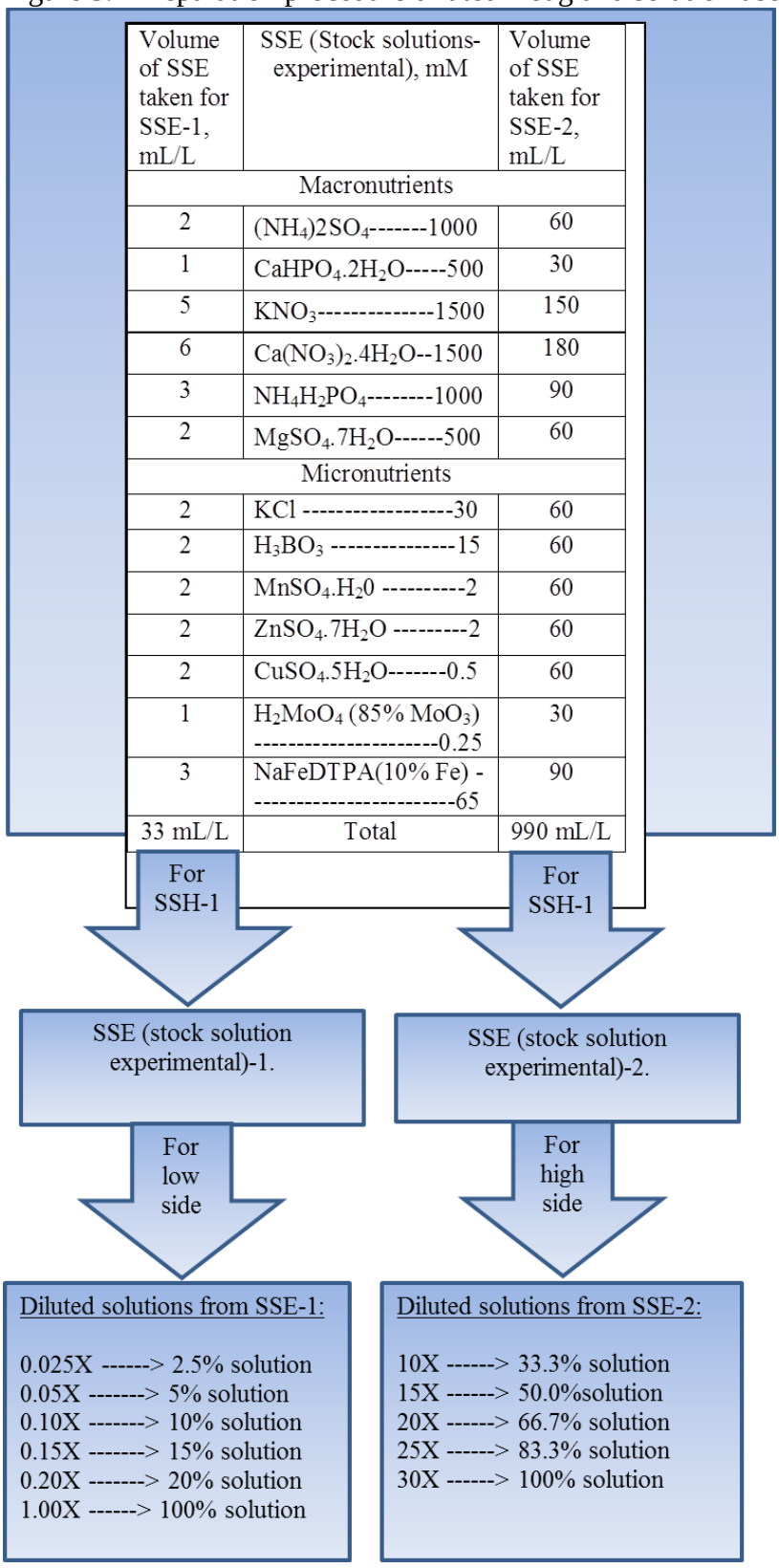
Diluted solutions from SSH-1:

0.025X ------> 2.5% solution
0.05X -------> 5% solution
0.10X -------> 10% solution
0.15X -------> 15% solution
0.20X -------> 20% solution
1.00X ------> 100% solution

Diluted solutions from SSH-2:

10X ------> 33.3% solution
15X ------> 50.0%solution
20X ------> 66.7% solution
25X ------> 83.3% solution
30X ------> 100% solution

Figure 3.1 Preparation procedure diluted Hoagland solution used in in all experiments.



| Volume of SSE taken for SSE-1, mL/L | SSE (Stock solutions-experimental), mM | Volume of SSE taken for SSE-2, mL/L |
|---|---|---|
| | Macronutrients | |
| 2 | $(NH_4)2SO_4$-------1000 | 60 |
| 1 | $CaHPO_4.2H_2O$-----500 | 30 |
| 5 | $KNO_3$----------1500 | 150 |
| 6 | $Ca(NO_3)_2.4H_2O$--1500 | 180 |
| 3 | $NH_4H_2PO_4$--------1000 | 90 |
| 2 | $MgSO_4.7H_2O$------500 | 60 |
| | Micronutrients | |
| 2 | KCl ----------30 | 60 |
| 2 | $H_3BO_3$ ----------15 | 60 |
| 2 | $MnSO_4.H_20$ ----------2 | 60 |
| 2 | $ZnSO_4.7H_2O$ ---------2 | 60 |
| 2 | $CuSO_4.5H_2O$-------0.5 | 60 |
| 1 | $H_2MoO_4$ (85% $MoO_3$) ----------0.25 | 30 |
| 3 | NaFeDTPA(10% Fe) - ----------65 | 90 |
| 33 mL/L | Total | 990 mL/L |

Figure 3.2 Preparation procedure diluted experimental solution used in in all experiments.

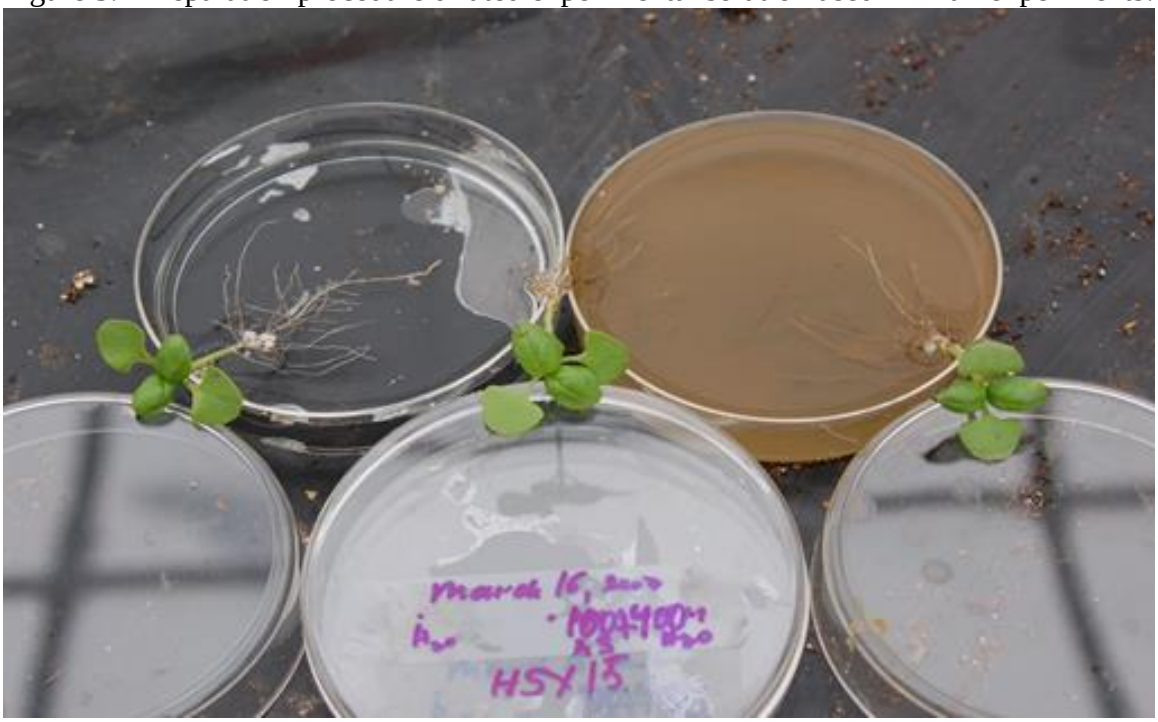


Figure 3.3 Preparing Petri dishes for Split root nutrition system. Initial trial.

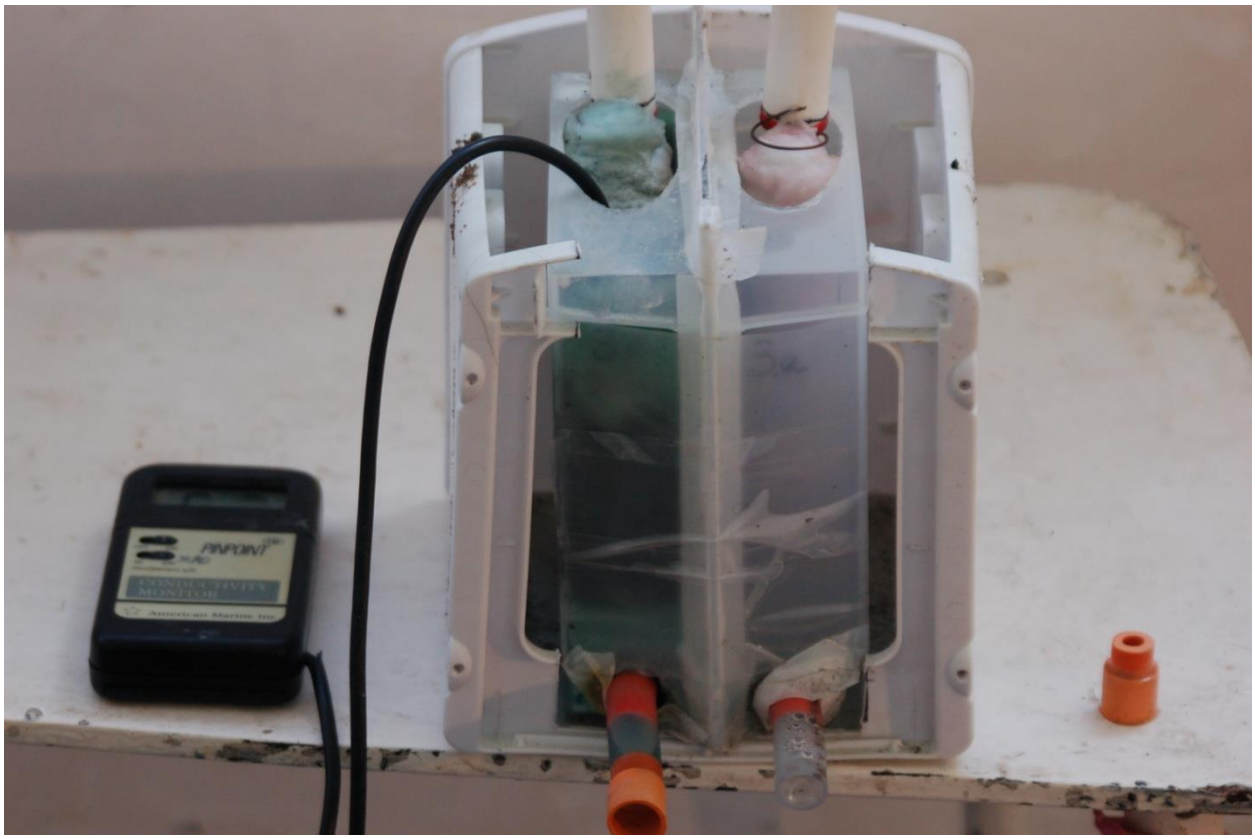


Figure 3.4 Preparing container box for Split root nutrition system: step 1

Figure 3.5 Preparing container box for Split root nutrition system: step 2

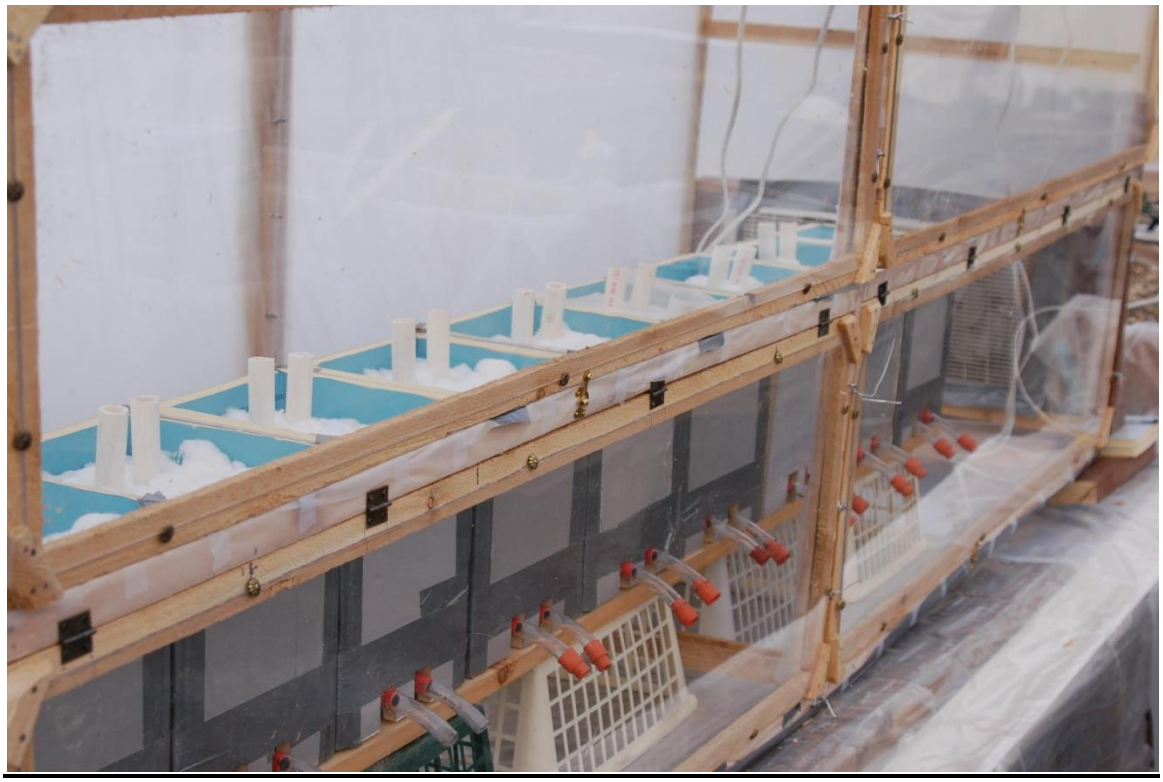


Figure 3.6 Preparing container box for experimental split root nutrition system: step 3

Figure 3.7 Using growing chamber to test experimental split root system

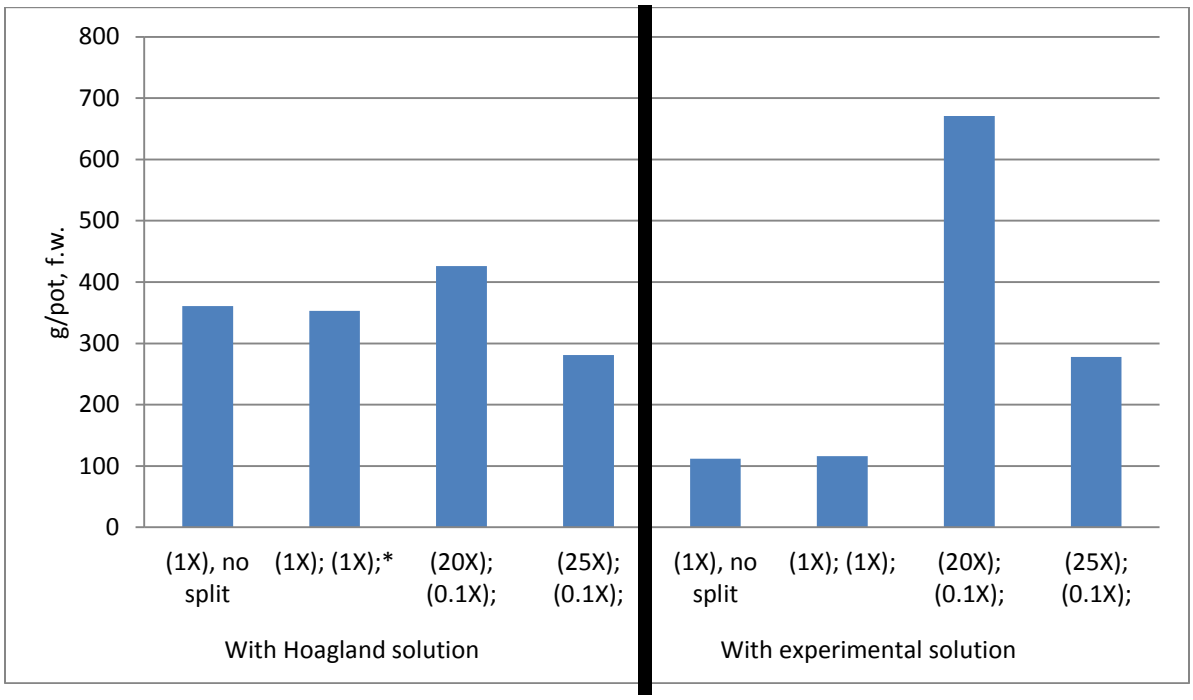



Figure 3.8 Effect of experimental split root nutrition on the basil productivity, g/pot, f.w.

Note:
*First bracket represents one side and second one represents other side of the basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

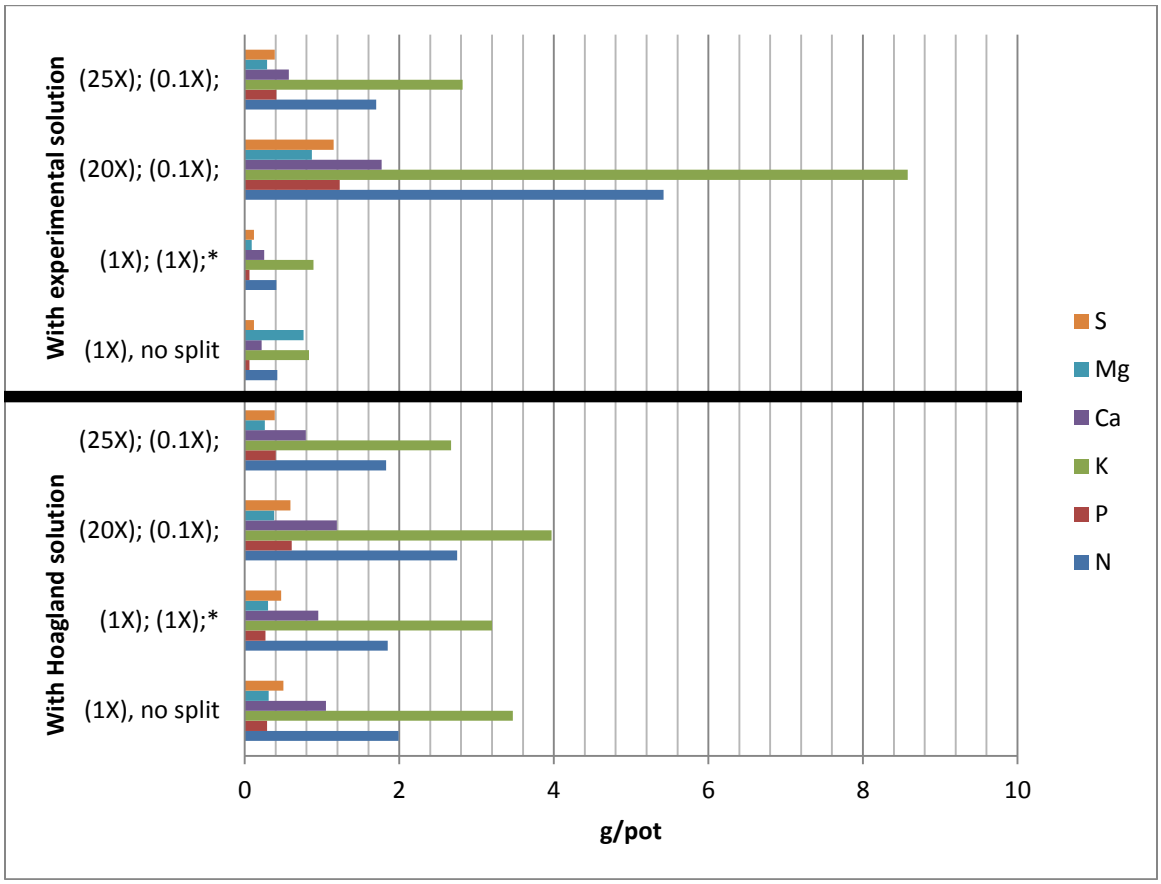



Figure 3.9 Effect of Split root nutrition on basil macro elements accumulation, g/pot

Note:
*First bracket represents one side and second one represents other side of the Basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

Figure 3.10 Toxicity, result of transplanting: part of root inserted in high and other part of root inverted in low concentrated media.


Figure 3.11 Result of seeding: Part of root located in high and part of root naturally located in low concentrated media, grow and development test in 2 day-May 27 to May 29 2010

Figure 3.12 Result of seeding: Part of root naturally located in high and part of root naturally located in low concentrated media, grow and development test in 4 day-May 27 to June 1

Figure 3.13 Comparison inserting part of root in high and other part in low concentrated media (right) and naturally splitting (left) with applied same amount of fertilizers

Figure 3.14 Comparison inserting part of root in high and other part in low concentrated media (right) and naturally splitting (left) with applied same amount of fertilizers, at the end of vegetation period


Figure 3.15 Basil plant in different root split conditions: beginning stage

Figure 3.16 Basil root in two different-High and Low concentrated media

Figure 3.17 Naturally separated Basil root in Split root nutrition system: from total root volume more root volume in low concentrated media and less volume of root in high concentrated media. Brown color area of root was in the high concentrated media

# CHAPTER 4

# FACTORS OF CONCENTRATED MEDIA FOR SPLIT ROOT NUTRITION SYSTEM

## 4.1 Introduction

Each nutrient element has a pH range at which the nutrient is more available to be absorbed by roots and participate in plant nutrition (Epstein 1963) same as described in Figure 2.1. To improve pH conditions in for the split root nutrition system, the high concentrated media was separated in two parts. In one part, the nutrients more available at a lower pH were placed and in the other part the nutrients more available at a higher pH were placed. For comparison with other studies, the total volume of high concentrated media was the same as in previous experiments (4 L in a container 10 cm x 20 cm x 20 cm). The volume was divided into two parts with each in a 2 L container (5 cm x 20 cm x 20 cm) with one volume adjusted to pH 7.5 and the second volume adjusted to pH 5.0.

Elements- K; Na; Cl; Mg; Ca; Mn; Fe; Zn and Cu serve to activate or control the activity of enzymes are classified as an enzyme activators (Epstein and Arnold 2005; Frausto and Williams 1991; Mengel and Kirkby 2001; Taiz and Zeiger 2002). Secondary metabolism has significant relationships with enzymes (Schubert and Yan 1999), for what it was an important to test increased enzyme activators in current experiment.

## 4.2 Materials and Methods

Plant materials. Basil (*Ocimum basilicum*) was used as the plant material in this study. Additionally in this experiment increased enzyme activators using divided pot

experiment as shown in Figures 4.1 and 4.2. As enzyme activators used Mg, Mn and Zn were tested 30, 50, and 100 % above norm of these elements than 20 doses of Hoagland and Experimental solution. Increased amount of these elements kept in high concentrated media.

## 4.3. Results and discussion.

### 4.3.1 Increased norm of enzyme activators in split root nutrition system and its relationships with *Ocimum Basilicum* L. agro chemistry

Increased norm of enzyme activators significantly increased basil productivity (Figure 4.3) with experimental solutions; however, enzyme activators decreased productivity with Hoagland solutions treatments. Especially with increasing dose of enzyme activators in Hoagland solution treatments drastically decreased productivity due to toxicity. With experimental solution productivity increased by increasing enzyme activators up to 50 % in '20 times more concentrated than base nutrient solution' treatment (Table 4.1.). It explains that when split-root nutrition system used, there is important to have right ratio of macro and micro elements (Table 3.4.) according to chemical constituents of plant. Importance of nutrients ratio mentioned with researchers Guodong et al. (2007), Groot et al. (2005), Haywood et al. (2003).

Results of this experiment did show that increasing enzyme activators up to 50 % than its 20 doses increased NPK uptake (Table 4.2. and Figures 4.4.; 4.5.; 4.6.), and Enzyme activator elements-Mg, Mn and Zn uptake (Table 4.3. and Figures 4.7.; 4.8). It is important to note that with traditional way of growing with this amount of Zn fertilization cause toxicity due to Zn mobility (Haslett B.S. et al. 2001). Reason of increasing norm of

those enzyme activators was impact to secondary metabolism of the basil. It is known that these enzyme activators play main role in Secondary metabolism (Malusa 2006). Essential oil of the basil is one of the secondary metabolites and significantly increased with increasing enzyme activators in split root nutrition system (Table 4.4).

### 4.3.2 Effect of splitting high concentrated media in Root nutrient selection system on pH of media, nutrient uptake and basil productivity

It is known that there is a relationship between nutrient availability and media pH (Epstein 1963; Yagodin 1990) and it is important to optimizing plant nutrition based on pH of the media. Results of this experiment did show that splitting high concentrated media in two-high and low pH can regulate pH in upper media of split root nutrition system (Table 4.7. and Figure 4.13.). However one part of high concentrated media had low pH-4.5 and other part of high concentrated media was high pH-7.5 (Figure 4.2.) and upper media had neutral pH-7 (Table 4.7) in all vegetation period. Optimum pH in the top media increased productivity (Table 4.5. and Figure 4.9). Splitting high concentrated media increased productivity not only comparing with main control, but increased productivity comparing with not splitting high concentrated media treatments as well (Figure 4.9.). As we know that there is the effect of nutrition spatial heterogeneity on root traits and carbon usage by roots (Gan et al. (2008) has relationships with grow and development of the plants. In this experiment also, treatments with experimental solution had higher productivity than treatments with Hoagland solutions. Accordingly in treatments with splitting high concentrated media increased nutrient uptake (Tables 4.6.; 4.7. and Figures 4.10.; 4.11.; 4.12.). Splitting high-concentrated media increased essential

oil yield of sweet basil (Table 4.8.), and it was 1.5 times more than treatments without splitting high concentrated media. Splitting high concentrated media with Hoagland solutions increased essential oil yield 24 % more than control-traditional growing hydroponics, and same time using experimental solution with splitting high concentrated media increased essential oil yield (Table 4.8.) up to 116 % more than traditional growing hydroponics.

### 4.4 Conclusions

Increasing Mg, Mn, Zn, Cu, and Fe 30% more in high concentrated side of the media can more activate enzymes in plant and consequently may increase productivity and essential oil of the basil due to relationships between secondary metabolism and enzymes. Researchers tried to increase norm of these elements, but results was unsuccessful due to toxicity. This work by using split-root nutrition system did avoid toxicity problems and increasing enzyme activators significantly increased productivity and essential oil of the sweet basil.

As we know that there are strong relationships between pH of media and nutrient availability and same time there is strong relationships between plant nutrition, productivity and essential oil yield. For what in this work splitting high concentrated media in two part-low pH-4.5 and high pH-7.5 significantly increased productivity and essential oil of the sweet basil by increasing nutrient uptake due to optimum pH in all media. So, other brief conclusion is: if let plant part of plant root be in low pH and part of

root be in high pH and part of root be in neutral pH, plant will develop well and will

increase its production and quality.

Table 4.1 Effect of increased norm of enzyme activators in split-root nutrition system on basil productivity, g/pot, f.w

| # | Treatments | g/pot, f.w. | Differences from control, % |
|---|---|---|---|
| With Hoagland solution | | | |
| 1 | (1X) no split | 347c | 0 |
| 2 | (1X);(1X);* | 305cd | -12 |
| 3 | (20X);(0.1X); | 376c | 8 |
| 4 | (20X+EA 30%); (0.1X) | 300cd | -13 |
| 5 | (20X+EA 50%); (0.1X); | 280d | -19 |
| 6 | (20X+EA 100%); (0.1X); | 253de | -27 |
| | Mean | 310 | |
| With experimental solution | | | |
| 7 | (1X) no split | 123e | -65 |
| 8 | (1X);(1X); | 127e | -63 |
| 9 | (20X);(0.1X); | 867ab | 150 |
| 10 | (20X+EA 30%); (0.1X) | 898a | 159 |
| 11 | (20X+EA 50%); (0.1X); | 923a | 166 |
| 12 | (20X+EA 100%); (0.1X); | 722b | 108 |
| | Mean | 610 | |

Mean separation in columns by Duncan's multiple range test at P=0.05.

Note:
*First bracket represents one side and second one represents other side of the basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

EA X%--Elements considered as enzyme activators (Mg, Mn, Zn, Cu and Fe) applied certain % (30%; 50% and 100%) more compared to 20 times more concentrate than base nutrient solution.

Table 4.2 Effect of increasing enzyme activators in split root nutrition system on basil nutrient accumulation, g/pot, f.w.

| Treatments | Accumulation, g/pot | | | | | |
|---|---|---|---|---|---|---|
| | N | | P | | K | |
| | g/pot | Differences from control, % | g/pot | Differences from control, % | g/pot | Differences from control, % |
| (1X) no split, control | 1.61cd | 0 | 0.23d | 0 | 2.85c | 0 |
| (1X);(1X);* | 1.48d | -8 | 0.21d | -9 | 2.58d | -9 |
| (20X);(0.1X); | 2.60b | 62 | 0.51b | 122 | 3.3c | 16 |
| (20X+EA 30%); (0.1X) | 1.73c | 8 | 0.33b | 44 | 2.6d | -9 |
| (20X+EA 50%); (0.1X); | 1.62cd | 1 | 0.31c | 35 | 2.38d | -16 |
| Mean | 1.81 | | 0.32 | | 2.74 | |
| (1X) no split | 0.43e | -73 | 0.06e | -74 | 0.84e | -70 |
| (1X);(1X); | 0.44e | -73 | 0.05e | -78 | 0.92e | -67 |
| (20X);(0.1X); | 4.93a | 206 | 0.97ab | 322 | 7.95b | 179 |
| (20X+EA 30%); (0.1X) | 5.12a | 218 | 1.22a | 430 | 8.02ab | 181 |
| (20X+EA 50%); (0.1X); | 5.93a | 268 | 1.31a | 460 | 9.53a | 234 |
| Mean | 3.37 | | 0.72 | | 5.45 | |

Mean separation in columns by Duncan's multiple range test at P=0.05

Note:
*First bracket represents one side and second one represents other side of the Basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

EA X%--Elements considered as enzyme activators (Mg, Mn, Zn, Cu and Fe) applied certain % (30%; 50% and 100%) more compared to 20 times more concentrate than base nutrient solution.

Table 4.3 Increasing enzyme activators application in basil split-root nutrition system and its effect to uptake of those enzyme activators

| # | Treatments | Mg | | Mn | | Zn | |
|---|---|---|---|---|---|---|---|
| | | g/pot | Differences from control, % | mg/pot | Differences from control, % | mg/pot | Differences from control, % |
| | | With Hoagland solution | | | | | |
| 1 | (1X) no split, control | 0.24d | 0 | 4.8d | 0 | 2.0e | 0 |
| 2 | (1X);(1X);* | 0.24d | -4 | 4.6d | -4 | 1.8e | -10 |
| 3 | (20X);(0.1X); | 0.30c | 25 | 7.8c | 63 | 6.3d | 215 |
| 4 | (20X+EA 30%); (0.1X) | 0.28c | 17 | 8.1c | 69 | 7.0d | 250 |
| 5 | (20X+EA 50%); (0.1X); | 0.26cd | 8 | 7.5cd | 56 | 6.8d | 240 |
| | Mean | 0.26 | | 6.6 | | 4.8 | |
| | | With experimental solution | | | | | |
| 6 | (1X) no split, control | 0.07e | -71 | 1.7e | -65 | 0.9e | -55 |
| 7 | (1X);(1X); | 0.09e | -62 | 1.9e | -60 | 0.9e | -55 |
| 8 | (20X);(0.1X); | 0.78b | 225 | 18.5b | 285 | 16.7c | 735 |
| 9 | (20X+EA 30%); (0.1X) | 0.89a | 271 | 22.5ab | 369 | 22.4b | 1020 |
| 10 | (20X+EA 50%); (0.1X); | 0.99a | 313 | 27.8a | 479 | 30.1a | 1405 |
| | Mean | 0.56 | | 14.5 | | 14.2 | |

Mean separation in columns by Duncan's multiple range test at P=0.05.

Note:
*First bracket represents one side and second one represents other side of the Basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

EA X%--Elements considered as enzyme activators (Mg, Mn, Zn, Cu and Fe) applied certain % (30%; 50% and 100%) more compared to 20 times more concentrate than base nutrient solution.

Table 4.4 Effect of increasing enzyme activators application in split-root nutrition system on basil essential oil, % in d.w.

| # | Treatments | Essential oil | | |
|---|---|---|---|---|
| | | %, in d.w. | Oil yield, mg/pot | Differences from control, % |
| | With Hoagland solution | | | |
| 1 | (1X) no split, control | 0.19 | 75.8d | 0 |
| 2 | (1X);(1X);* | 0.22 | 74.4d | -1.8 |
| 3 | (20X);(0.1X); | 0.23 | 106.4c | 40.4 |
| 4 | (20X+EA 30%); (0.1X) | 0.32 | 118.1c | 55.8 |
| 5 | (20X+EA 50%); (0.1X); | 0.29 | 96.6cd | 27.4 |
| | Mean | | 94.3 | |
| | With experimental solution | | | |
| 6 | (1X) no split, control | 0.25 | 37.2e | -50.9 |
| 7 | (1X);(1X); | 0.23 | 35.1e | -53.7 |
| 8 | (20X);(0.1X); | 0.37 | 407.4b | 437.5 |
| 9 | (20X+EA 30%); (0.1X) | 0.45 | 521.3a | 587.7 |
| 10 | (20X+EA 50%); (0.1X); | 0.42 | 484.6b | 539.3 |
| | Mean | | 297.1 | |

Mean separation in columns by Duncan's multiple range test at P=0.05.

Note:
*First bracket represents one side and second one represents other side of the Basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

EA X%--Elements considered as enzyme activators (Mg, Mn, Zn, Cu and Fe) applied certain % (30%; 50% and 100%) more compared to 20 times more concentrate than base nutrient solution.

Table 4.5 Effect of splitting high concentrated media in split-root nutrition system on basil productivity in 2006-2010

| # | Treatments | Productivity | | | |
|---|---|---|---|---|---|
| | | g/pot, f.w. | % d.w. | g/pot, dw | Differences from control, % |
| | With Hoagland solution | | | | |
| 1 | (1X), no split, control | 345c | 10 | 33d | 0 |
| 2 | (1X); (1X); | 337c | 10 | 34d | -2 |
| 3 | (20X);(0.1X); | 402bc | 10.3 | 41cd | 17 |
| 4 | (20X-ST);(0.1X); | 471b | 11.9 | 56c | 36 |
| 5 | (25X);(0.1X); | 268d | 12.2 | 33d | -22 |
| 6 | (25X-ST);(0.1X); | 381c | 12.1 | 46c | 10 |
| | Mean | 367 | | 41 | |
| | With experimental solution | | | | |
| 7 | (1X), no split, control | 105e | 9.5 | 10e | -70 |
| 8 | (1X); (1X); | 112e | 9.7 | 11e | -68 |
| 9 | (20X);(0.1X); | 619ab | 11.3 | 70b | 79 |
| 10 | (20X-ST);(0.1X); | 702a | 12.8 | 90a | 103 |
| 11 | (25X);(0.1X); | 467b | 11.8 | 55c | 35 |
| 12 | (25X-ST);(0.1X); | 503b | 12.4 | 62b | 46 |
| | Mean | 418 | | 50 | |

Mean separation in columns by Duncan's multiple range test at P=0.05.

Note:
*First bracket represents one side and second one represents other side of the Basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

ST-Split, so M1 split in two separate parts: M1a (filled with nutrient solution which has nutrient elements available in higher pH) and M1b (filled with nutrient solution which has nutrient elements available in low pH).

Table 4.6 Effect of splitting high concentrated media in split-root nutrition system on basil nutrient-NPK accumulation, g/pot

| # | Treatments | Nutrient accumulation | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Yield, g/pot d.w | N | | P | | K | |
| | | | % in d.w | g/pot | % in d.w | g/pot | % in d.w | g/pot |
| | With Hoagland solution | | | | | | | |
| 1 | (1X), no split, control | 33d | 4.12 | 1.36 | 0.43 | 0.14 | 7.12 | 2.35 |
| 2 | (1X); (1X); | 34d | 3.98 | 1.35 | 0.41 | 0.14 | 7.11 | 2.42 |
| 3 | (20X);(0.1X); | 41cd | 5.11 | 2.1 | 0.46 | 0.19 | 7.97 | 3.27 |
| 4 | (20X-ST);(0.1X); | 56c | 5.25 | 2.94 | 0.68 | 0.38 | 8.12 | 4.55 |
| 5 | (25X);(0.1X); | 33d | 5.15 | 1.7 | 0.71 | 0.23 | 7.5 | 2.48 |
| 6 | (25X-ST);(0.1X); | 46c | 5.13 | 2.36 | 0.69 | 0.32 | 7.47 | 3.44 |
| | With experimental solution | | | | | | | |
| 7 | (1X), no split, control | 10e | 4.22 | 0.42 | 0.42 | 0.04 | 7.03 | 0.7 |
| 8 | (1X); (1X); | 11e | 3.99 | 0.44 | 0.39 | 0.04 | 7.14 | 0.79 |
| 9 | (20X);(0.1X); | 70b | 4.87 | 4.53 | 1.01 | 0.94 | 8.17 | 7.6 |
| 10 | (20X-ST);(0.1X); | 90a | 5.37 | 6.23 | 1.22 | 1.42 | 9.43 | 10.94 |
| 11 | (25X);(0.1X); | 55c | 5.15 | 1.65 | 1.23 | 0.39 | 9.31 | 2.98 |
| 12 | (25X-ST);(0.1X); | 62b | 5.11 | 3.17 | 1.21 | 0.75 | 9.29 | 5.76 |

Mean separation in columns by Duncan's multiple range test at P=0.05.

Note:
*First bracket represents one side and second one represents other side of the Basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

ST-Split, so M1 split in two separate parts: M1a (filled with nutrient solution which has nutrient elements available in higher pH) and M1b (filled with nutrient solution which has nutrient elements available in low pH).

Table 4.7 Effect of splitting high concentrated media in split root nutrition system on dynamics of pH of upper media

| # | Treatments | pH | | | | |
|---|---|---|---|---|---|---|
| | | 1st day | 15th day | 30th day | 45th day | 60th day |
| | With Hoagland solution | | | | | |
| 1 | (1X), no split, control | 6.3 | 6.2 | 6.2 | 6.4 | 6.7 |
| 2 | (1X); (1X); | 6.5 | 6.1 | 6.5 | 6.6 | 6.6 |
| 3 | (20X);(0.1X); | 6.4 | 6.2 | 5.9 | 5.7 | 5.4 |
| 4 | (20X-ST);(0.1X); | 6.3 | 7.3 | 7.1 | 7.3 | 7.6 |
| 5 | (25X);(0.1X); | 6.2 | 5.3 | 4.7 | 4.2 | 4.4 |
| 6 | (25X-ST);(0.1X); | 6.5 | 7.2 | 7.6 | 7.5 | 7.3 |
| | With Experimental solution | | | | | |
| 7 | (1X), no split, control | 6.3 | 6.7 | 6.4 | 6.5 | 6.7 |
| 8 | (1X); (1X); | 6.6 | 6.1 | 6.2 | 6.6 | 6.9 |
| 9 | (20X);(0.1X); | 6.4 | 6.4 | 6.6 | 6.4 | 6.3 |
| 10 | (20X-ST);(0.1X); | 6.7 | 7.4 | 7.3 | 7.6 | 7.5 |
| 11 | (25X);(0.1X); | 6.1 | 5.2 | 4.5 | 4.2 | 4.3 |
| 12 | (25X-ST);(0.1X); | 6.3 | 7.2 | 7.6 | 7.1 | 7.6 |

Note:
*First bracket represents one side and second one represents other side of the Basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

ST-Split, so M1 split in two separate parts: M1a (filled with nutrient solution which has nutrient elements available in higher pH) and M1b (filled with nutrient solution which has nutrient elements available in low pH).

Table 4.8 Effect of splitting high concentrated media in split-root nutrition system on essential oil of basil

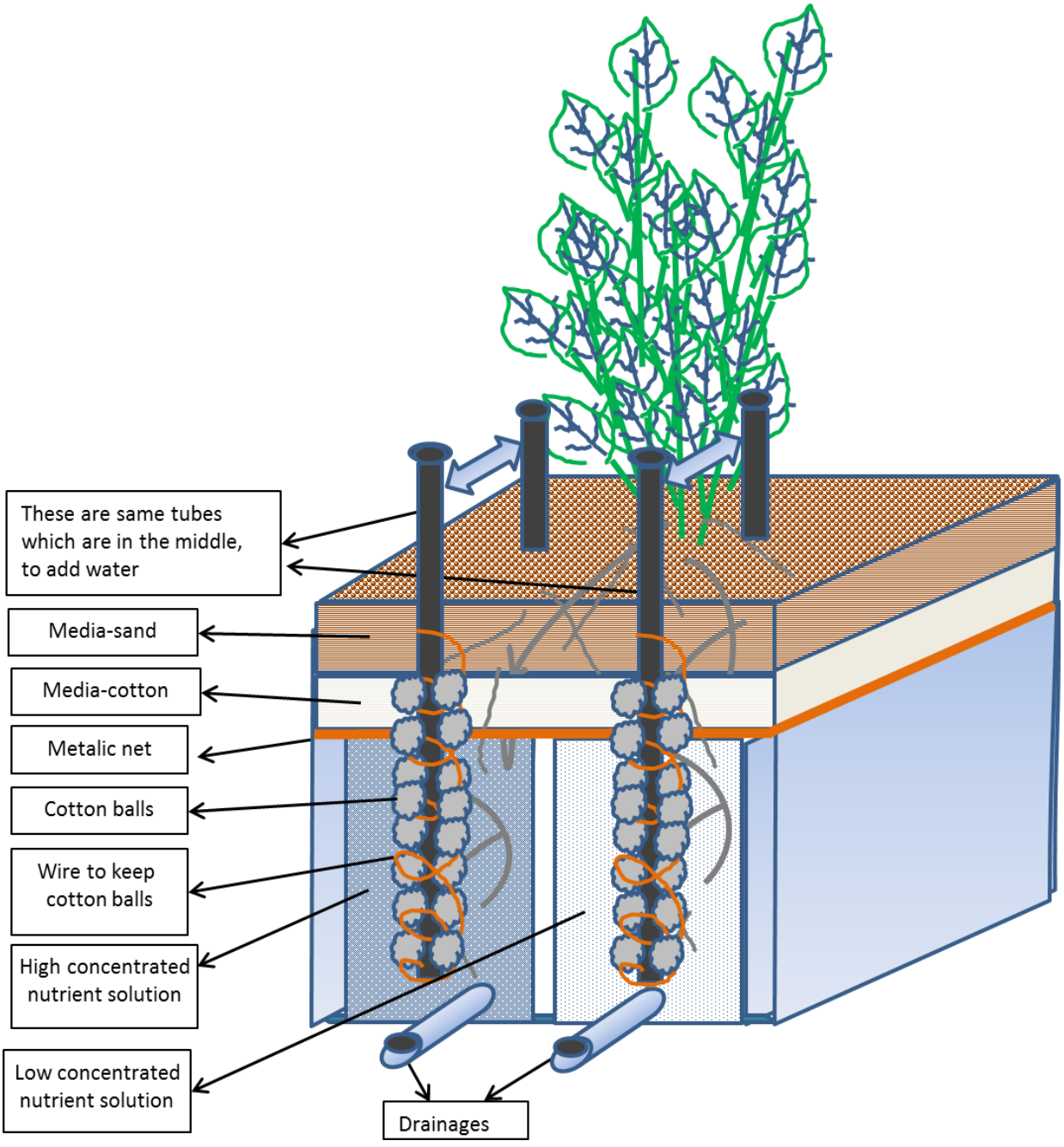
| # | Treatments | Essential oil | |
|---|---|---|---|
| | | % in d.w. | Differences from control, % |
| | With Hoagland solution | | |
| 1 | (1X), no split, control | 0.25d | 0 |
| 2 | (1X); (1X); | 0.23d | -8 |
| 3 | (20X);(0.1X); | 0.28cd | 12 |
| 4 | (20X-ST);(0.1X); | 0.31c | 24 |
| 5 | (25X);(0.1X); | 0.19e | -24 |
| 6 | (25X-ST);(0.1X); | 0.23d | -8 |
| | Mean | 0.25 | |
| | With experimental solution | | |
| 7 | (1X), no split, control | 0.27cd | 8 |
| 8 | (1X); (1X); | 0.25d | 0 |
| 9 | (20X);(0.1X); | 0.54b | 116 |
| 10 | (20X-ST);(0.1X); | 0.83a | 232 |
| 11 | (25X);(0.1X); | 0.35c | 40 |
| 12 | (25X-ST);(0.1X); | 0.71ab | 184 |
| | Mean | 0.50 | |

Mean separation in columns by Duncan's multiple range test at P=0.05.

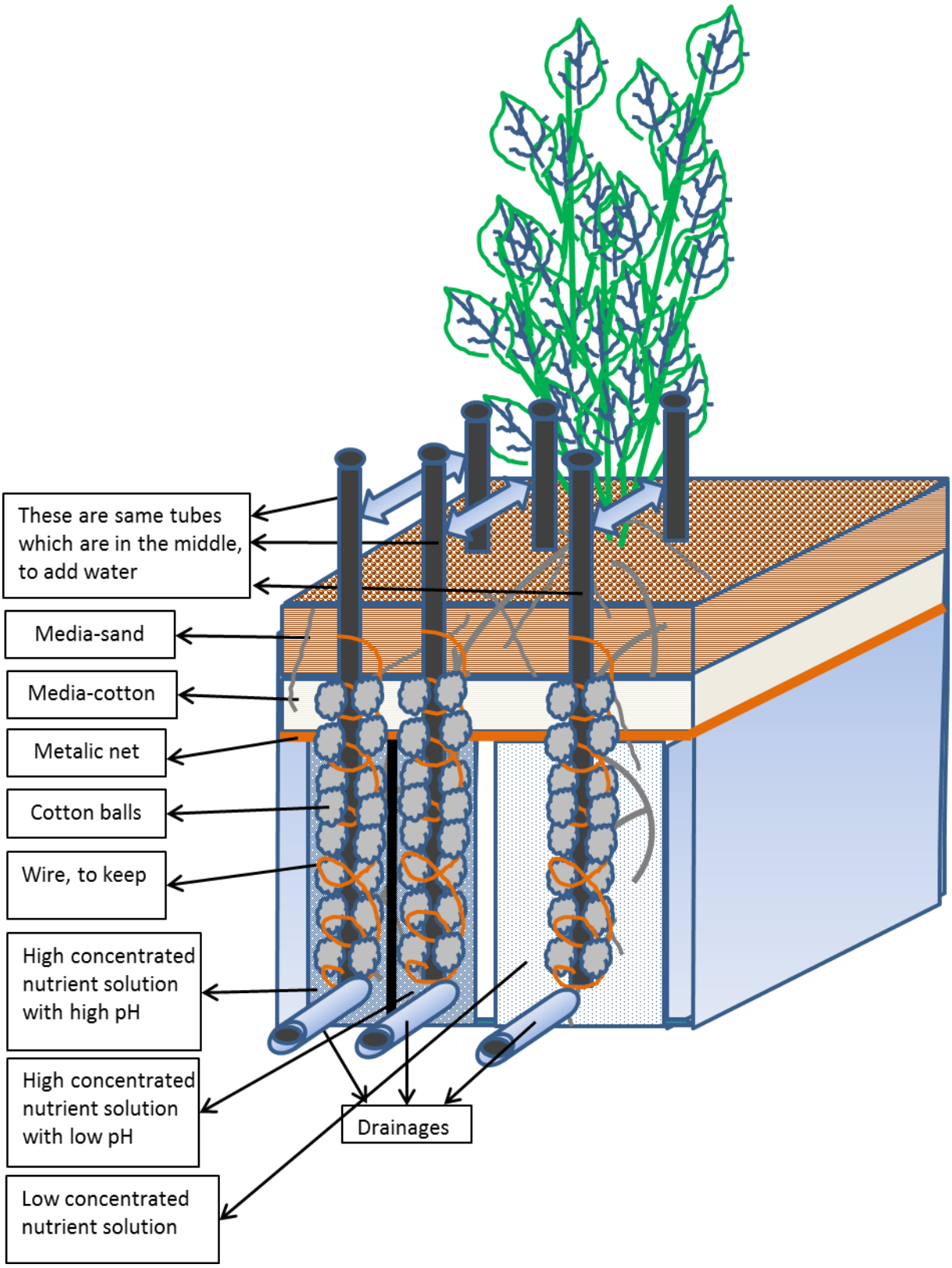
Note:
*First bracket represents one side and second one represents other side of the Basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

ST-Split, so M1 split in two separate parts: M1a (filled with nutrient solution which has nutrient elements available in higher pH) and M1b (filled with nutrient solution which has nutrient elements available in low pH).




4.1. Sketch of container experiment, high concentrated media not divided.




4.2. Sketch of container experiment, high concentrated media divided.

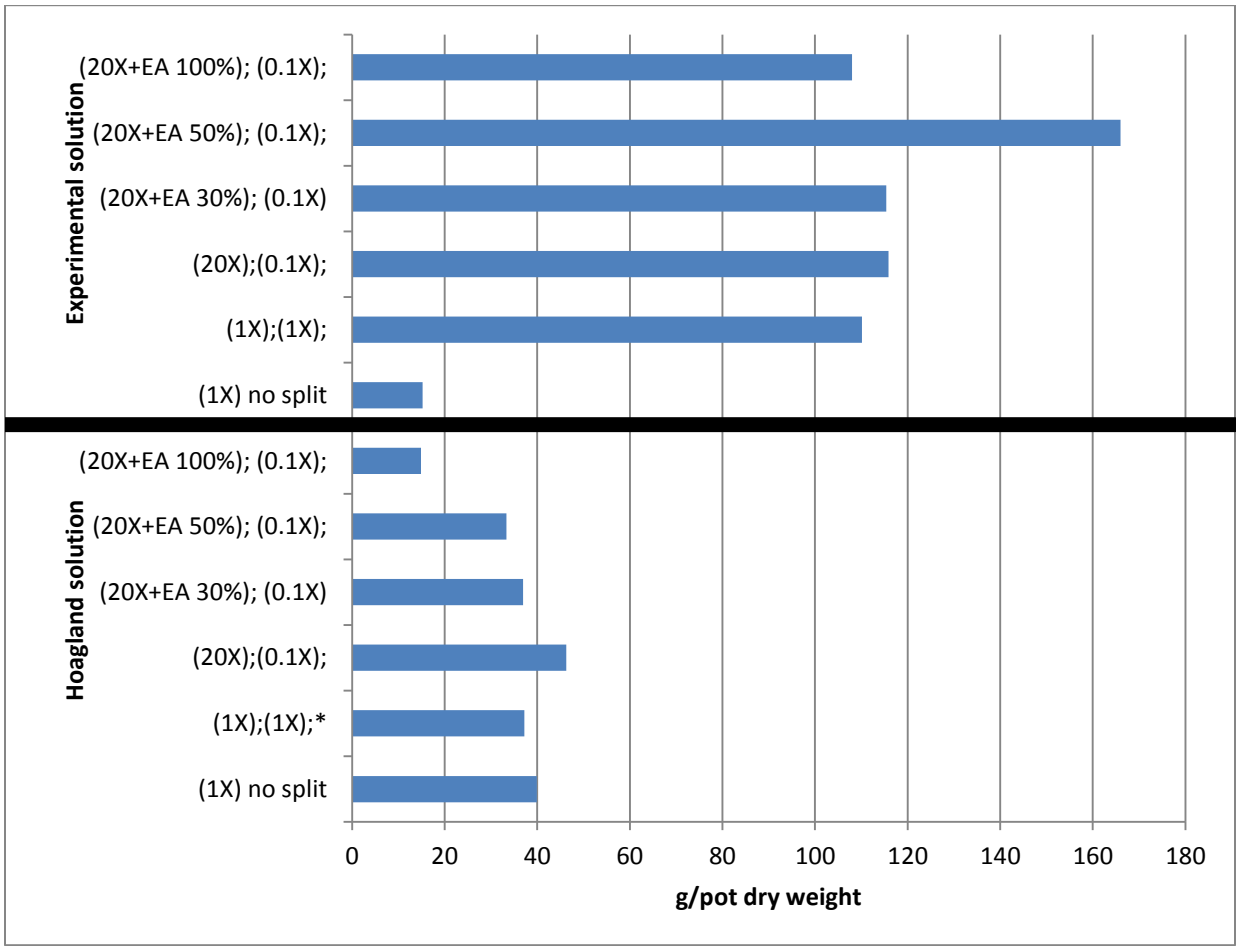



Figure 4.3 Effect of increased norm of enzyme activators in split root nutrition system on Basil productivity.

Note:
*First bracket represents one side and second one represents other side of the Basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

EA X%--Elements considered as enzyme activators (Mg, Mn, Zn, Cu and Fe) applied certain % (30%; 50% and 100%) more compared to 20 times more concentrate than base nutrient solution.

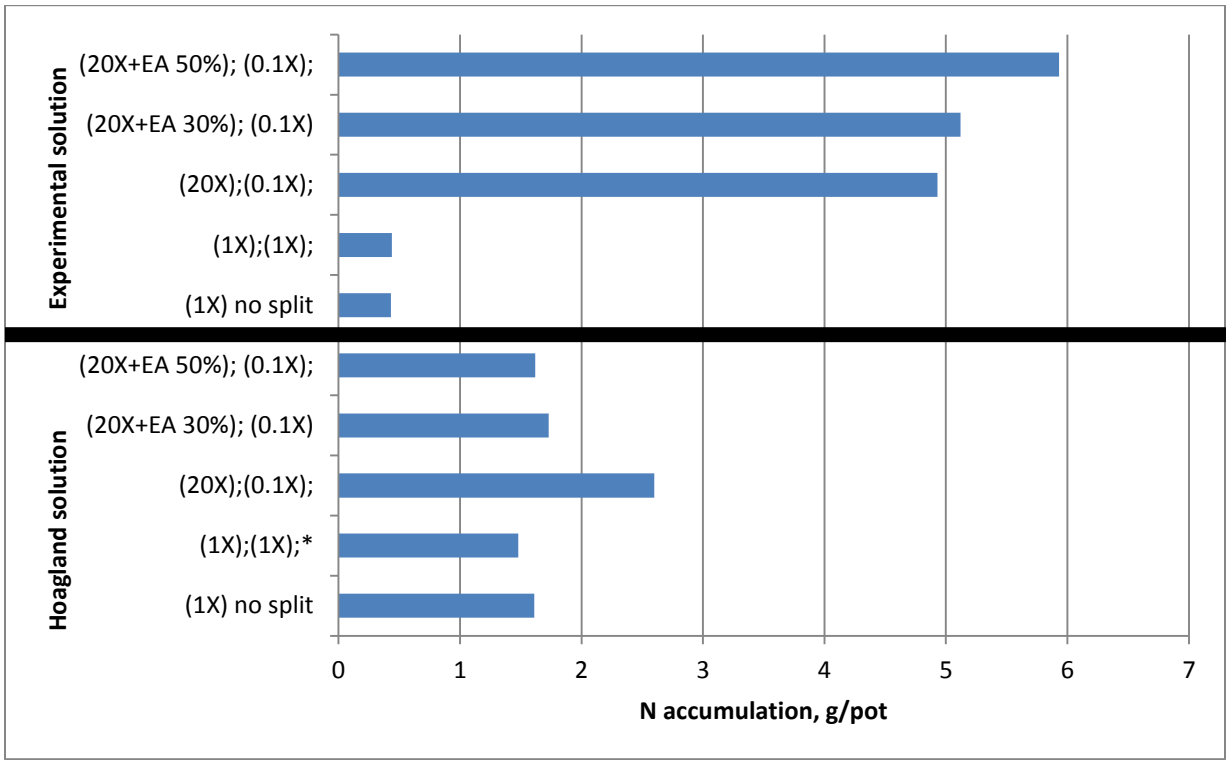



Figure 4.4 Effect of increased norm of enzyme activators in split-root nutrition system on basil N accumulation, g/pot

Note:
*First bracket represents one side and second one represents other side of the basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

EA X%--Elements considered as enzyme activators (Mg, Mn, Zn, Cu and Fe) applied certain % (30%; 50% and 100%) more compared to 20 times more concentrate than base nutrient solution.

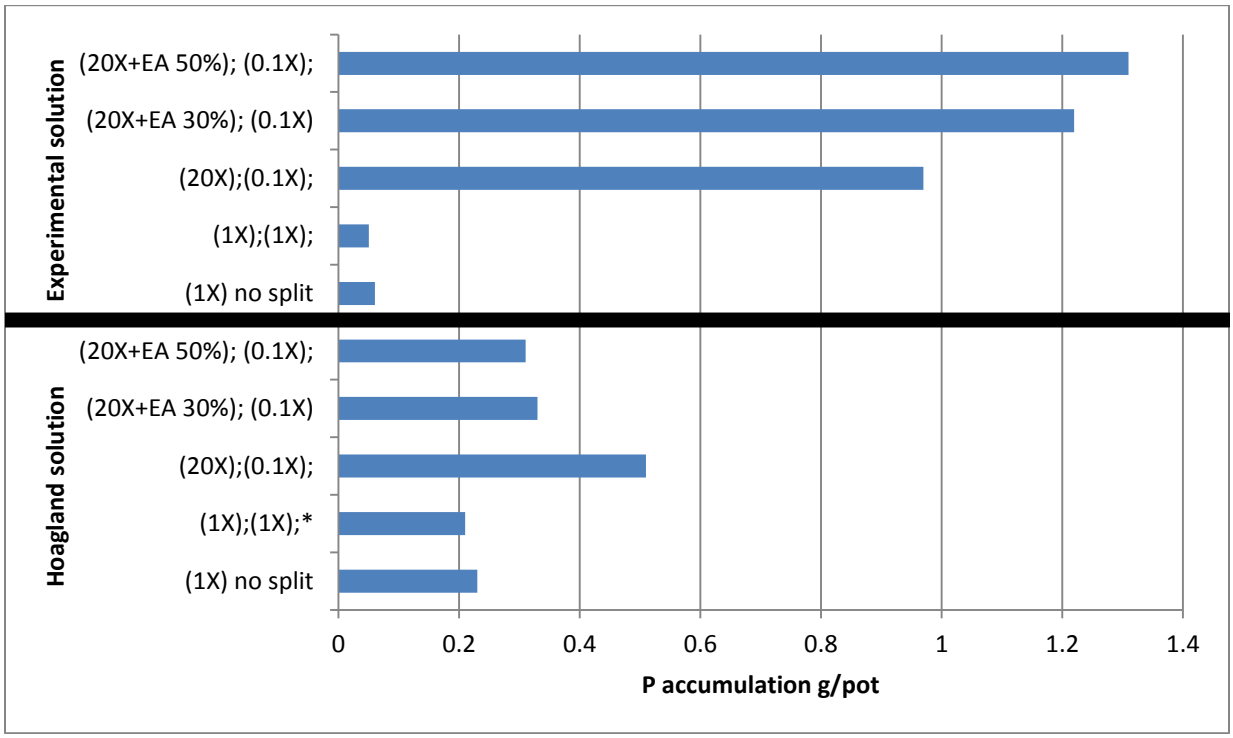



Figure 4.5 Effect of increased norm of enzyme activators in split-root nutrition system on basil P accumulation, g/pot

Note:
*First bracket represents one side and second one represents other side of the basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

EA X%--Elements considered as enzyme activators (Mg, Mn, Zn, Cu and Fe) applied certain % (30%; 50% and 100%) more compared to 20 times more concentrate than base nutrient solution.

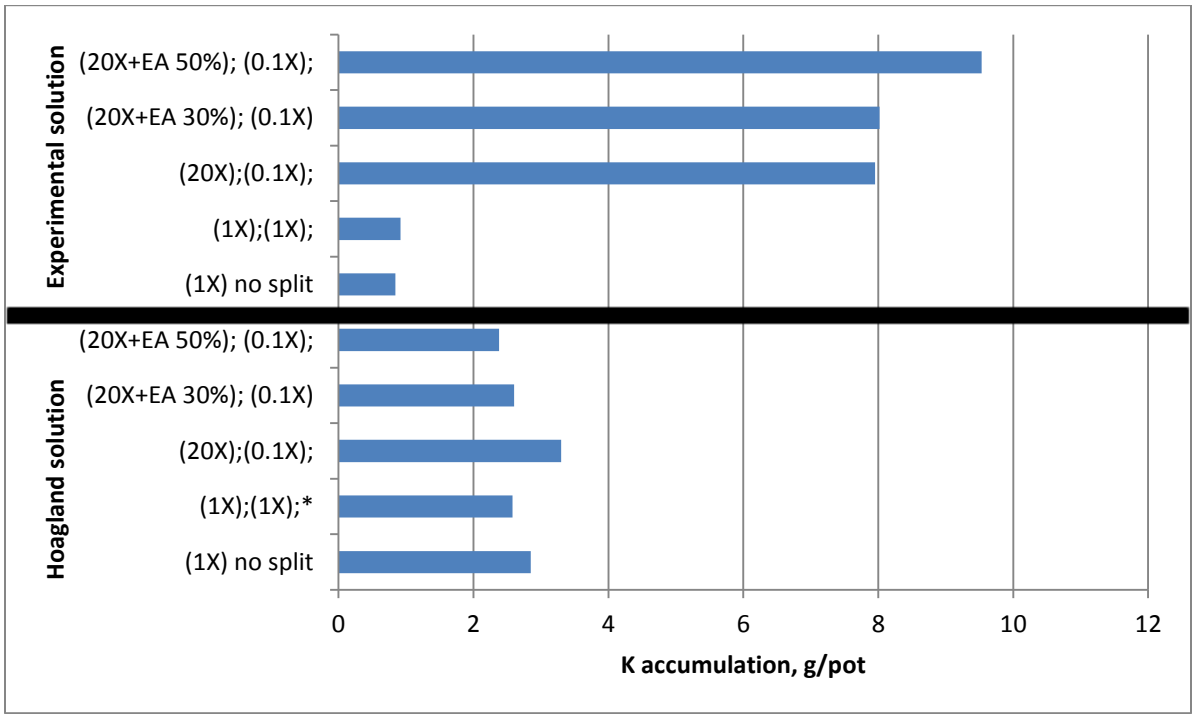



Figure 4.6 Effect of increased norm of enzyme activators in split-root nutrition system on basil K accumulation, g/pot

Note:
*First bracket represents one side and second one represents other side of the basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

EA X%--Elements considered as enzyme activators (Mg, Mn, Zn, Cu and Fe) applied certain % (30%; 50% and 100%) more compared to 20 times more concentrate than base nutrient solution.

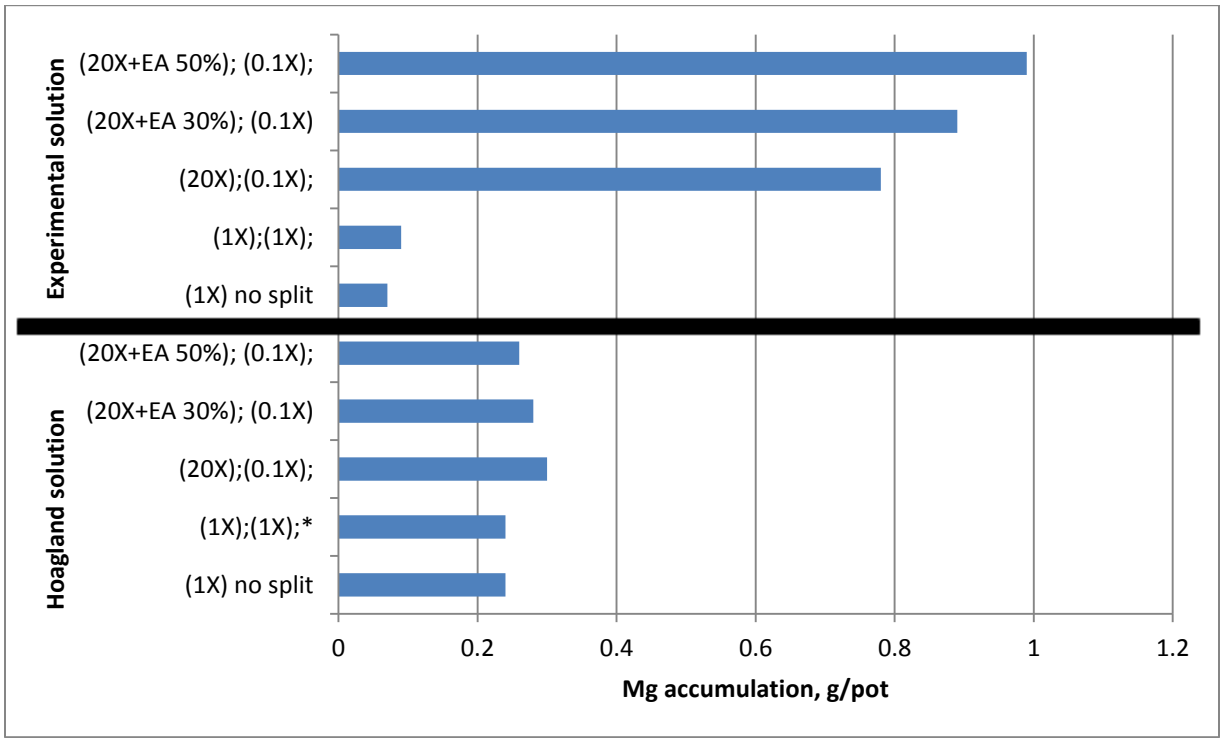



Figure 4.7 Increased norm of enzyme activators used in basil split-root nutrition system and its effect to accumulation of those enzyme activator-Mg, g/pot
Note:
*First bracket represents one side and second one represents other side of the basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

EA X%--Elements considered as enzyme activators (Mg, Mn, Zn, Cu and Fe) applied certain % (30%; 50% and 100%) more compared to 20 times more concentrate than base nutrient solution.

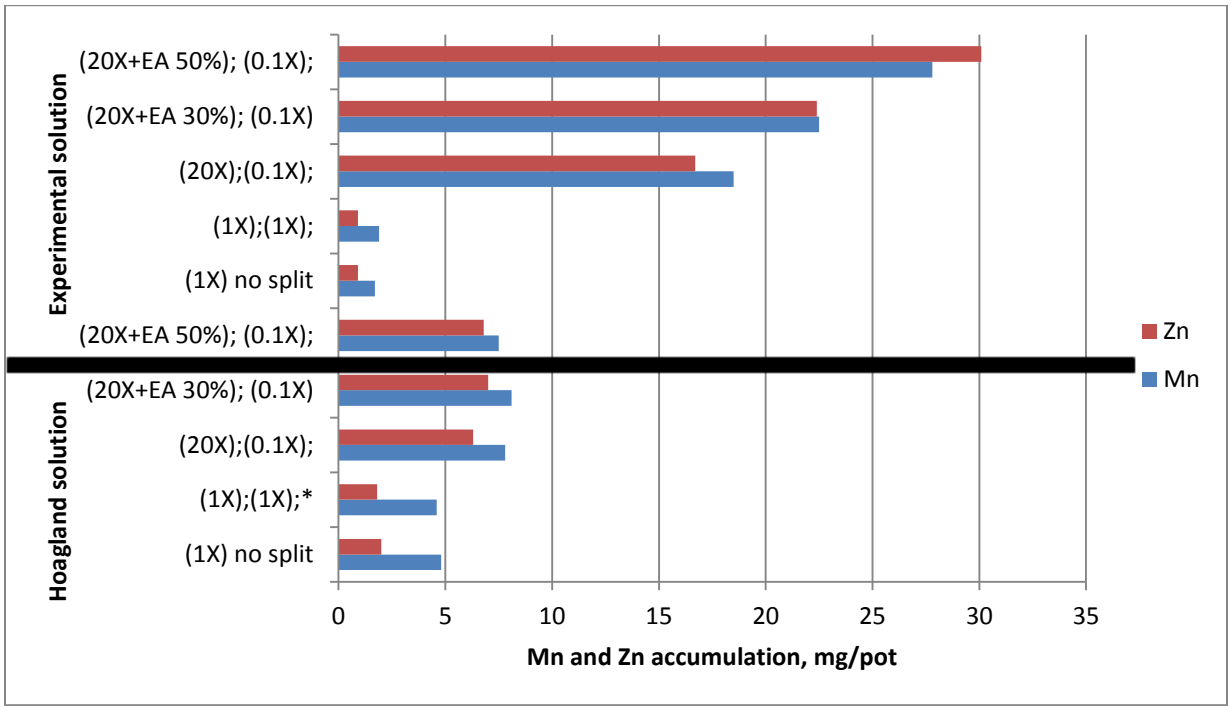



Figure 4.8 Increased norm of enzyme activators used in basil split-root nutrition system and its effect to accumulation of those enzyme activators-Mn and Zn, mg/pot

Note:
*First bracket represents one side and second one represents other side of the Basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

EA X%--Elements considered as enzyme activators (Mg, Mn, Zn, Cu and Fe) applied certain % (30%; 50% and 100%) more compared to 20 times more concentrate than base nutrient solution.

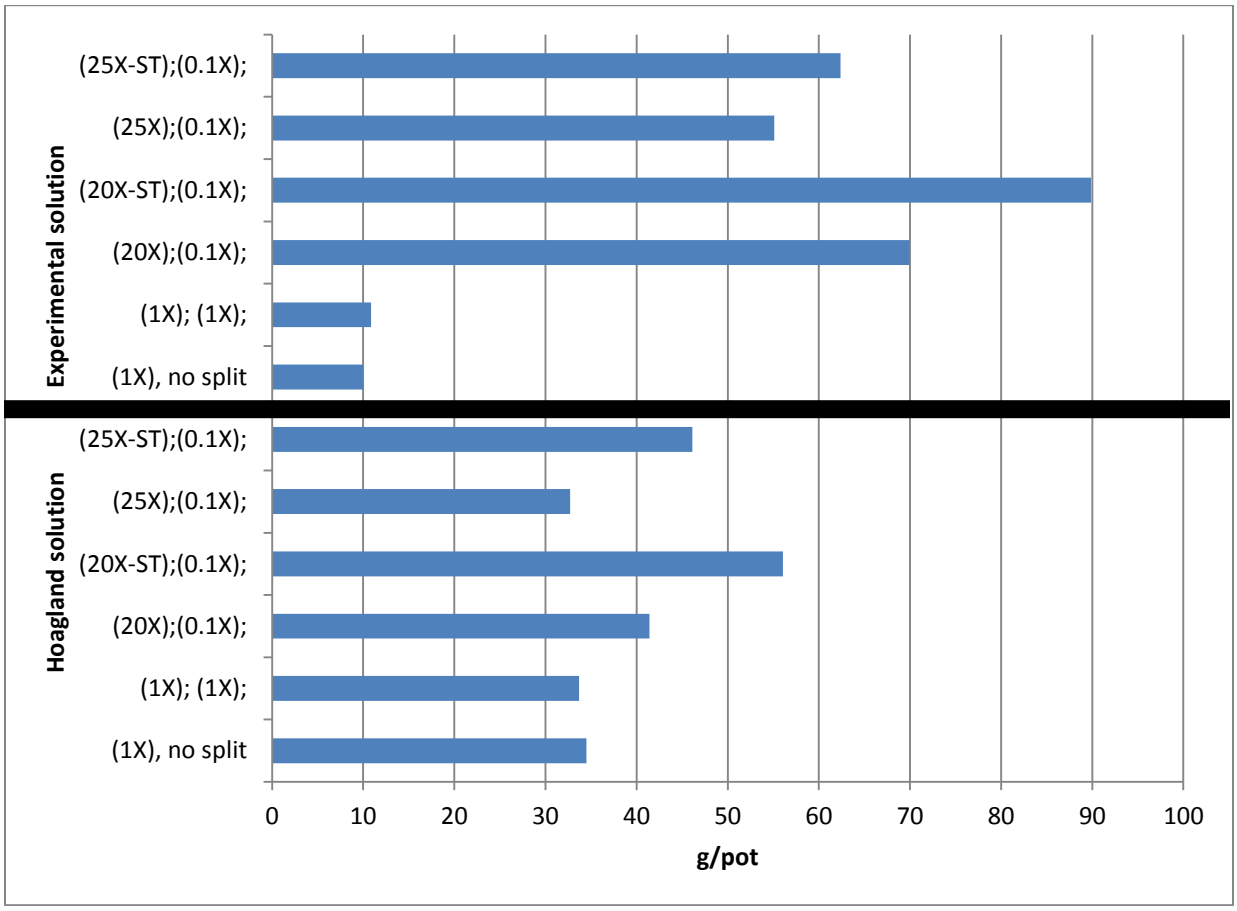



Figure 4.9 Effect of splitting high concentrated media in split-root nutrition system on basil productivity-g/pot d.w.

Note:
*First bracket represents one side and second one represents other side of the Basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

ST-Split, so M1 split in two separate parts: M1a (filled with nutrient solution which has nutrient elements available in higher pH) and M1b (filled with nutrient solution which has nutrient elements available in low pH).

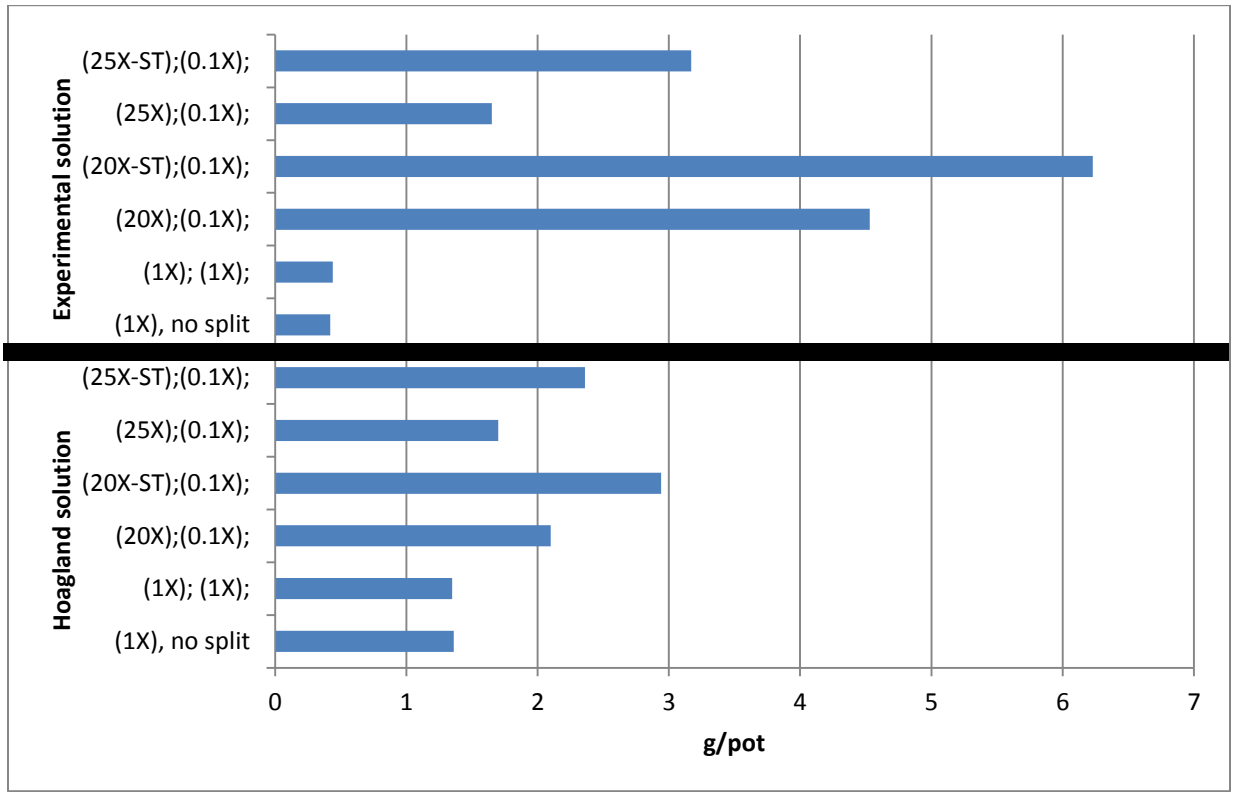



Figure 4.10 Effect of splitting high concentrated media in split root nutrition system on basil nutrient N accumulation, g/pot

Note:
*First bracket represents one side and second one represents other side of the Basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

ST-Split, so M1 split in two separate parts: M1a (filled with nutrient solution which has nutrient elements available in higher pH) and M1b (filled with nutrient solution which has nutrient elements available in low pH).

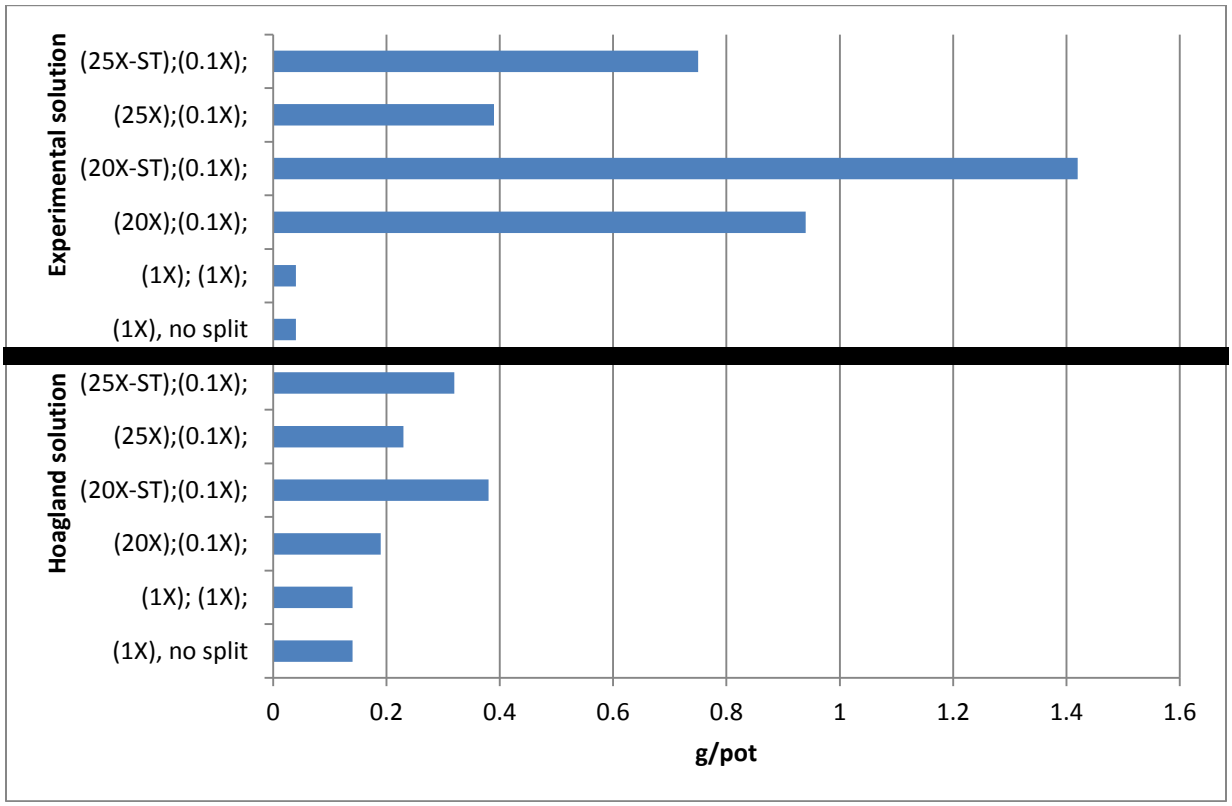



Figure 4.11 Effect of splitting high concentrated media in split root nutrition system on Basil nutrient P accumulation, g/pot

Note:
*First bracket represents one side and second one represents other side of the Basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

ST-Split, so M1 split in two separate parts: M1a (filled with nutrient solution which has nutrient elements available in higher pH) and M1b (filled with nutrient solution which has nutrient elements available in low pH).

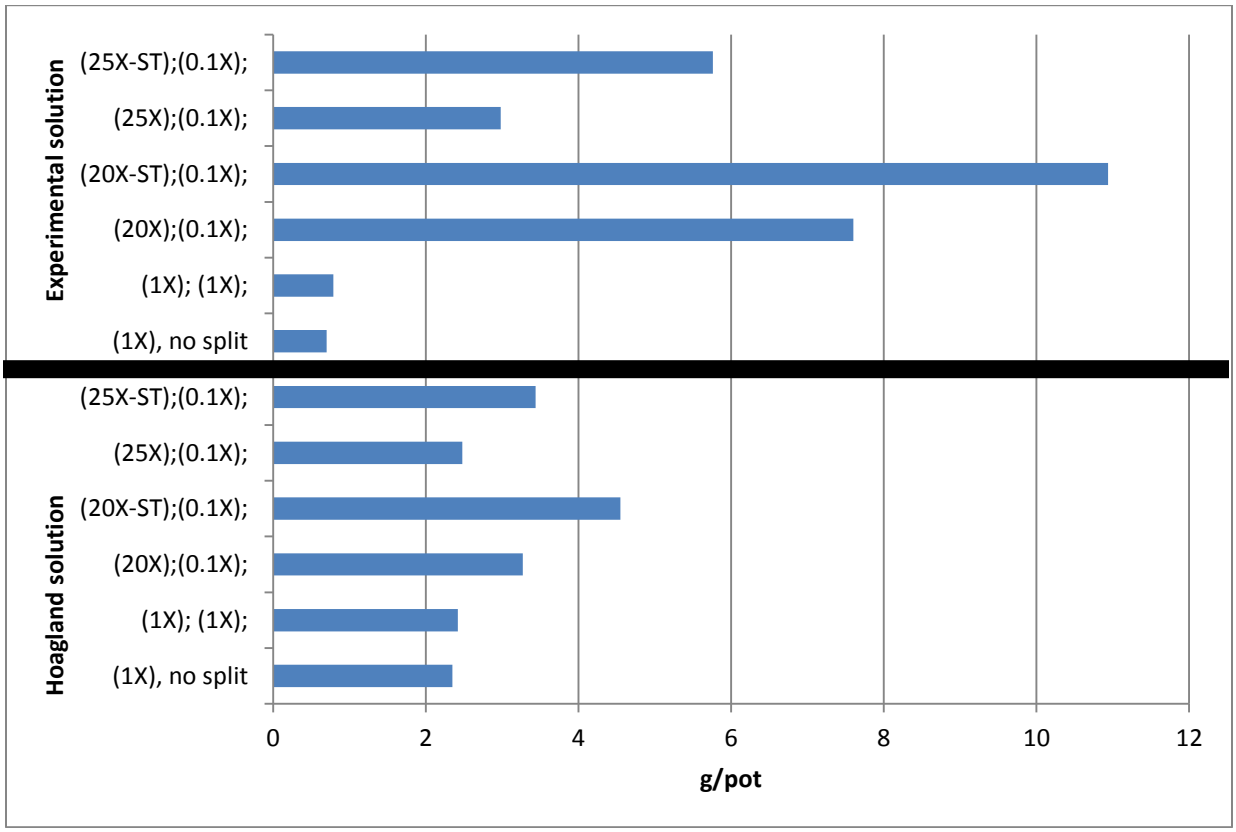



Figure 4.12 Effect of splitting high concentrated media in split root nutrition system on Basil nutrient K accumulation, g/pot

Note:
*First bracket represents one side and second one represents other side of the Basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

ST-Split, so M1 split in two separate parts: M1a (filled with nutrient solution which has nutrient elements available in higher pH) and M1b (filled with nutrient solution which has nutrient elements available in low pH).

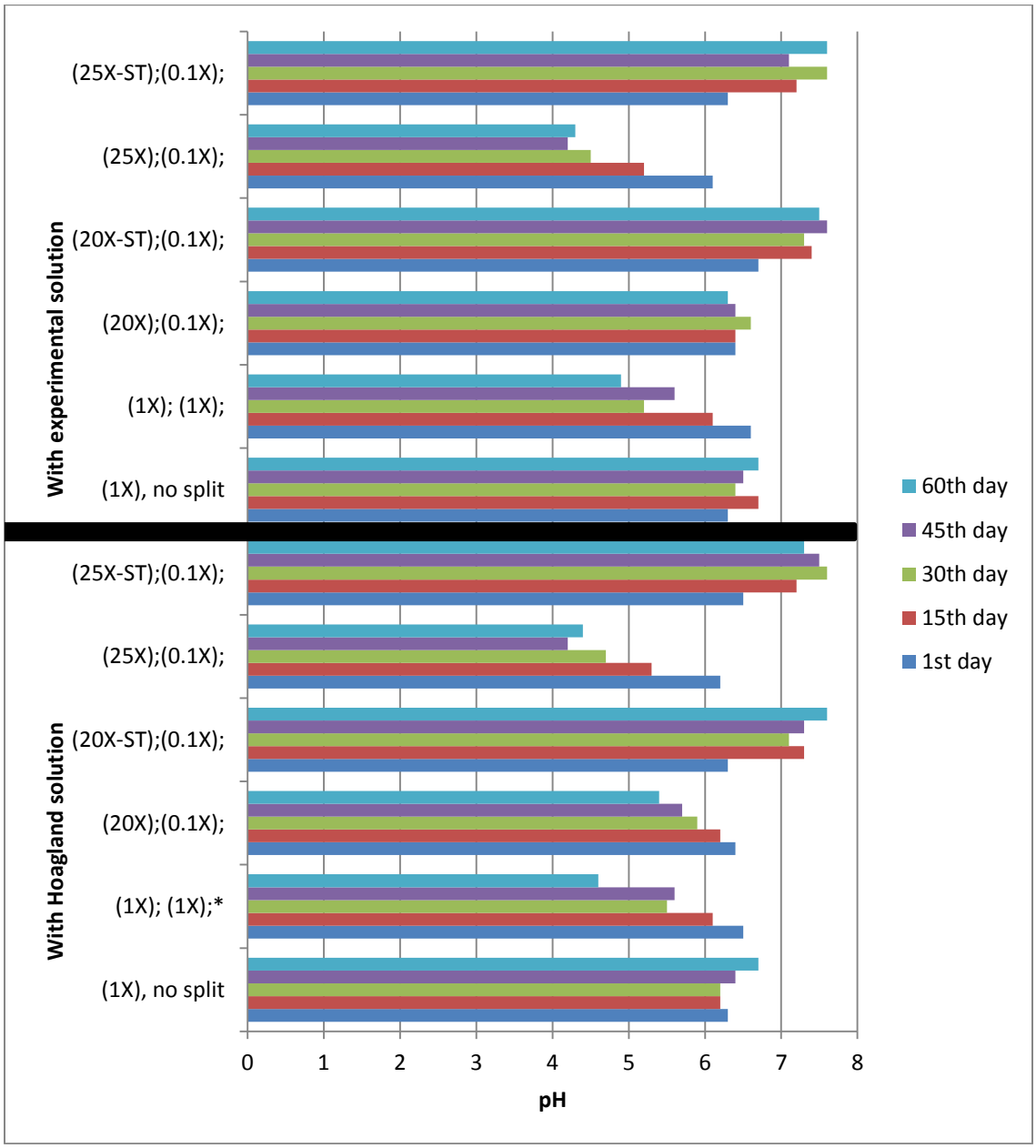



Figure 4.13 Effect of splitting high concentrated media in split root nutrition system on dynamics of pH of top media

Note:

*First bracket represents one side and second one represents other side of the Basil roots for all treatments.

X-dose, 1X considered equal to normal concentration of Hoagland solution or Experimental solution;

ST-Split, so M1 split in two separate parts: M1a (filled with nutrient solution which has nutrient elements available in higher pH) and M1b (filled with nutrient solution which has nutrient elements available in low pH)

# CHAPTER 5

# SPLIT ROOT NUTRITION SYSTEM USING SELECTED NUTRIENT APPICATION SYSTEMS.

## 5.1 Introduction

This experiment was done to determine the necessity of the split root system (High-Low) was necessary for increased growth and yield of basil plants. The design (Figure 5.2.; 5.3.) was to insure that all plants had the same quantity and type of mineral nutrition (Table 5.2.) available, but applied differently to match the common method used to fertilize basil and alternatives to focus on the split root methodology. Medium used were: growing mix SUNSHINE #8, produced by "SUN GRO Horticulture Canada Ltd." The control common system applied all nutrients to the top of the growing medium as a nutrient solution. The split root system consisted of plants with a high concentration of nutrients available to all roots, plants with a high concentration on one side of the plants and a Low concentration of nutrients on the opposite of the row, and plants with a low concentration of nutrients on both sides of the row with additional nutrient solution added at the top of the media (as in the common control). All plants had available equal amounts of nutrients. All roots were allowed to grow naturally (no physical separation was applied). Roots were growing everywhere in all media and we have a high and low concentrated media into all media so, naturally some roots were in high and some roots were in low concentrated medium.

## 5.2. Materials and Methods

This experiment studied the treatments described below (Table 5.2.):

1. Control (all nutrients applied as an irrigation with nutrient solution from above the ground),
2. Root separation into ‘high and high’ (both troughs had a high concentrated nutrient solution, for what it called high and high) nutrient media in troughs at the bottom of the media.
3. Root separation into ‘high and low’ (one troughs had a high concentrated nutrient solution and other had low concentrated nutrient solution, for what it called high and low) nutrient media in separate troughs at the bottom of the media, and
4. Root separation into ‘low and low’ (both troughs had a low concentrated nutrient solution, for what it called low and low) nutrient media in separate troughs at the bottom of the growth media, and with extra nutrients applied as an irrigation with nutrient solution from above the ground.

Root zone nutrients were as described in Table 5.1.

Plant material. The plant material used in this study was sweet basil (*Ocimum basilicum* L.). Basil seeds were seeded into growing mix contained in a prebuilt plot. Prebuilt plots were prepared with wood and plastic (Figure 5.3).

Nutrient solutions prepared as indicated in (Table 5.2.) in the troughs at the bottom of the container according to the treatments described above. The boxes were filled with moisturized growing mix-SUNSHINE #8, produced by “SUN GRO Horticulture Canada Ltd.”. The seeds were planted 2 to 3 mm deep.

Irrigation and fermentation were through the installed tube in the corner of the box.

The ‘high and high’ treatment had the applied fertilizer evenly divided between the two troughs (Figure 5.1.). The ‘high and low’ treatment had the applied fertilizer divided between the two troughs with one side having a high amount and one side with a low amount (Table 5.4.). The low – low treatment had the low level fertilizer placed in each of the troughs. The control treatment had the nutrients applied at the

media surface periodically as a liquid nutrient solution. All plantings received the same amount of fertilizer during the growing period. The same growing conditions were maintained for all plots (temperature 25 ± 3 $^{o}$ C. Irrigation was based on maintaining normal field moisture conditions-65 to 70% from OFMC (overall field moisture capacity). Root development and location were a function of plant growth (no physical division was made: roots were growing everywhere in all media and we have a high and low concentrated media into all media so, naturally some roots were in high and some roots were in low concentrated medium).

Weekly measurements were made of plant growth using ruler to measure from the soil level to the plant tip. After five weeks growth, the basil plants were harvested by cutting the stem with a shears at the point at the medium level. The fresh weight of each plant top (stem and leaves) was immediately measured using an electronic balance, and the samples were bagged for drying. After drying in a mechanical dryer at 45$^{o}$C to a constant weight, the samples were reweighed using an electronic balance. Root and media samples also were dried in the same mechanical dryer at the same temperature as the foliage samples.

The dried foliar, root, and media samples were subsequently analyzed for mineral content using a plasma spectrophotometer for macroelements P, K, Ca, Mg, S and microelements B, Mn, Zn, Mo, Fe, Cu and Kjeldahl method for nitrogen at University Soil Testing Laboratory (Sparks 1996). The media pH were also measured. The roots of each plant and the media within the root growing area were sampled as indicated in Figure 5.2.

The experiment had four replicates.

### 5.3. Results and Discussions.

#### 5.3.1. Effect of split root nutrition system on basil growth rate.

In the treatment where plants were seeded above troughs with one having a low nutrient concentration and one having a high concentration, the plants grew at a significantly faster rate than plants in any of the other nutrient treatments (Figure 5.3.). Root development in high and low concentration growth rate significantly increased compared with control treatment that had no root separation. As shown in Figure 5.2**.** Growth rate in 'high and low' treatment was 4 to 23 cm during the 20 days. Same time 'no split' treatments had only 3 to 15 cm. Increased growth rate explains with optimizing nutrition in the media due to better root formation. Better root formation due to optimum nutrients confirmed by researchers Morgan (1984), Papadopoulos *et al*. (1983), Kobayashi *et al.* (2010). Growth rate in 'high and high' treatment was close to zero, due to toxicity. However 'low and low' treatment's growth rate was very close to 'no split' treatment.

Better growth rate of the studying treatment explains with optimizing plant nutrition. As we see from the Figures 5.19.; 5.20.; 5.21.; 5.22.; 5.23.; 5.24.; 5.25.; 5.26.; 5.27.; 5.28.; 5.29.; 5.30. , "high and low" treatment had about several times more nutrient in the central part of the root, comparing with "no split" treatment. However it did not cause toxicity problems due to fewer nutrients at other side of the root (Table 5.2.). Same phenomenon can be seen for "high and high" treatment too. So, in "high and high" treatment had very high amount-1100 mg/kg potassium (Figure 5.22.) comparing about 70 mg/kg potassium in "no split" treatment and other nutrients were much higher amount

than control which leading to toxicity. Nutrient contents in different stages studied by researcher Zhu Y. (2000) and confirmed that it is important to have less nutrient concentration in the media than nutrient concentration in the root. Basil plant had toxicity stress in “high and high” treatment due to high nutrient concentration in the media. Same phenomenon confirmed by researcher Zekri (1990).

### 5.3.2. Effect of split root nutrition system on basil productivity

Productivity of the basil plants significantly increased where used treatment “high and low” root separation. Researcher Qifu and Zed (2008) confirmed that there are the relationships between nutrient acquisition and nutrient distribution, but it is important to how distribute. We believe that our “high and low” treatment specific nutrient distribution which can optimize plant nutrition and this is one of the reason increasing productivity (Table 5.3.). As shown Table 5.3 productivity increased up to 58% than “no split” treatment, however “low and low” treatment’s, productivity increased 4% more than “no split” treatment. Increasing productivity in “low and low” treatment, evidence that naturally split root in two solutions more effective than traditional growing system- “no split” treatment. It is important to note that when splitting root combined with osmotic regulation, same as our treatment “high and low”- plant develop well and productivity will increase significantly. As shown in Table 5.3., splitting root can’t be as both side splitting in high concentration due to toxicity. Results of media, root and shoot mineral analysis are as a function of Growth rate and Biomass of the Basil plant.

### 5.3.3. Effect of split root nutrition system on basil chemical constituents

The mineral analysis of basil shoots demonstrated the largest amount of nutrient was in “high and high” treatment (Tables 5.5.; 5.6.). The treatment “high and low” nutrients had certain elements same as “no split” treatment and other, such as potassium (Figure 5.5.), zinc (Figure 5.8.), cupper (Figure 5.11.) and iron (Figure 5.12) significantly increased than “no split” treatment. However phosphorus in the shoot of study treatment was same as “no split” treatment, but root’s phosphorus was the significantly higher than “no split” treatment (Figure 5.15.). In study treatment-“high and low” elements which are less than “no split” treatments are due to dilution of them due to high volume of the biomass of the “high and low” treatment. For example, Ca (Figure 5.6), Mg (Figure 5.7) and B (Figure 5.9.) was the lower in study treatment than control. Root mineral analysis show that where “high and low” treatment content of Sulfur is higher than control (Figure 5.15.). If we look to results of shoot mineral analysis (Figure 5.13) it is the opposite-control treatments has more sulfur than “high and low” treatment. It is known that increased amount of the sulfur in the root one of the direct proportion of the growth rate and biomass due to nitrogen and sulfur tale, unless if it is in toxic amount. This phenomenon confirmed by researcher Hitsuda (2005) by studying sulfur requirement of crops at early stages of growth.

Zinc also increased in the root (Figure 5.16.) where is treatment “high and low” compared to “no split” treatment. Increasing Zn, B and other nutrients in our experiment confirms with results of optimum pH, EC (Table 5.4., and Figures 5.17.; 18.) and with

work of other researchers, such as Jackson *et al*. (2000). They studied nitrogen and sulfur on canola yield and nutrient accumulation. They report that sulfur will increase nutrient accumulation, especially Zn, if it has right ratio with nitrogen, but exact right ratio is never known. Results of our experiment show that they statement is true when nutrients are everywhere of the media. Our study treatment with "high and low" concentration is exception of their statement, and we believe that letting root be in two different media allows naturally exact right ratio no matter how incorrect ratio will be prepared in nutrient solution.

Media analysis show that control treatment has nutrients all over places are almost the same amount and where is the spitting root as the "high and low" has significantly lower nutrient in the middle of the root (Tables 5.5.; 5.6.). "High and High" treatment had less nutrients in the middle of the root same as "high and low" treatment (Table 5.5.) but its total amount was much higher than "high and low" treatments, for what most of the plant are didn't survive. A media nutrient in the "low and low" treatment (Tables 5.5.; 5.6.) was the similar to the "no split" treatment.

### 5.4. Conclusion.

According to results of experiments it is concluded that split root nutrition system with High and Low concentrated nutrient media improve growth and development of the basil due to optimized mineral nutrition. Overall, results of all experiments can be expressed by formula below and it can be used for growing any crops using tubes with high and low concentrated nutrients (Figure 5.32 and Figure 5.33).

$$\mathbf{pA\ or\ \Delta Y_{asr}} \approx k\,\frac{[\mathbf{S}]_{\mathbf{1}} * (\mathbf{V_{total}} - \mathbf{V_1})}{[\mathbf{S}]_{\mathbf{2}} * \mathbf{V_1}}$$

$\mathbf{pA}$ – power of absorption or namely $\mathbf{\Delta Y_{asr}}$-deference yield of nutrient absorption due to poly-media nutrition (or wide range split-root nutrition)
$[\mathbf{S}]_{\mathbf{1}}$ -nutrient concentration of high concentrated part of the root zone, % from total nutrient in overall root zone: $50 < [\mathbf{S}]_{\mathbf{1}} < 100$
$\mathbf{V_{total}}$- volume of total root zone, %.
$[\mathbf{S}]_{\mathbf{2}}$-nutrient concentration of low concentrated part of the root zone, % from total nutrient in overall root zone: $0 < [\mathbf{S}]_{\mathbf{2}} < 50$
$\mathbf{V_1}$- volume of high nutrient concentrated part of the root zone, % from total root zone volume: $0 < \mathbf{V_1} < 50$
*k*-constant, for basil-0.01; and different for each crops

Briefly it can be expressed that power of nutrient absorption (*pA*) is the similar to "energy and force" (Appendix 1): direct proportional to concentration of high concentrated part and volume of low concentrated part of the plant root's poly-media.

Table 5.1 Root zone nutrient combinations.

| | Treatments | Combinations | |
|---|---|---|---|
| # | | Concentration in root zone 1 | Concentration in root zone 2 |
| 1 | Control | None | None |
| 2 | High and high | High | High |
| 3 | High and low | High | Low |
| 4 | Low and low | Low | Low |

Table 5.2 Total application and application rate of the mineral elements during all growing period, macroelements including microelement-Cl in g, and microelements in mg.

| Treatments | Elements | Total in all troughs | Total in media | Total in all growing period |
|---|---|---|---|---|
| Control | N | 0 | 62 | 62 |
| | P | 0 | 19 | 19 |
| | K | 0 | 94 | 94 |
| | Ca | 0 | 48 | 48 |
| | Mg | 0 | 15 | 15 |
| | S | 0 | 32 | 32 |
| | Cl | 0 | 43 | 43 |
| | B | 0 | 378 | 378 |
| | Mn | 0 | 275 | 275 |
| | Zn | 0 | 170 | 170 |
| | Cu | 0 | 54 | 54 |
| | Mo | 0 | 19 | 19 |
| | Fe | 0 | 1200 | 1200 |
| High and High | N | 56 | 6 | 62 |
| | P | 17 | 2 | 19 |
| | K | 84 | 10 | 94 |
| | Ca | 43 | 5 | 48 |
| | Mg | 13 | 2 | 15 |
| | S | 29 | 3 | 32 |
| | Cl | 39 | 4 | 43 |
| | B | 340 | 38 | 378 |
| | Mn | 248 | 27 | 275 |

| | | | | |
|---|---|---|---|---|
| | Zn | 153 | 17 | 170 |
| | Cu | 49 | 5 | 54 |
| | Mo | 17 | 2 | 19 |
| | Fe | 1080 | 120 | 1200 |
| High and Low | N | 34 | 28 | 62 |
| | P | 10 | 9 | 19 |
| | K | 51 | 43 | 94 |
| | Ca | 26 | 22 | 48 |
| | Mg | 8 | 7 | 15 |
| | S | 17 | 15 | 32 |
| | Cl | 23 | 20 | 43 |
| | B | 206 | 172 | 378 |
| | Mn | 149 | 126 | 275 |
| | Zn | 92 | 78 | 170 |
| | Cu | 29 | 25 | 54 |
| | Mo | 10 | 9 | 19 |
| | Fe | 652 | 548 | 1200 |
| Low and Low | N | 7 | 55 | 62 |
| | P | 2 | 17 | 19 |
| | K | 10 | 84 | 94 |
| | Ca | 5 | 43 | 48 |
| | Mg | 2 | 13 | 15 |
| | S | 4 | 28 | 32 |
| | Cl | 5 | 38 | 43 |
| | B | 41 | 337 | 378 |
| | Mn | 30 | 245 | 275 |
| | Zn | 19 | 151 | 170 |
| | Cu | 6 | 48 | 54 |
| | Mo | 2 | 17 | 19 |
| | Fe | 131 | 1069 | 1200 |

Table 5.3 Effect of different split root nutrition on basil productivity, f.w. and d.w. of each plot.

| Treatments | f.w. g/plot | % from control | Differences from control % | d.w. g/plot | % from control | Differences from control % |
|---|---|---|---|---|---|---|
| Control | 507.8b | - | - | 123.8b | - | - |
| High and high | 166.7c | 32.8 | -67.2 | 21.6a | 17.8 | -82.2 |
| High and low | 791.3a | 155.8 | 55.8 | 146.8c | 120.5 | 20.5 |
| Low and low | 520b | 103.6 | 3.6 | 126.7b | 103.9 | 4 |

Mean separation in columns by Duncan's multiple range test at P=0.05

Table 5.4 Effect of different split root nutrition on media pH and EC.

| Treatments | Place media taken for analysis | pH | EC ( dS /m ) |
|---|---|---|---|
| Control | Side 1-none | 5.7 | 0.86 |
| | Middle | 5.7 | 0.74 |
| | Side 2-none | 5.7 | 0.88 |
| High and high | Side 1-high | 4.8 | 6.89 |
| | Middle | 5.1 | 2.75 |
| | Side 2-high | 4.8 | 6.83 |
| High and low | Side 1-high | 5.0 | 4.26 |
| | Middle | 5.3 | 3.08 |
| | Side 2-low | 3.7 | 3.84 |
| Low and Low | Side 1-low | 5.6 | 0.87 |
| | Middle | 5.7 | 0.72 |
| | Side 2-low | 5.6 | 0.80 |

Table 5.5 Effect of different split root nutrition on macroelements of overall root zone, mg/kg

| Treatments | Place medium taken for analysis | Macroelements | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | $NO_3$ | $NH_4$ | $P_2O_5$ | $K_2O$ | Ca | Mg | $SO_4$ |
| Control | Side 1-none | 11.0 | 7.0 | 13.0 | 55.3 | 51.3 | 59.8 | 30.3 |
| | Middle | 8.5 | 6.5 | 9.75 | 39.5 | 45.5 | 55.0 | 26.5 |
| | Side 2-none | 11.5 | 6.5 | 13.5 | 57.1 | 52.9 | 61.9 | 31.4 |
| High and high | Side 1-high | 238.3 | 180.0 | 159.0 | 941.8 | 251.8 | 229.3 | 209.5 |
| | Middle | 42.8 | 25.5 | 75.0 | 392.0 | 122.8 | 94.8 | 131.8 |
| | Side 2-high | 293.4 | 162.0 | 155.5 | 937.6 | 253.9 | 229.6 | 201.5 |
| High and low | Side 1-high | 127.3 | 78.0 | 91.0 | 538.0 | 175.5 | 158.5 | 119.4 |
| | Middle | 95.8 | 54.9 | 67.0 | 383.2 | 131.1 | 125.5 | 106.3 |
| | Side 2-low | 146.3 | 79.5 | 77.6 | 451.9 | 154.0 | 153.7 | 93.4 |
| Low and Low | Side 1-low | 14.8 | 11.8 | 12.2 | 58.8 | 51.3 | 60.0 | 28.0 |
| | Middle | 10.3 | 8.8 | 9.0 | 41.0 | 46.5 | 55.3 | 23.5 |
| | Side 2-low | 11.8 | 7.8 | 11.3 | 52.0 | 50.0 | 58.3 | 26.8 |

Table 5.6 Effect of different split root nutrition on microelements of overall root zone, mg/kg

| Treatments | Place medium taken for analysis | Microelements | | | | | |
|---|---|---|---|---|---|---|---|
| | | Zn | B | Mn | Cu | Fe | Na |
| Control | Side 1-none | 0.0 | 0.2 | 0.1 | 0.0 | 0.4 | 31.9 |
| | Middle | 0.0 | 0.2 | 0.0 | 0.0 | 0.4 | 29.9 |
| | Side 2-none | 0.0 | 0.2 | 0.1 | 0.0 | 0.3 | 31.7 |
| High and high | Side 1-high | 1.2 | 3.4 | 0.9 | 0.1 | 5.4 | 105.4 |
| | Middle | 0.9 | 2.7 | 0.4 | 0.1 | 3.3 | 60.6 |
| | Side 2-high | 1.2 | 3.2 | 0.8 | 0.1 | 5.4 | 103.2 |
| High and low | Side 1-high | 0.8 | 1.9 | 0.5 | 0.1 | 3.5 | 71.3 |
| | Middle | 0.5 | 1.6 | 0.4 | 0.1 | 2.5 | 62.0 |
| | Side 2-low | 0.5 | 1.2 | 0.4 | 0.1 | 2.5 | 61.0 |
| Low and Low | Side 1-low | 0.0 | 0.2 | 0.0 | 0.0 | 0.4 | 30.2 |
| | Middle | 0.0 | 0.2 | 0.0 | 0.0 | 0.4 | 27.3 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Side 2-low | 0.0 | 0.2 | 0.0 | 0.0 | 0.4 | 28.6 |

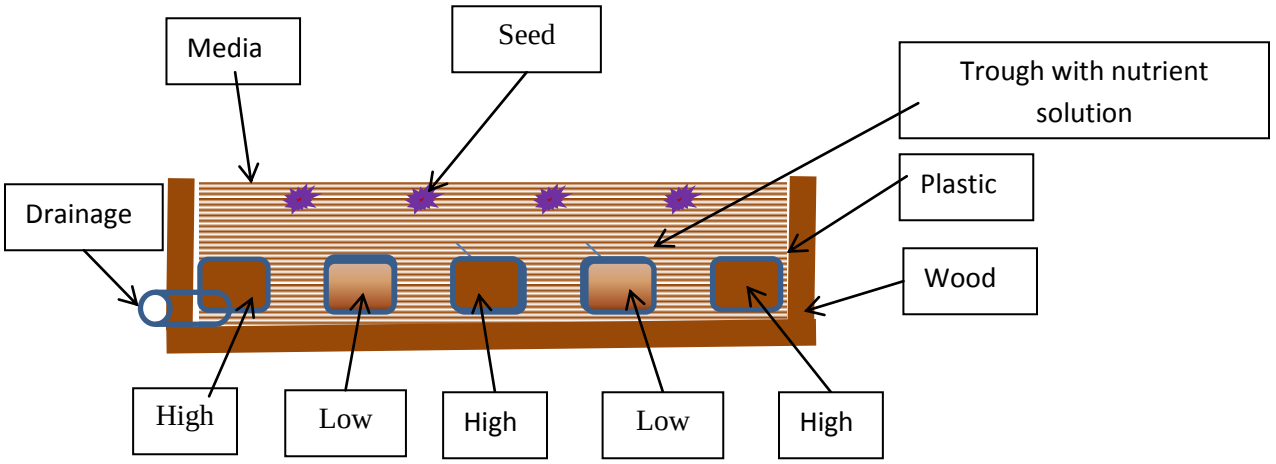



Figure 5.1 Sketch of experimental plot and place of seeding.

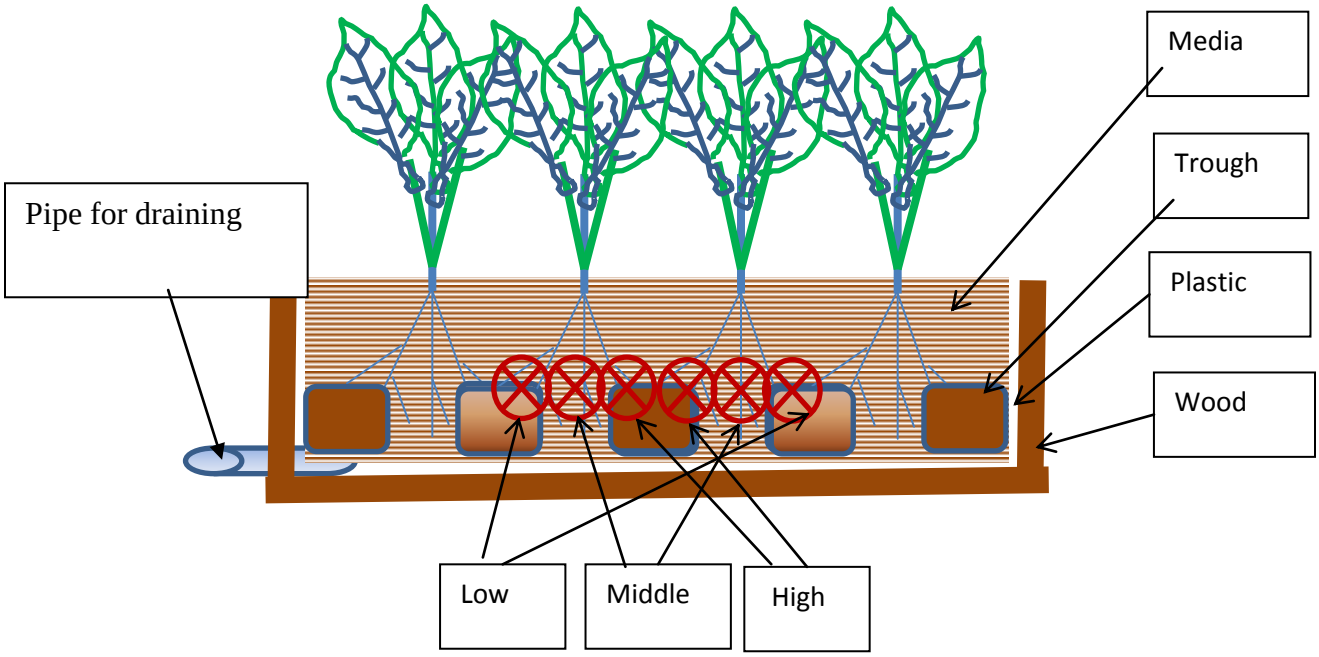



Figure 5.2 Sketch of experimental plot and place taken samples for analysis.
Abbreviations:

Sympol ⊗ showing place where were taken media samples for analysis.

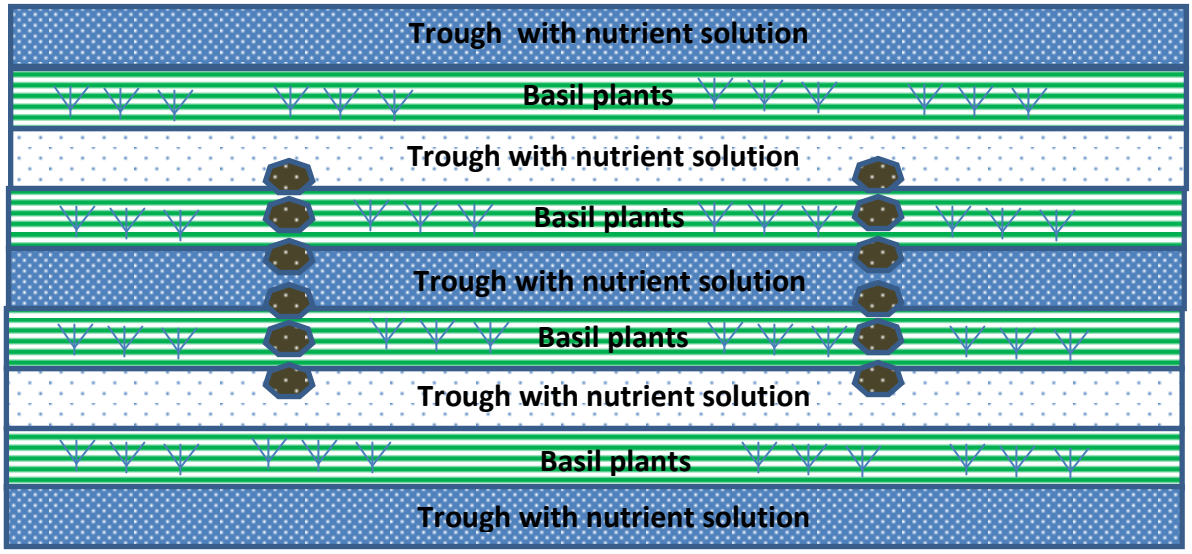



Figure 5.3 Sketch of experimental plot and place taken samples for analysis.
Abbreviations:
Sympol showing place where were taken media and plant samples for laboratory analysis.

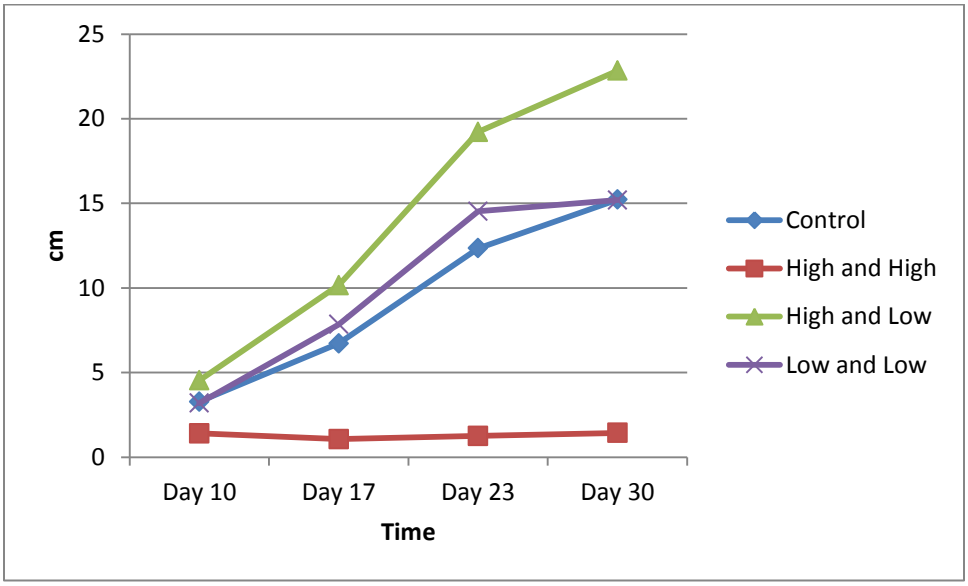



Figure 5.4 Growth rate of basil depending on different root split, cm (note: "control" is "no split" treatment)

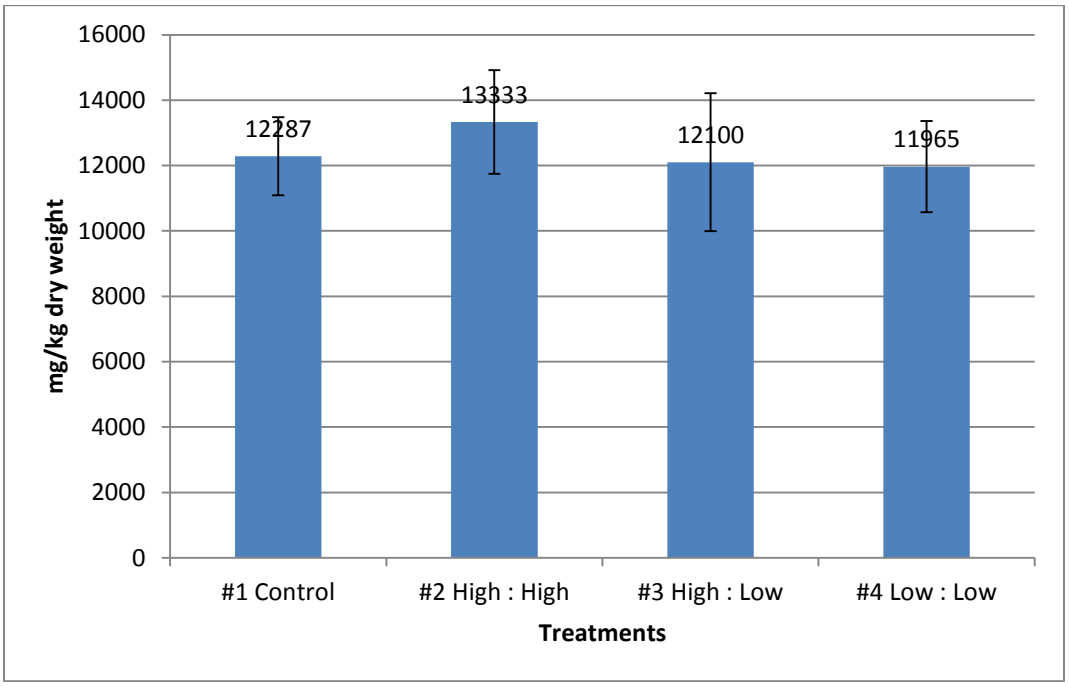



Figure 5.5 Phosphorus content of the basil shoot depending on different split-root (note: “control” is “no split” treatment)

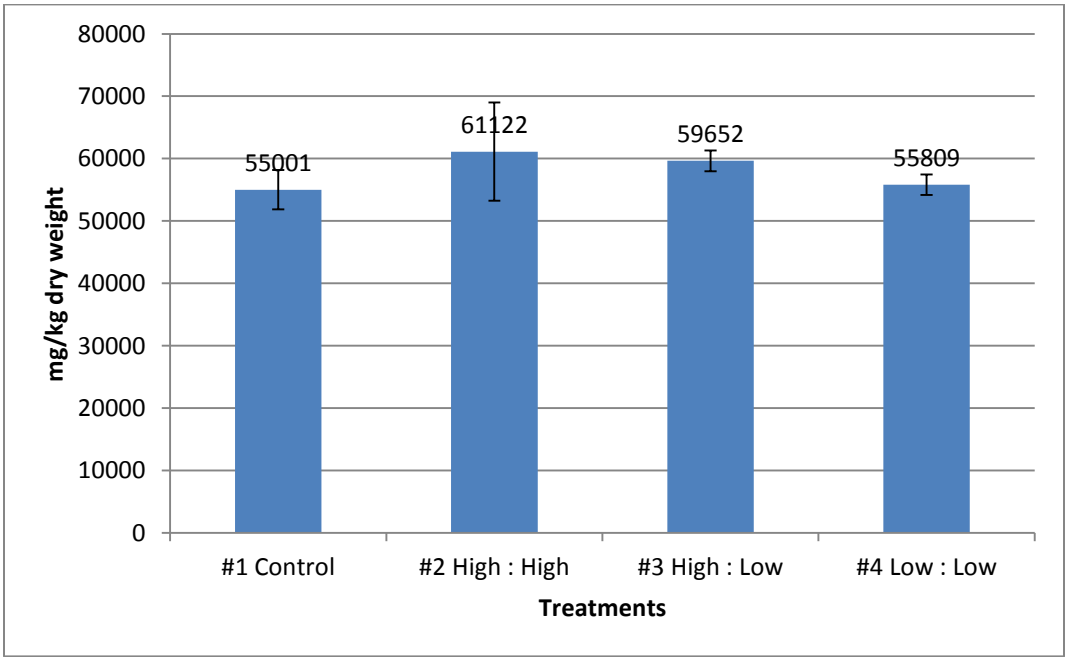



Figure 5.6 Potassium content of the basil shoot depending on different split-root (note: “control” is “no split” treatment)

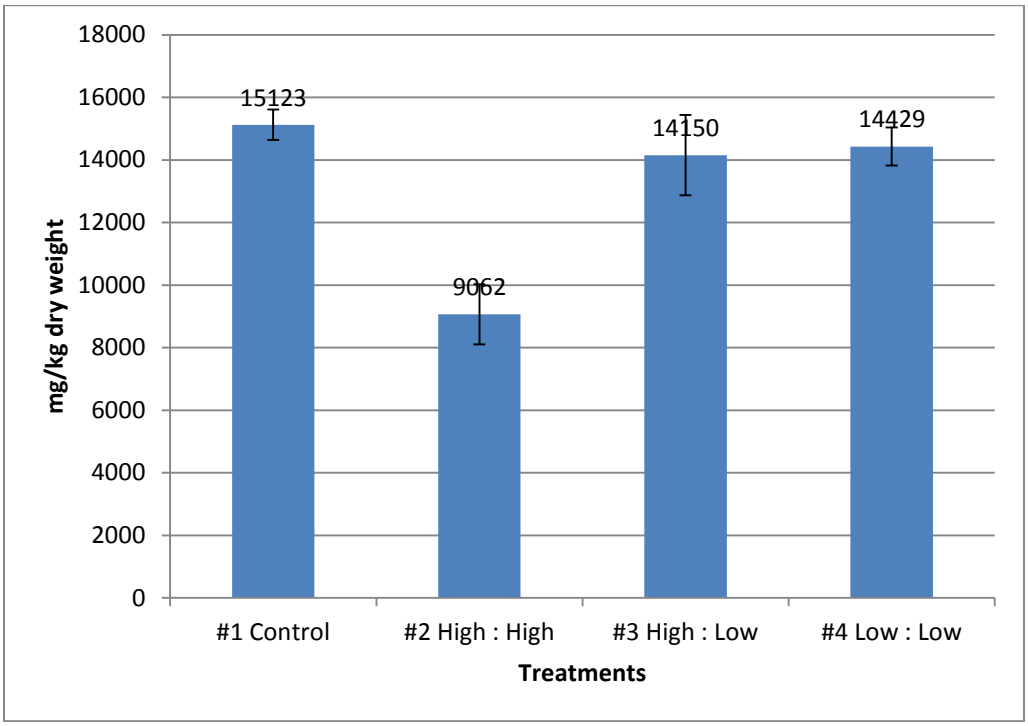



Figure 5.7 Calcium content of the basil shoot depending on different split-root (note: “control” is “no split” treatment)

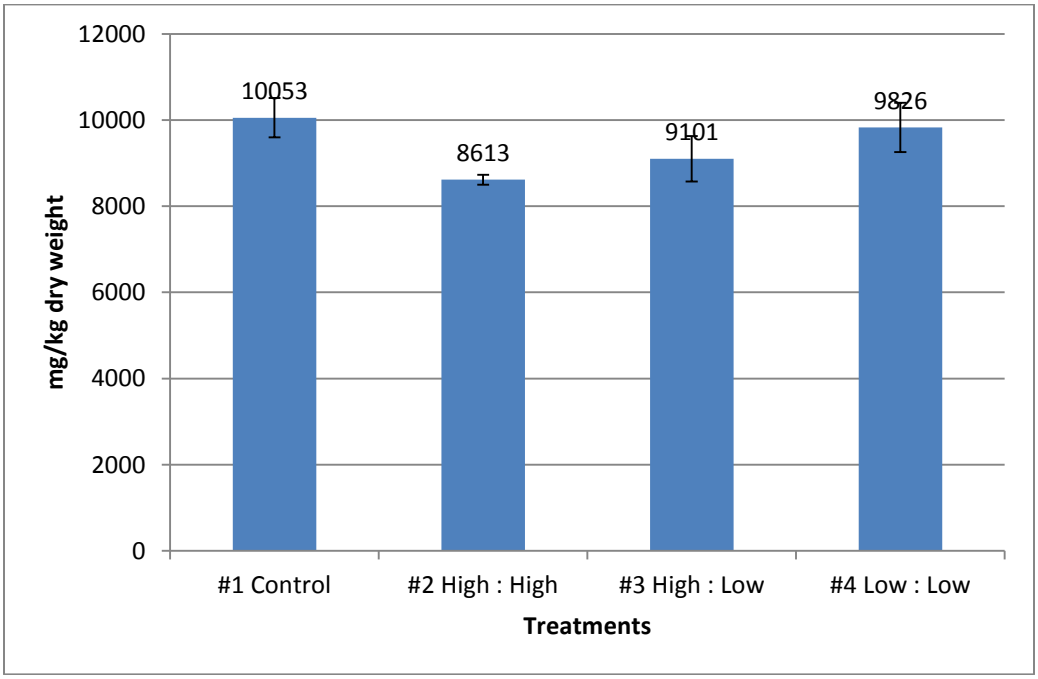



Figure 5.8 Magnesium content of the basil shoot depending on different split-root (note: “control” is “no split” treatment)

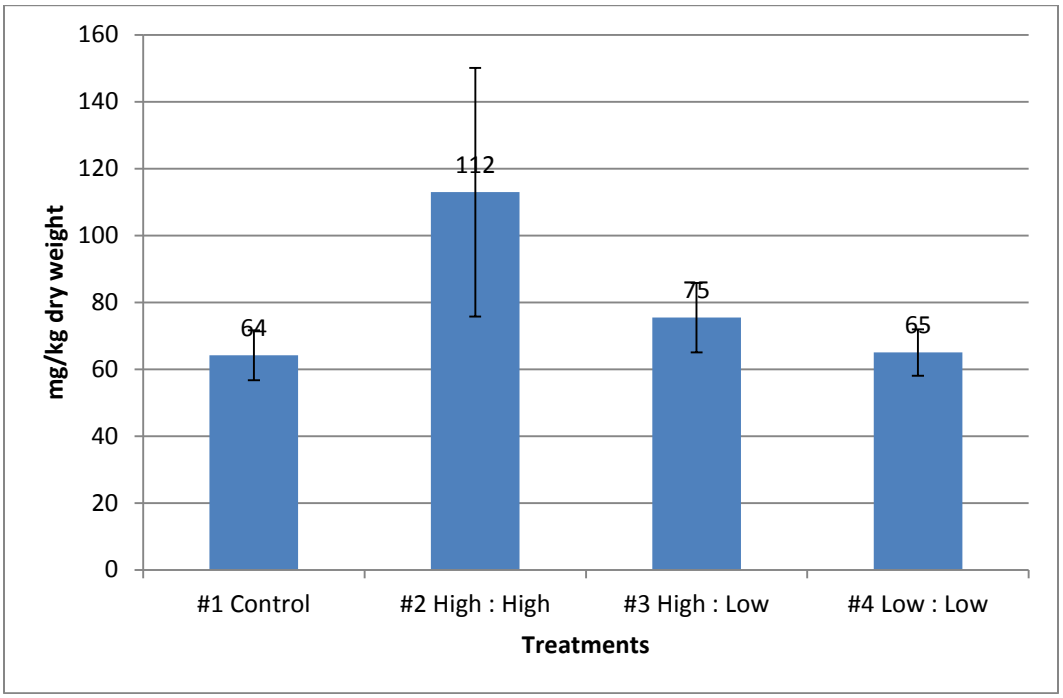



Figure 5.9 Zinc content of the basil shoot depending on different split-root (note: "control" is "no split" treatment)

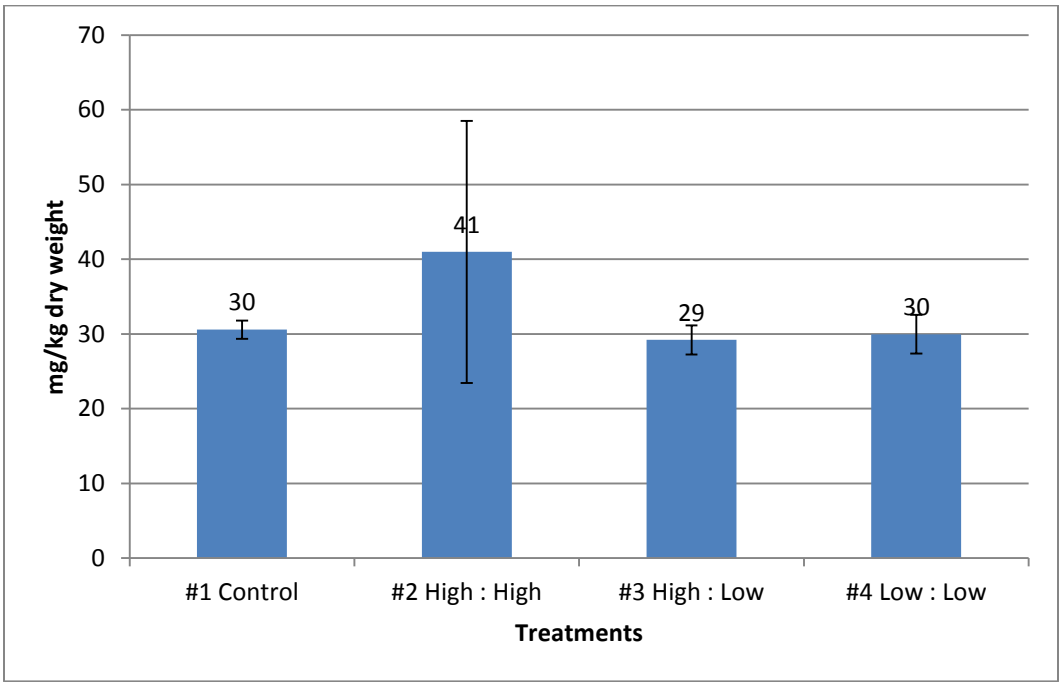



Figure 5.10 Boron content of the basil shoot depending on different split-root (note: "control" is "no split" treatment)

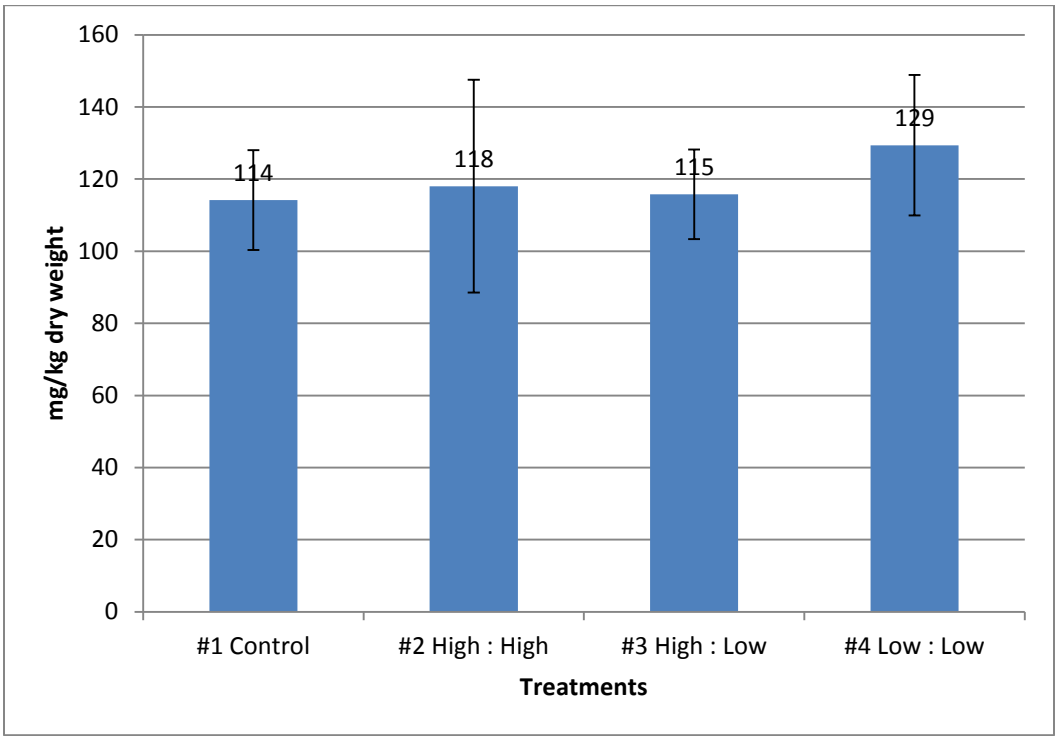



Figure 5.11 Manganese content of the basil shoot depending on different split-root (note: "control" is "no split" treatment)

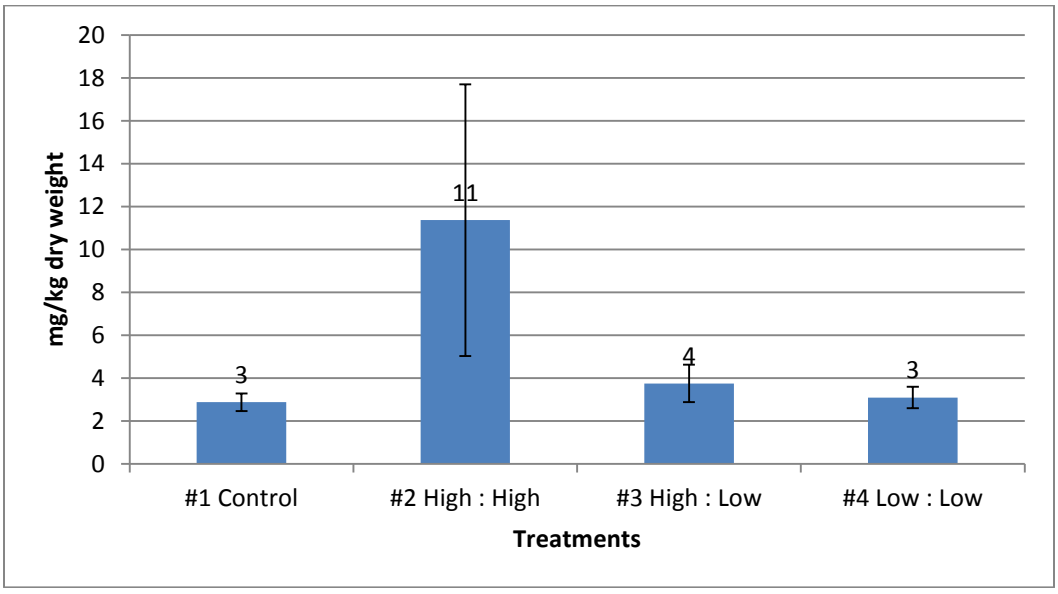



Figure 5.12 Cupper content of the basil shoot depending on different split-root (note: "control" is "no split" treatment)

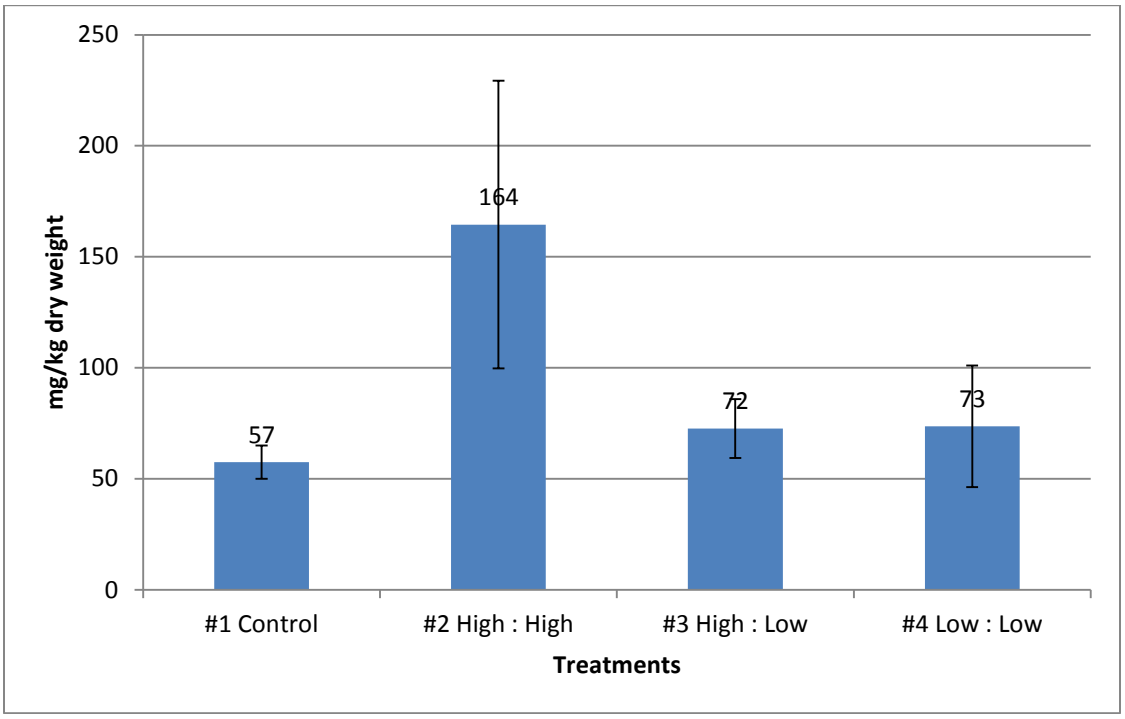



Figure 5.13 Iron content of the basil shoot depending on different split-root (note: "control" is "no split" treatment)

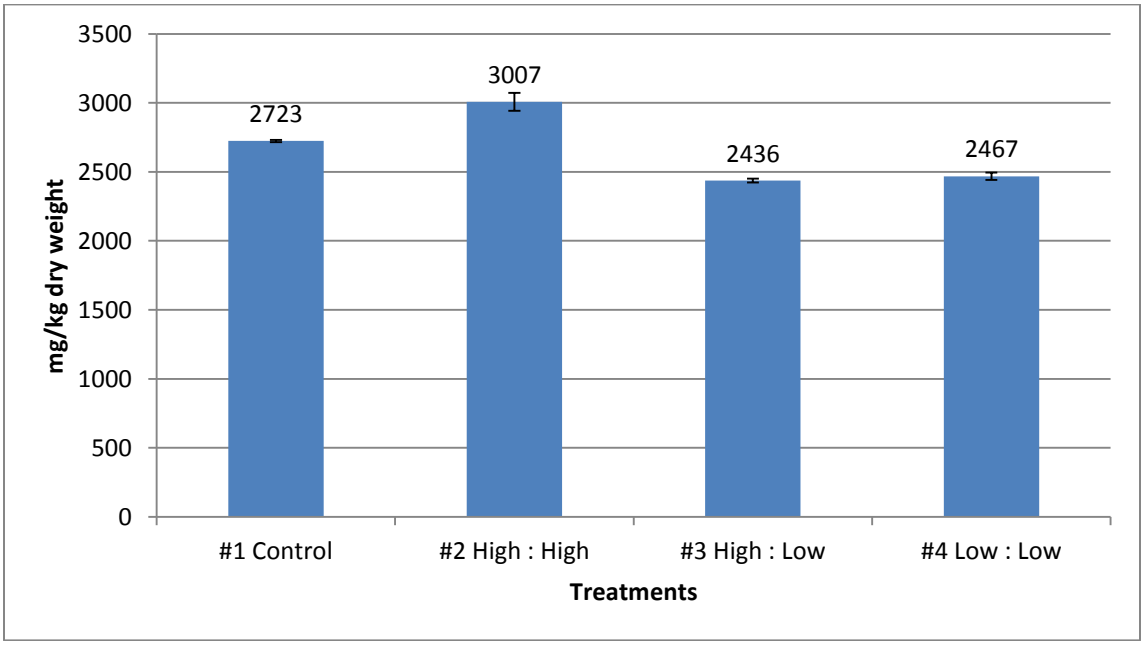



Figure 5.14 Sulfur content of the basil shoot depending on different split-root (note: "control" is "no split" treatment)

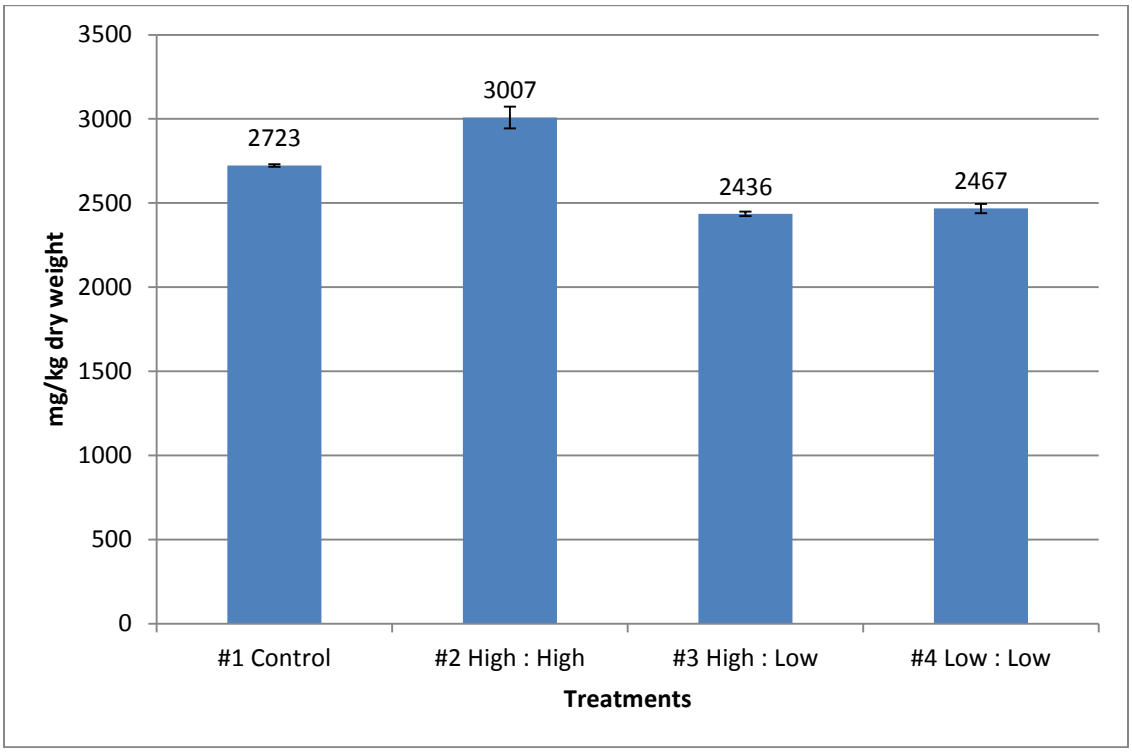



Figure 5.15 Sodium content of the basil shoot depending on different split-root (note: “control” is “no split” treatment)

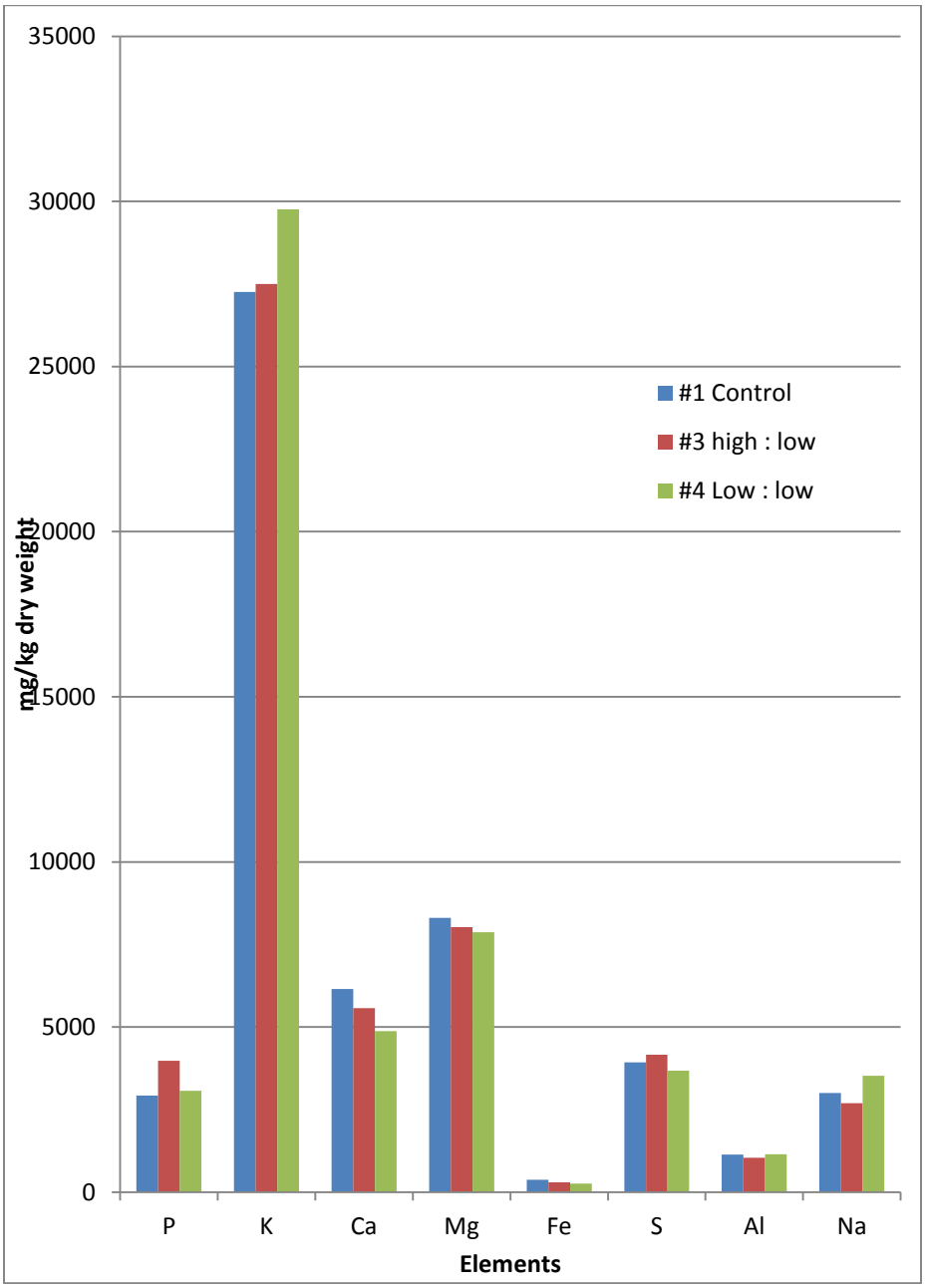



Figure 5.16 Mineral element content of the basil root depending on different split-root (note: “control” is “no split” treatment)

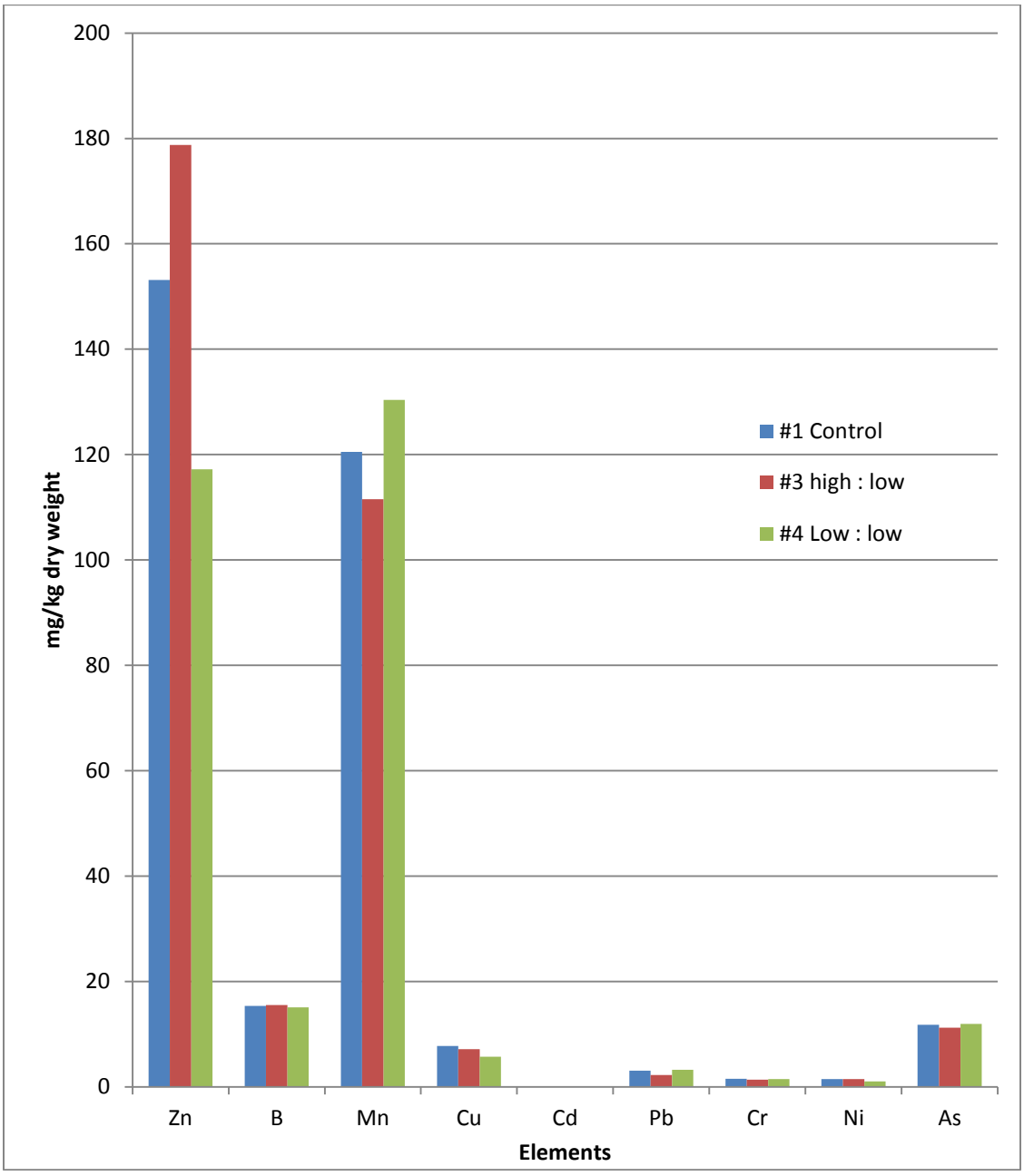



Figure 5.17 Mineral element content of the basil root depending on different split-root (note: “control” is “no split” treatment)

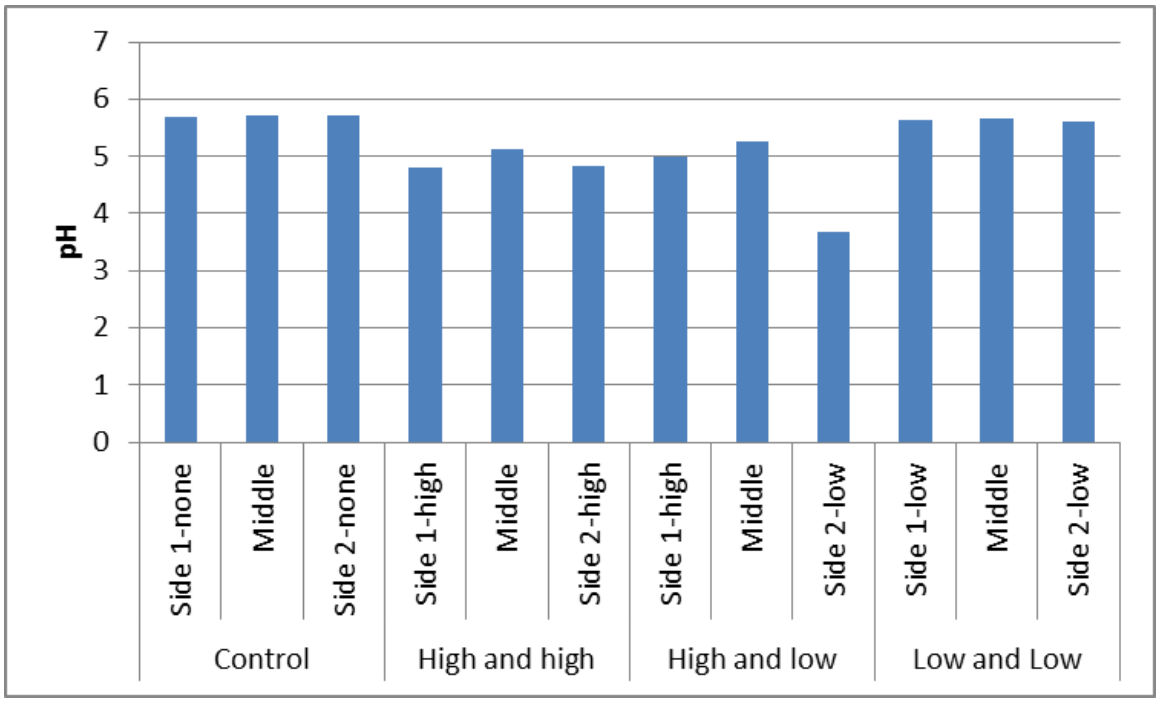



Figure 5.18 Effect of different split-root nutrition on media pH (note: "control" is "no split" treatment)

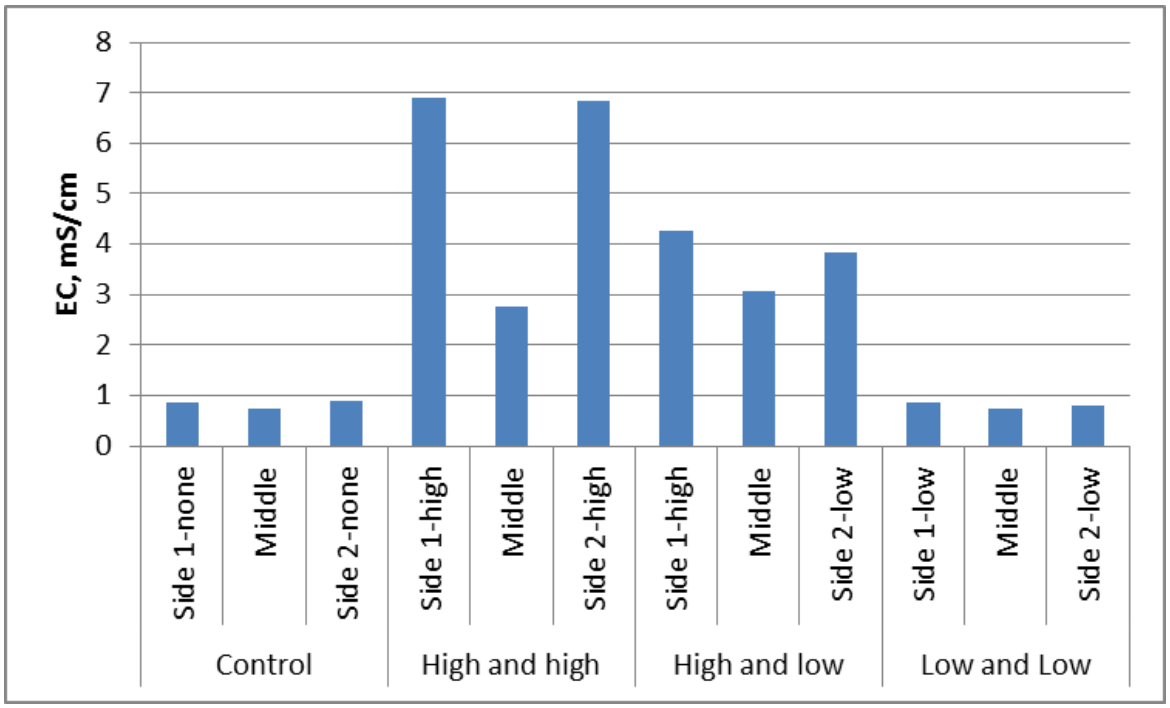



Figure 5.19 Effect of different split-root nutrition on media EC (note: "control" is "no split" treatment)

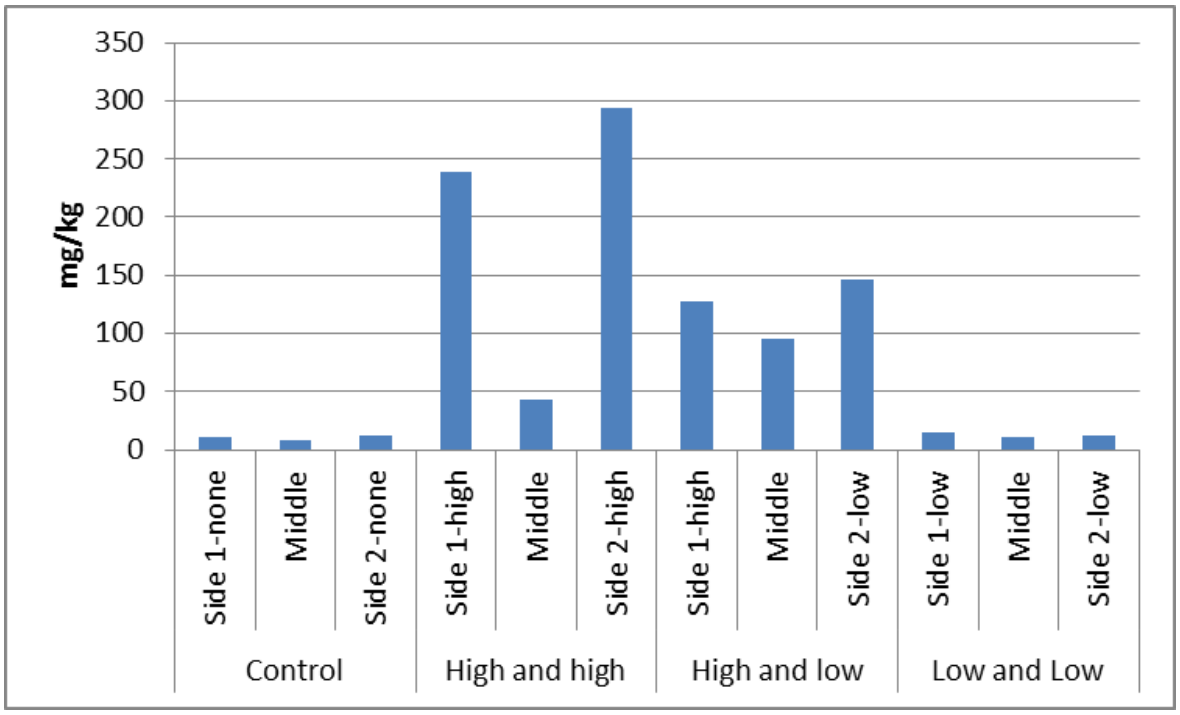



Figure 5.20 Effect of different split-root nutrition on media $NO_3$ (note: "control" is "no split" treatment),

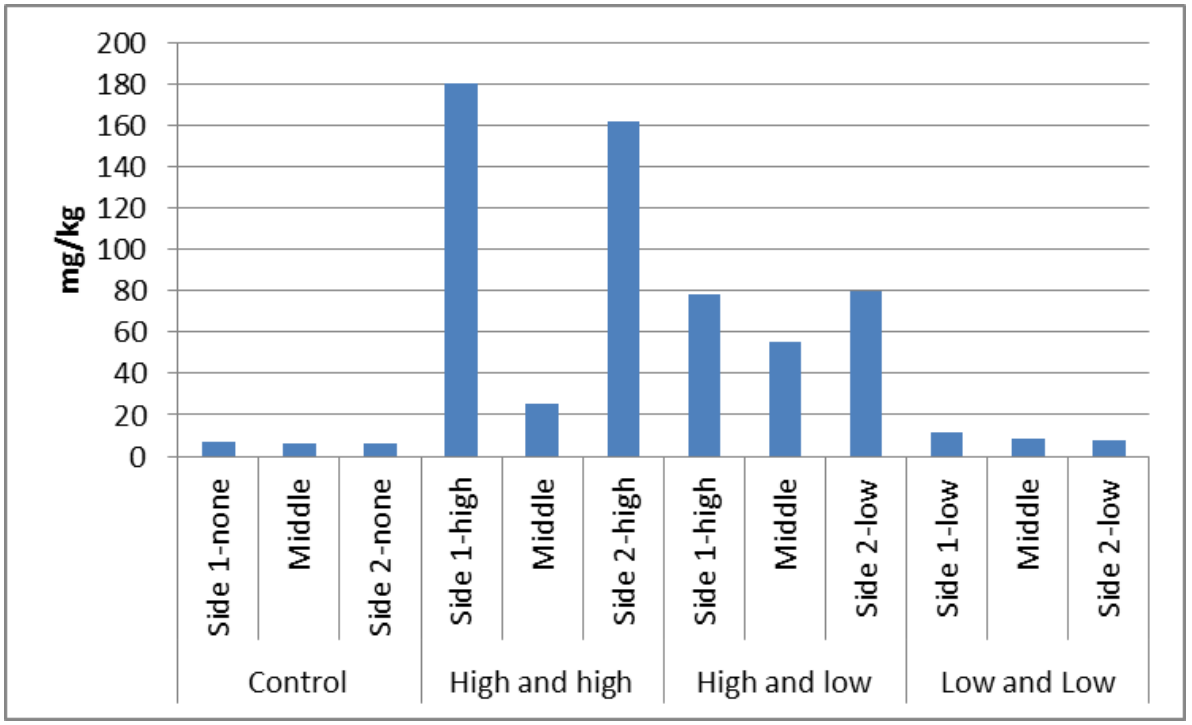



Figure 5.21 Effect of different split-root nutrition on media $NH_4$–N (note: "control" is "no split" treatment)

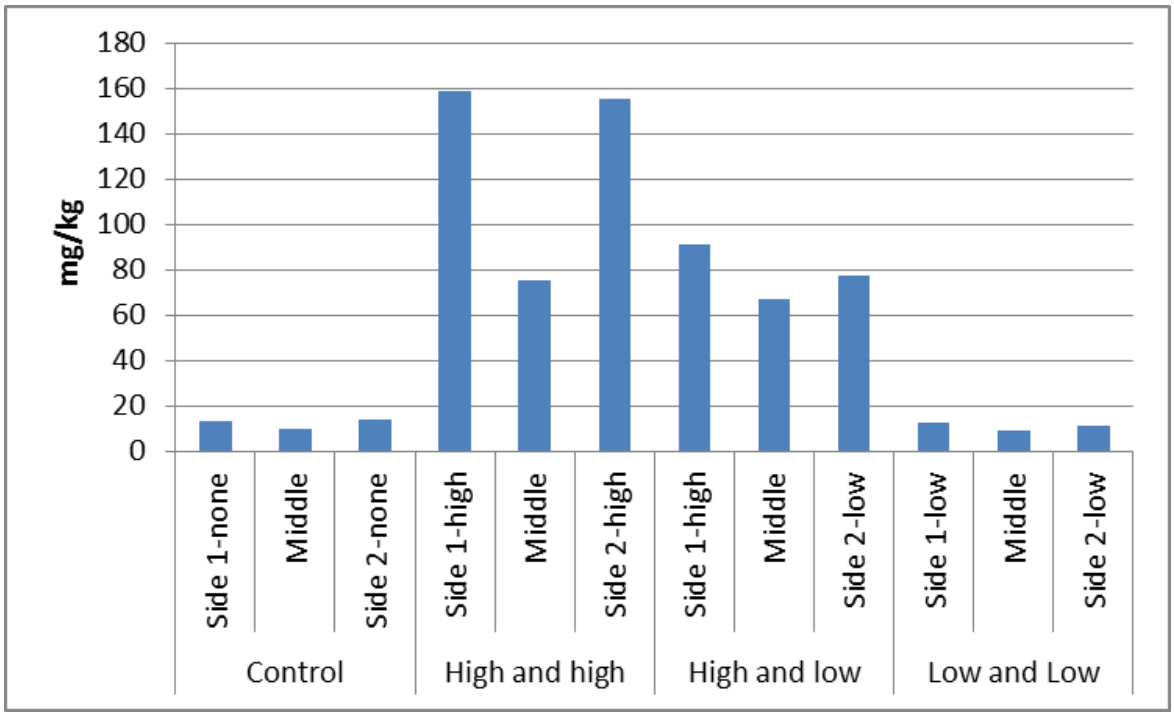



Figure 5.22 Effect of different split-root nutrition on media P (note: "control" is "no split" treatment)

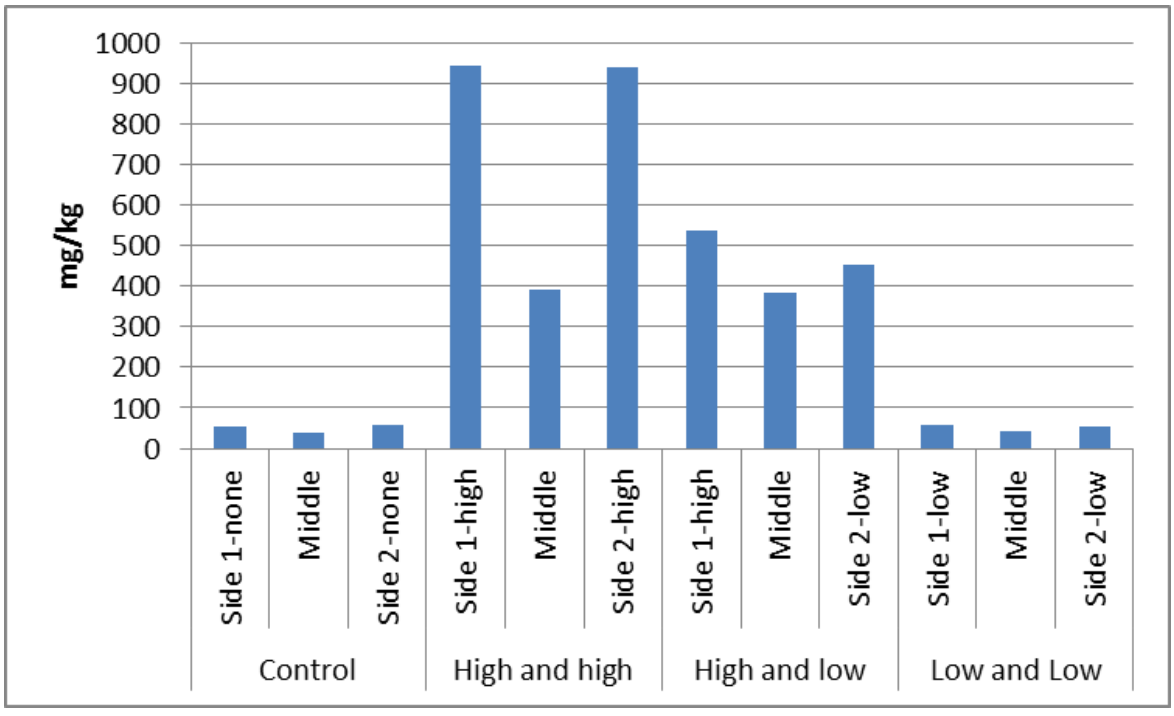



Figure 5.23 Effect of different split-root nutrition on media K (note: "control" is "no split" treatment)

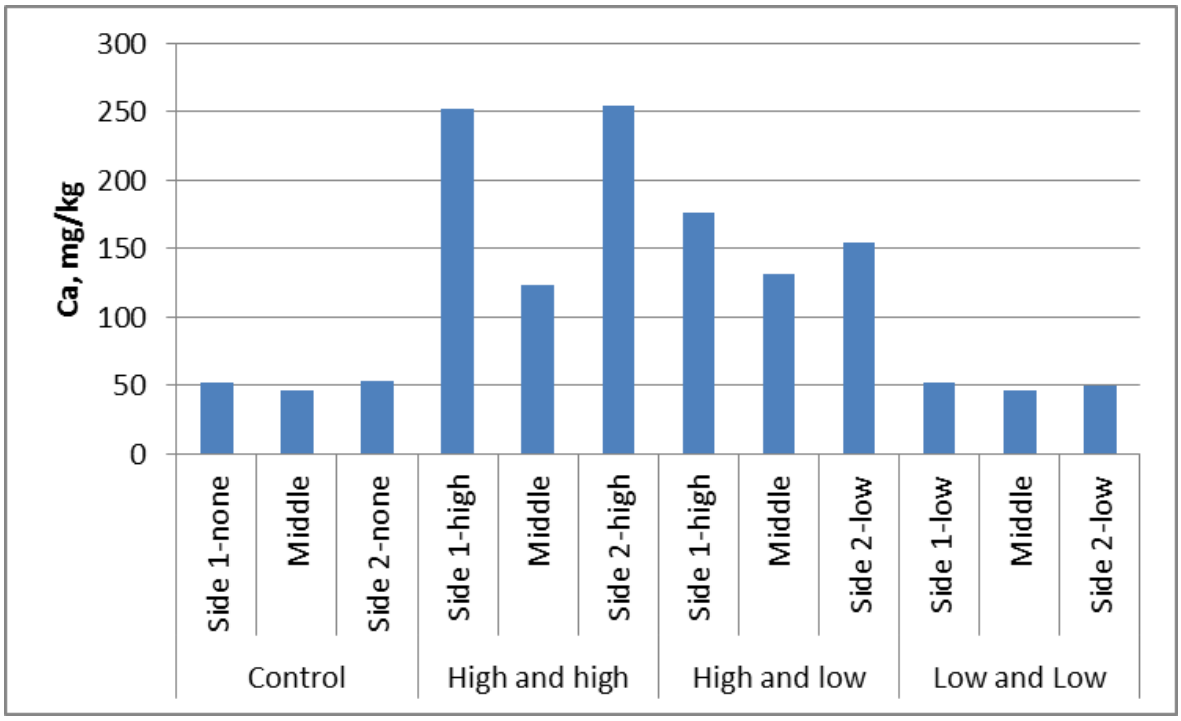



Figure 5.24 Effect of different split-root nutrition on media Ca (note: "control" is "no split" treatment)

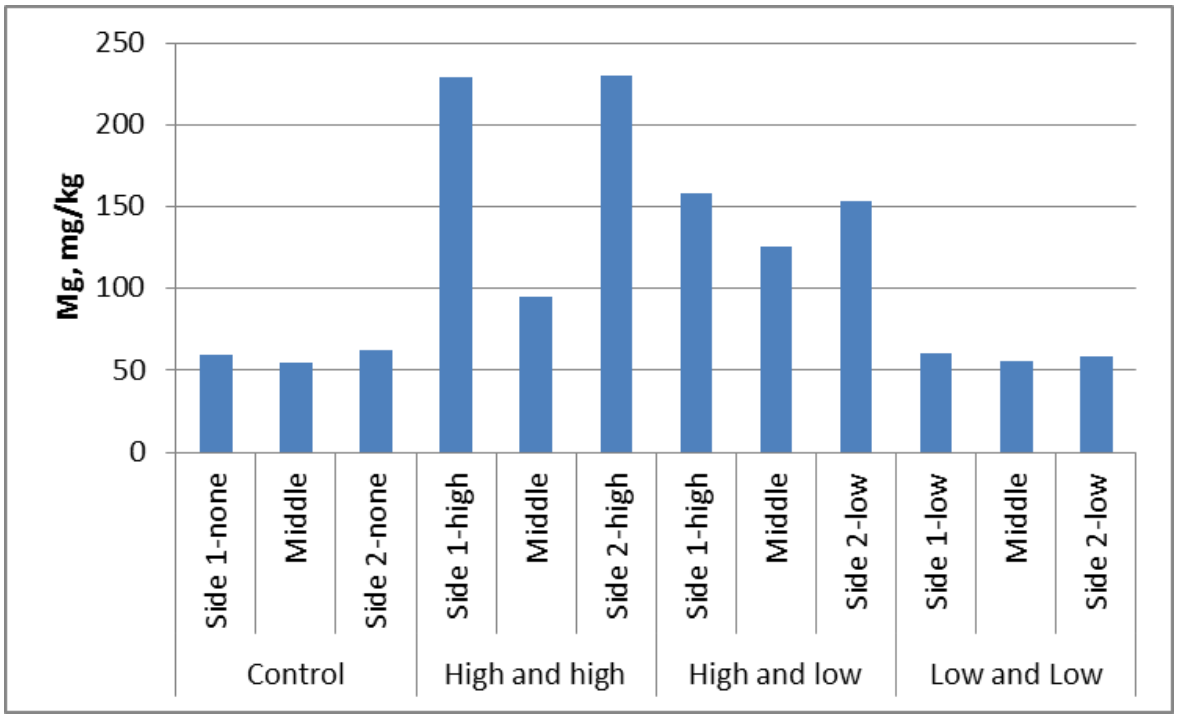



Figure 5.25 Effect of different split-root nutrition on media Mg, mg/kg

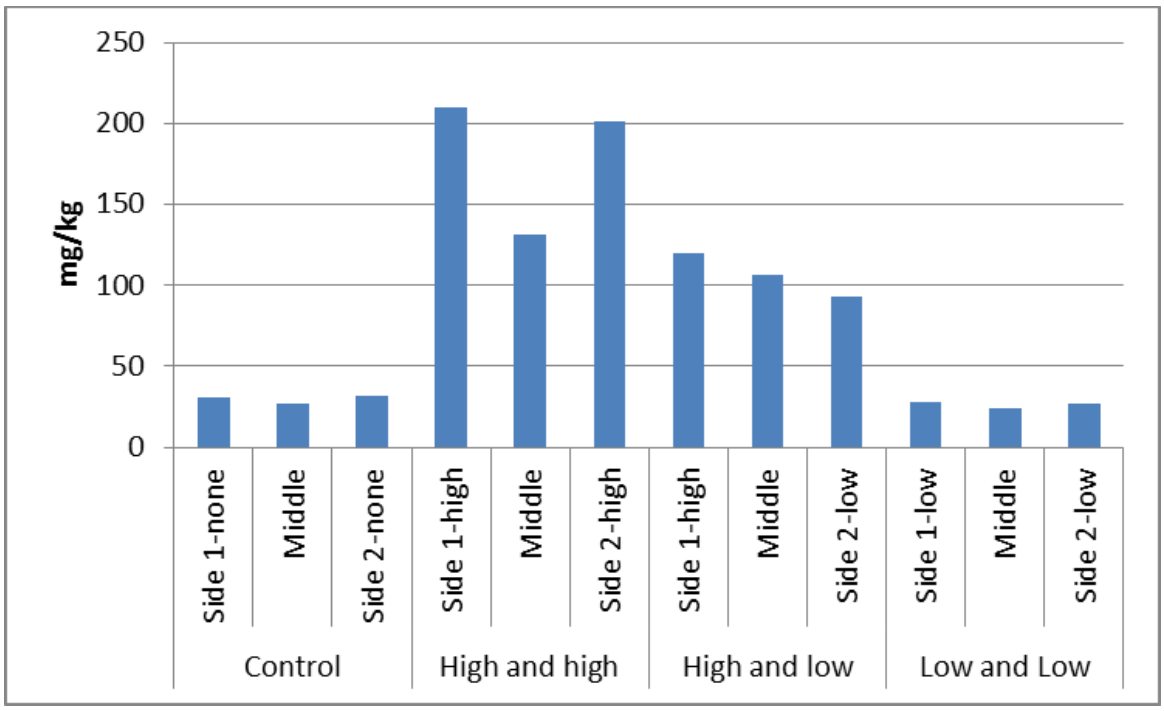



Figure 5.26 Effect of different split-root nutrition on media S (note: "control" is "no split" treatment)

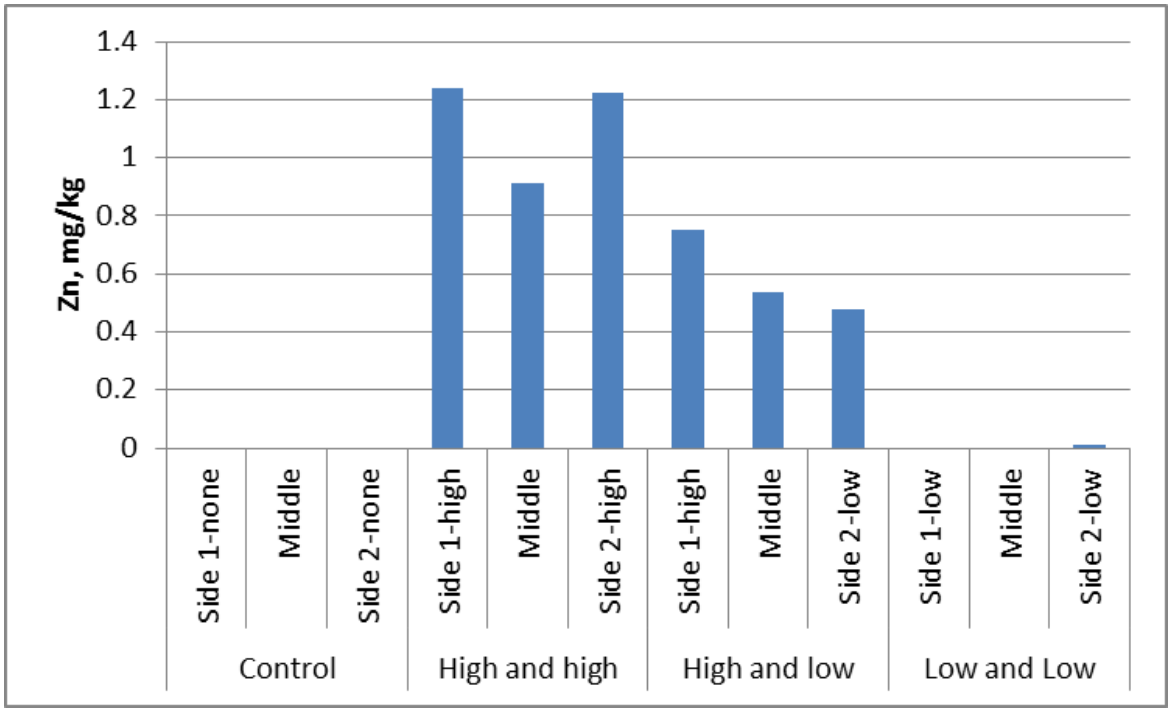



Figure 5.27 Effect of different split-root nutrition on media Zn (note: "control" is "no split" treatment)

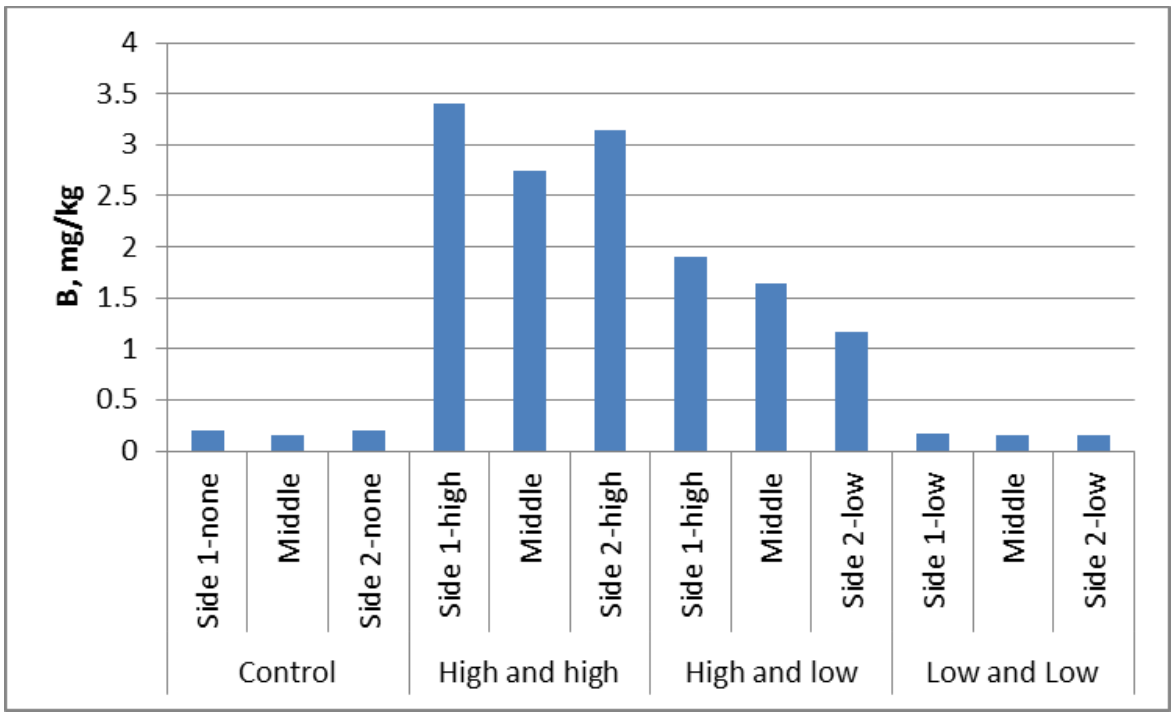



Figure 5.28 Effect of different split-root nutrition on media B (note: "control" is "no split" treatment)

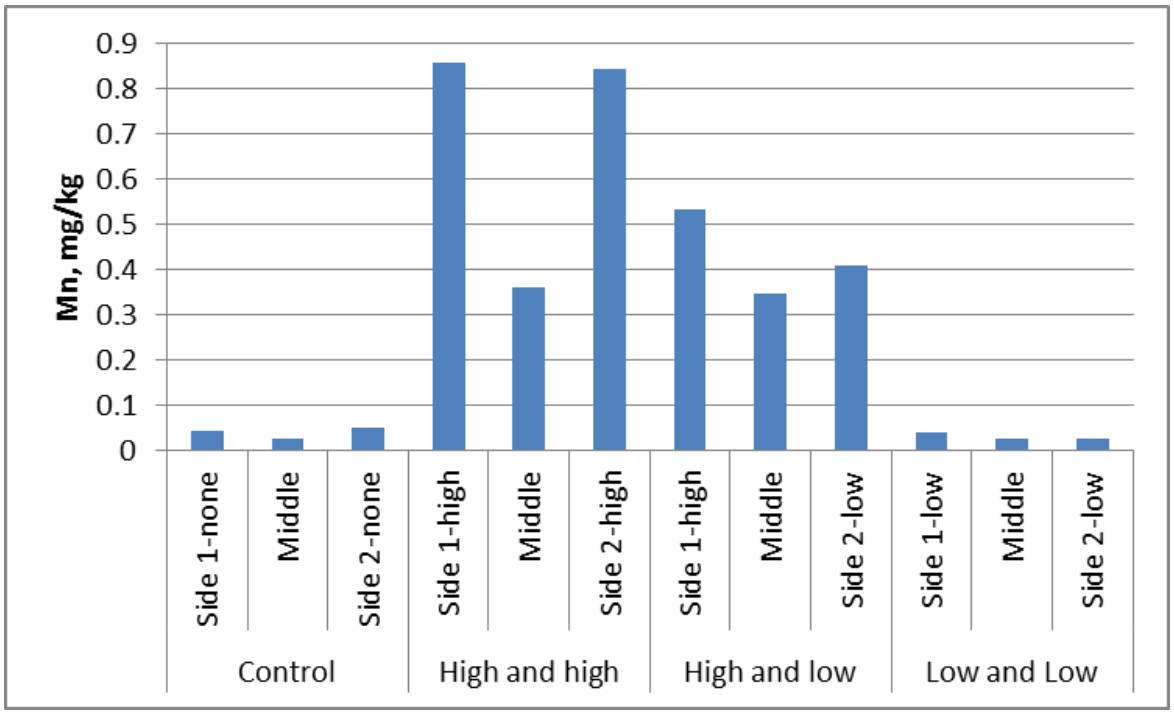



Figure 5.29 Effect of different split-root nutrition on media Mn (note: "control" is "no split" treatment)

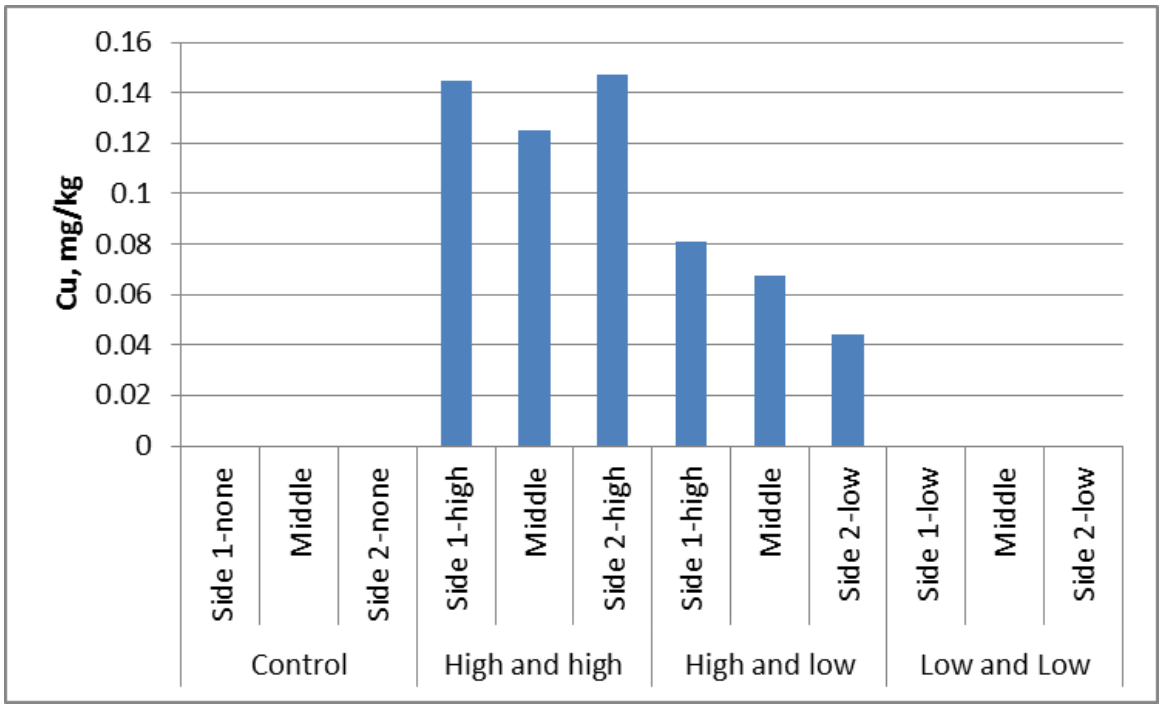



Figure 5.30 Effect of different split-root nutrition on media Cu (note: "control" is "no split" treatment)

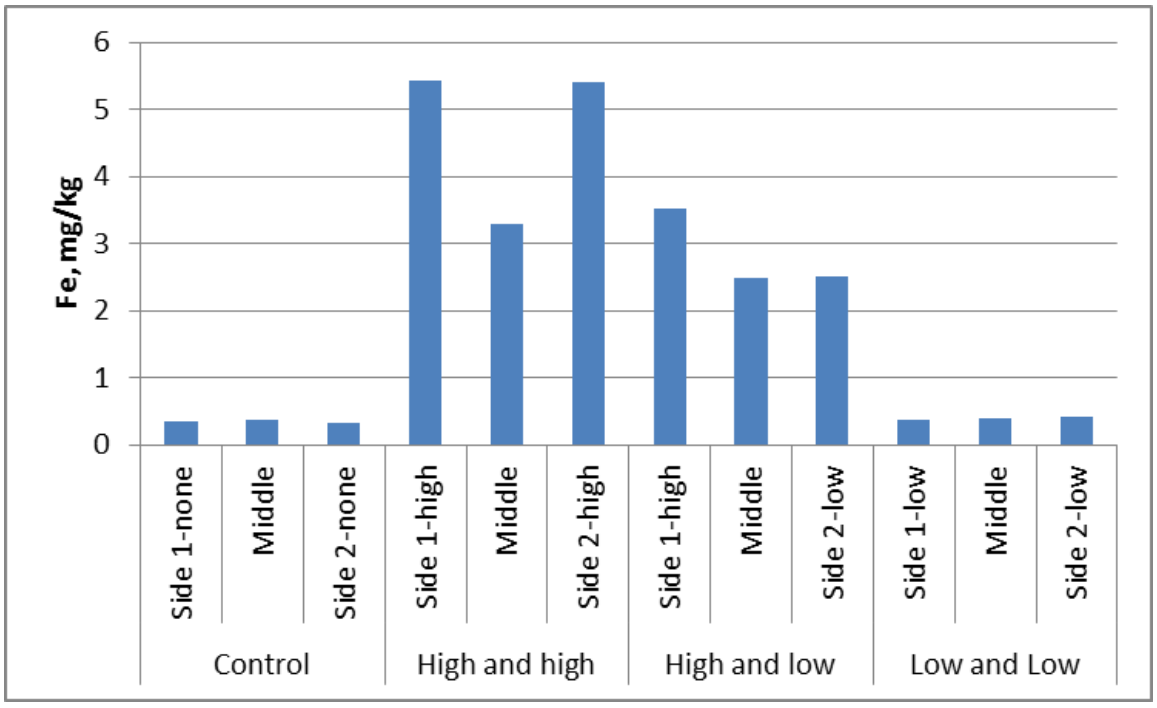



Figure 5.31 Effect of different split root-nutrition on media Fe (note: "control" is "no split" treatment)


Figure 5.32 Preliminary experiments with other crops; high nutrient concentration part of the root zone using tubes with experimental colloid nutrient solution.

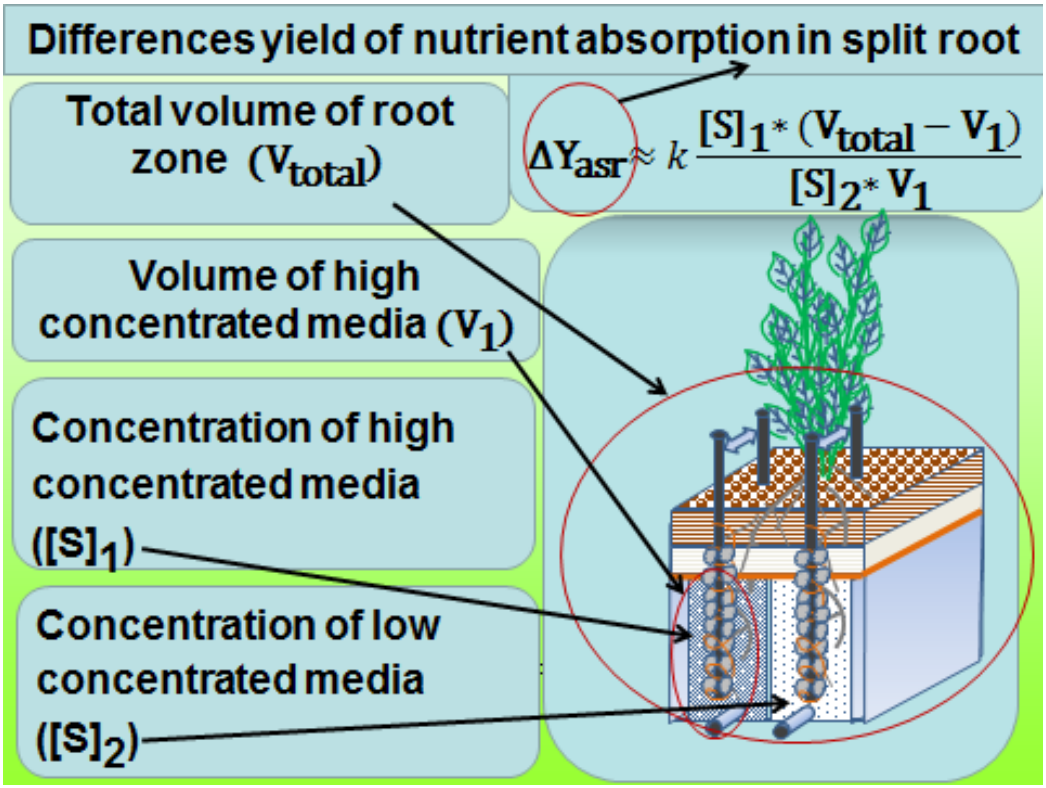



Figure 5.33 Differences yield of nutrient absorption in split root nutrition system

# APPENDIX

## MORE DETAILED EXPLANATION ABOUT THE FORMULA INVENTED IN THIS RESEARCH

Einstein A., who awarded with Nobile prize, made grate discovery by his formula $E=mc^2$ and Newton's second law $F=ma$ is important in science. Hoagland D., who also Nobile prize winner discovered important "plant nutrient solution".

Whoever making powerful bomb, they are using Einstein and Newton's formula. This is how it works: to make powerful bomb needs more energy ($E$), more force ($F$) and to have more energy needs more mass ($m$) and/or more speed, acceleration ($a$) of that mass. Of course increasing mass has limitation and we can't increase it much, but we may increase speed of that mass. Increasing speed of that mass also has limitation, but we may increase its' speed by blowing up that mass as a particle and in result have a more energy, more force. Certainly it is very bad making bomb, killing millions of people which happen in history in Japan in WWII.

Whoever trying to make more food, they are using Hoagland's nutrient formula, but food production rate is not high enough in our century, because population growing and climate is changing, which may cause food shortage in our planet in near future.

However combining Einstein's, Newton's and Hoagland's news can drastically increase food production in our planet and in this research short version of discovered formula $pA=[S_1]V_2$ combining these news which can make more food for billions of people.

This is how it works: for growing plant faster and for having more products needs more energy, more force. Keeping part of media with high concentrated nutrient solution, namely having poly-media will act same as a bomb in a very small scale. Mass ($m$) is increased by one time application of all nutrients. Mass as a particle moves faster from high to low concentrated media, which increasing speed of that mass's particles as a nutrients. Keeping part of media with low concentrated nutrient solution giving possibility for plant regulate its nutrition, instead human regulated plant nutrition in traditional mono-media.

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