

Evaluation of the Efficiency of Dual Chelation Technology in Foliar Nutrient Absorption and Site-specific Translocation of Different Nutrients within Plants

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1. INTRODUCTION

Foliar applied nutrition is becoming more popular and widely used in the Almond industry due to its ability to correct nutrient deficiencies relatively quickly and effectively^{1,2}. However, differing suppliers offer foliar nutrients which vary in formulation, form and chelation agents. Most suppliers compare product efficiency in terms of treated and control trials, so there are a limited number of studies which evaluate nutrient translocation between different plant parts (such as leaves, kernels and developing buds). Nutrient transport and accumulation into the kernel is directly correlated with kernel yield and quality. Better understanding of the active role of Dual Chelation Technology (DCT) in promoting site specific nutrient accumulation will aid the Almond industry in its decision making on nutrient application scheduling and formulations. As such this study aimed to understand nutrient accumulation into kernels via Dual Chelation Technology, and the potential return on investment for foliar nutrients in the Almond industry.

Dual Chelation Technology

Dual Chelation Technology is a patented and unique fertilizer formulation technology that was developed to deliver plant nutrients and minerals efficiently into plant tissues where nutrient corrections are required. The Dual Chelated products contain uniquely formulated minerals and plant nutrients together with organically derived amino-acids, and biologically highly active molecules (BAOM – patented product). Organically derived amino-acid chelated minerals have a lower level of phytotoxicity and a higher level of penetration into plant tissues. Biologically active molecules drive the nutrition to where it is required within the plant. The ultimate result efficiently delivers plant nutrition and minerals to address nutrition deficiencies and increase the productivity of plants.

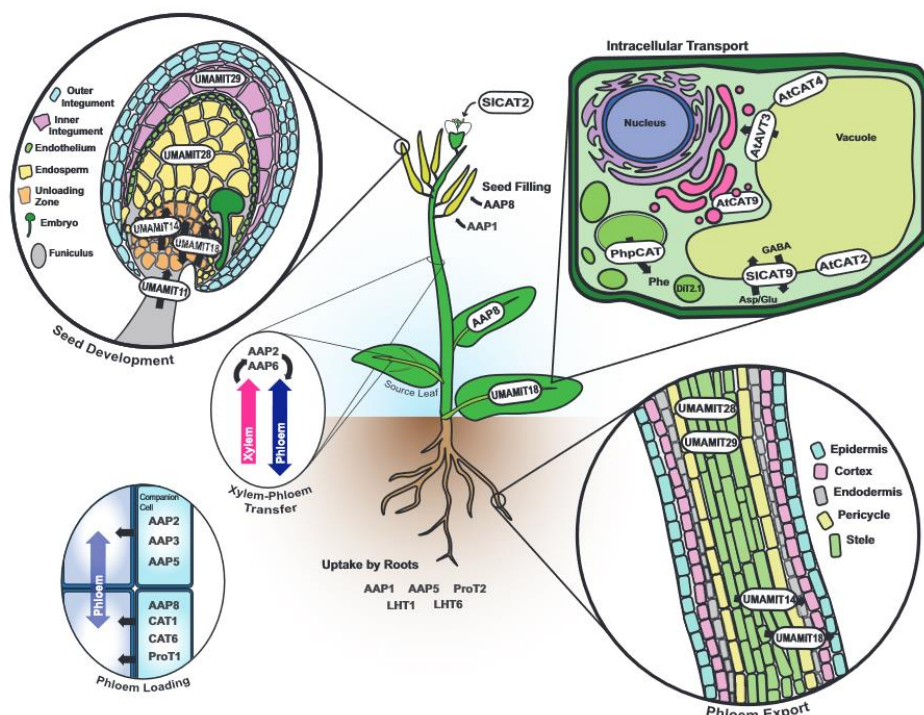


Figure 1. Amino acid transport pathways within plants, and summary of role of amino acid transporters in plants (Dinkeloo et al., 2017).

2. OBJECTIVE

The specific objectives of this study are:

1. To study the efficacy of Dual Chelation Technology (amino acid chelated nutrients + CPPA) in nutrient absorption via leaves.
2. To study the site-specific translocation of different nutrients as per the nutrient requirement at nut maturing stage.
3. To examine the impacts of foliar applied nutrients on the yield parameters: nut weight, hull weight and kernel weight, and the outturn and return on investment.

3. MATERIALS AND METHOD

3.1. Site Selection and Trial Design

Site selection was done according to the OLAM orchards annual trial plan, and Campbells farm in Victoria was selected as the trial site. A block in the new development (5th leaf) with relatively small trees was selected, to conduct manual foliar spray and to ensure 100% spray coverage. The trial design was Randomised Complete Block Design with three (3) trees for each treatment, replicated three times. Water volume used was 1200 L/ha. Spray volume per tree was 4.76 L (1200 L/ha water, 252 Trees/ ha). Every step of the trial was supervised by the OLAM technical agronomist.

Table1. Treatments and application rate

Treatment	Rate/ ha	Active Ingredient/Ha
Transit Mg	2Kg	134g/ha
Transit Ca	2Kg	210g/ha
Transit Zn	2Kg	210g/ha
Transit Cu	2Kg	336g/ha
Transit Fe	2Kg	210g/ha
Premium Trace (Cu, B, Zn, Mn & Fe)	2Kg	210g/ha
Control	0	0

Figure1. Trial Layout –Randomised Complete Block Design

		Tree Numbers from South Side of the Block																						
		South																						
NP	ROW 361	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
	ROW 360	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
NP	ROW 359	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
	ROW 358	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
NP	ROW 357	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
	ROW 356	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
NP	ROW 355	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
	ROW 354	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
NP	ROW 353	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
	ROW 352	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
NP	ROW 351	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
	ROW 350	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
NP	ROW 349	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
	ROW 348	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
NP	ROW 347	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23

Treatments	1	Magnesium			2kg/ha
	2	Calcium			2kg/ha
	3	Zinc			2kg/ha
	4	Copper			2kg/ha
	5	Iron			2kg/ha
	6	Coumpound			2kg/ha
	7	Control 1 & 2			0kg/ha

252 Trees/Ha
Water Rate 1,200L/Ha
Trial application rate -> 4.76L water + 8g of product per Tree

4. OBSERVATIONS

4.1. Kernel and Leaf Nutrient Analysis

After fourteen days of treatment, twenty leaves per plant were collected from three plants per treatment, and washed leaves were analysed for elements N, P, K, Ca, Mg, B, Zn, Fe, Mo, Cu and S at Phosyn Analytical Laboratory, QLD.

After fourteen days of treatment, twenty nuts per plant were collected from three plants per treatment. Nuts were manually cut open and kernels were analyzed for elements N, P, K, Ca, Mg, B, Zn, Fe, Mo, Cu and S at Phosyn Analytical Laboratory, QLD.

4.2. Kernel Weight, Hull Weight and Nut Weight

At harvest, sixty nuts were randomly collected from the whole harvested nuts from the three plants that received each treatment. Nut weight, hull weight and kernel weight were separately recorded.

4.3. Statistical analysis

Analysis of variance was performed using Prism 7 (Graph Pad Software). Significant difference between the treatments was determined by comparing the replicate means using Tukey's test ($P < 0.05$). *t*-test was performed to determine the significant difference between the control versus treated, a *P* value < 0.15 was considered to be significant.



Figure 2. Olam orchards Campbells farm trial site



Figure 3. Growth stage of almonds when treatments were applied



Figure 4, 5 Shows trees marked with different spray paints for different treatments



Figure 6, 7. Preparing samples: removing kernels from nut for nutrient analysis.

5. RESULTS

Figure 6. Analysis of Ca in the leaves and kernels with reference to Control vs Transit Ca treatment.

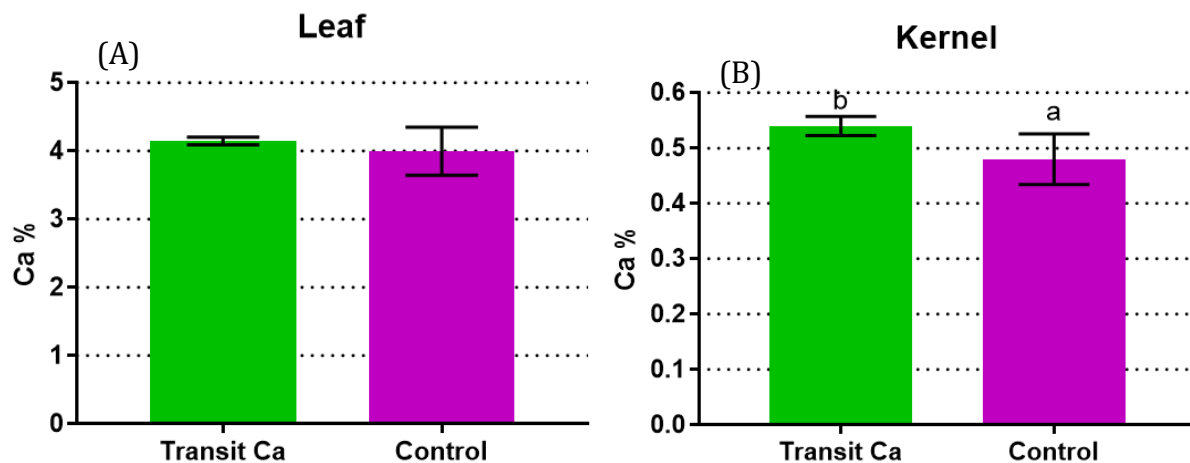


Figure 6 shows that significantly higher levels of Ca were present in the kernels of Transit Ca treated plants compared to control plants (B), while there was no significant difference in the leaf Ca levels between control and Transit Ca treated plants (A). This indicates that the foliar applied Ca was actively translocated into the kernel during the kernel development stage. An increase of 12.5% in the levels of kernel Ca was observed due to Transit Ca treatment (Table 2).

Figure 7. Analysis of Mg in the leaves and kernels with reference to Control vs Transit Mg treatment.

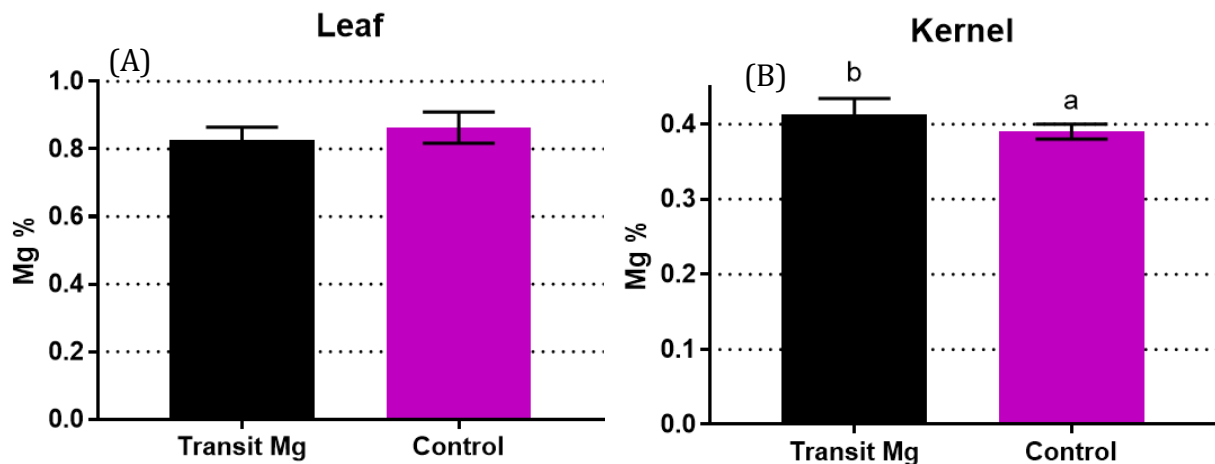


Figure 7 shows that significantly higher levels of Mg were present in the kernels of Transit Mg treated plants compared to control plants (B), while there was no significant difference in the leaf Mg levels between control and Transit Mg treated plants (A). This indicates that the foliar applied Mg was actively translocated into the kernel during the kernel development stage. An increase of 6% in the levels of kernel Mg was observed in the Transit Mg treated plants compared to the control plants (Table 2).

Figure 8. Analysis of Cu in the leaves and kernels with reference to Control vs Transit Cu treatment.

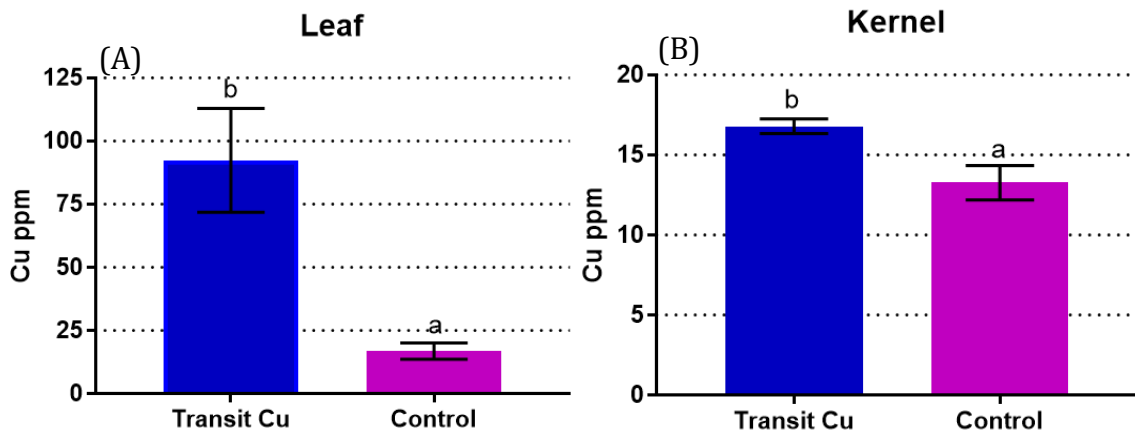


Figure 8 shows that significantly higher levels of Cu were present both in the leaves and kernels of Transit Cu treated plants compared to control plants (A) and (B). A 450% increase in the levels of leaf Cu and a 21% increase in the levels of kernel Cu was observed in the Transit Cu treated plants compared to the control plants (Table 2).

Figure 9. Analysis of Zn in the leaves and kernels with reference to Control vs Transit Zn treatment.

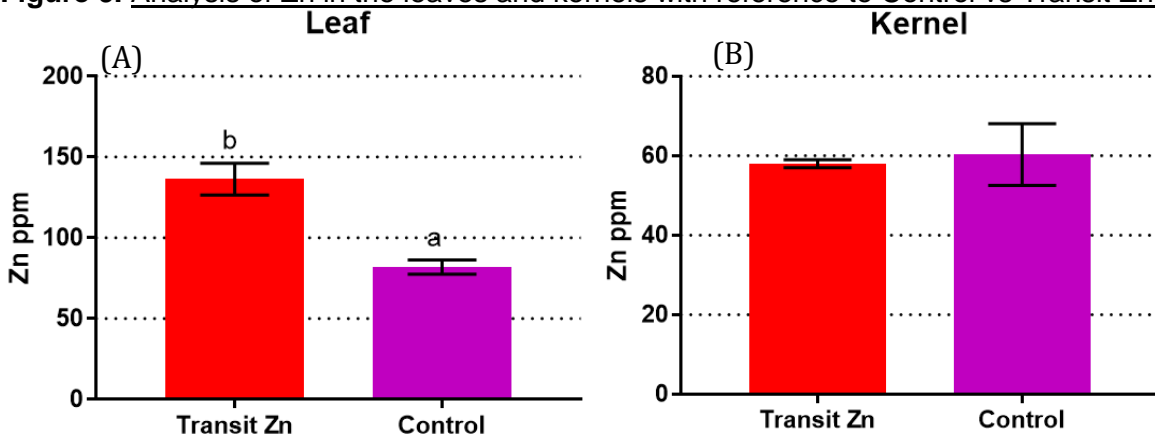


Figure 9 shows that significantly higher levels of Zn were present in leaves of Transit Zn treated plants compared to control plants (A), while there was no significant difference in the kernel Zn levels between control and Transit Zn treated plants (B). Leaf Zn levels increased by 66.2% in the Transit Zn treatment compared to control (Table 2).

Figure 10. Analysis of Fe in the leaves and kernels with reference to Control vs Transit Fe treatment.

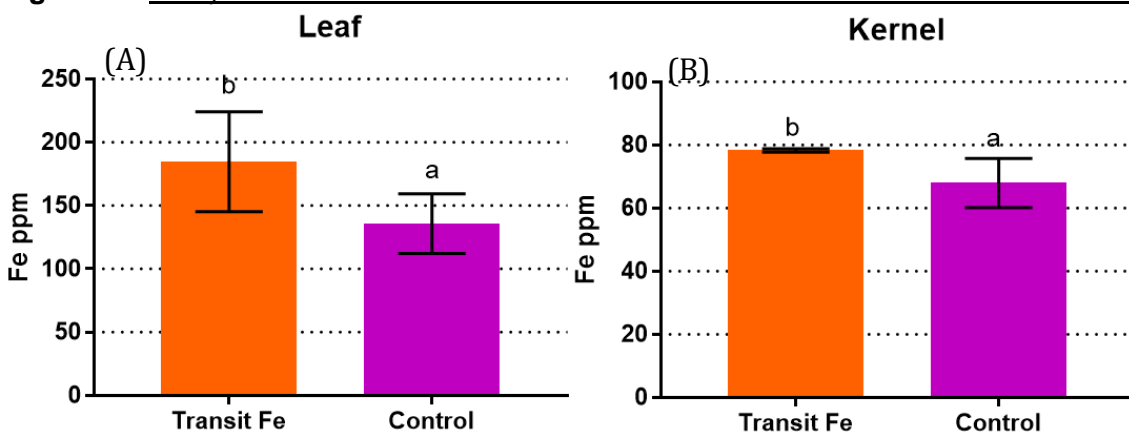


Figure 10 shows significantly higher levels of Fe both in the leaves and kernels of Transit Fe treated plants compared to control plants (A) and (B). Leaf Fe levels increased by 36.1% and kernel Fe levels increased by 15.2% in the Transit Fe treated plants compared to the control plants (Table 2).

Figure 11. Analysis of yield parameters with reference to Control vs Premium Trace treatment.

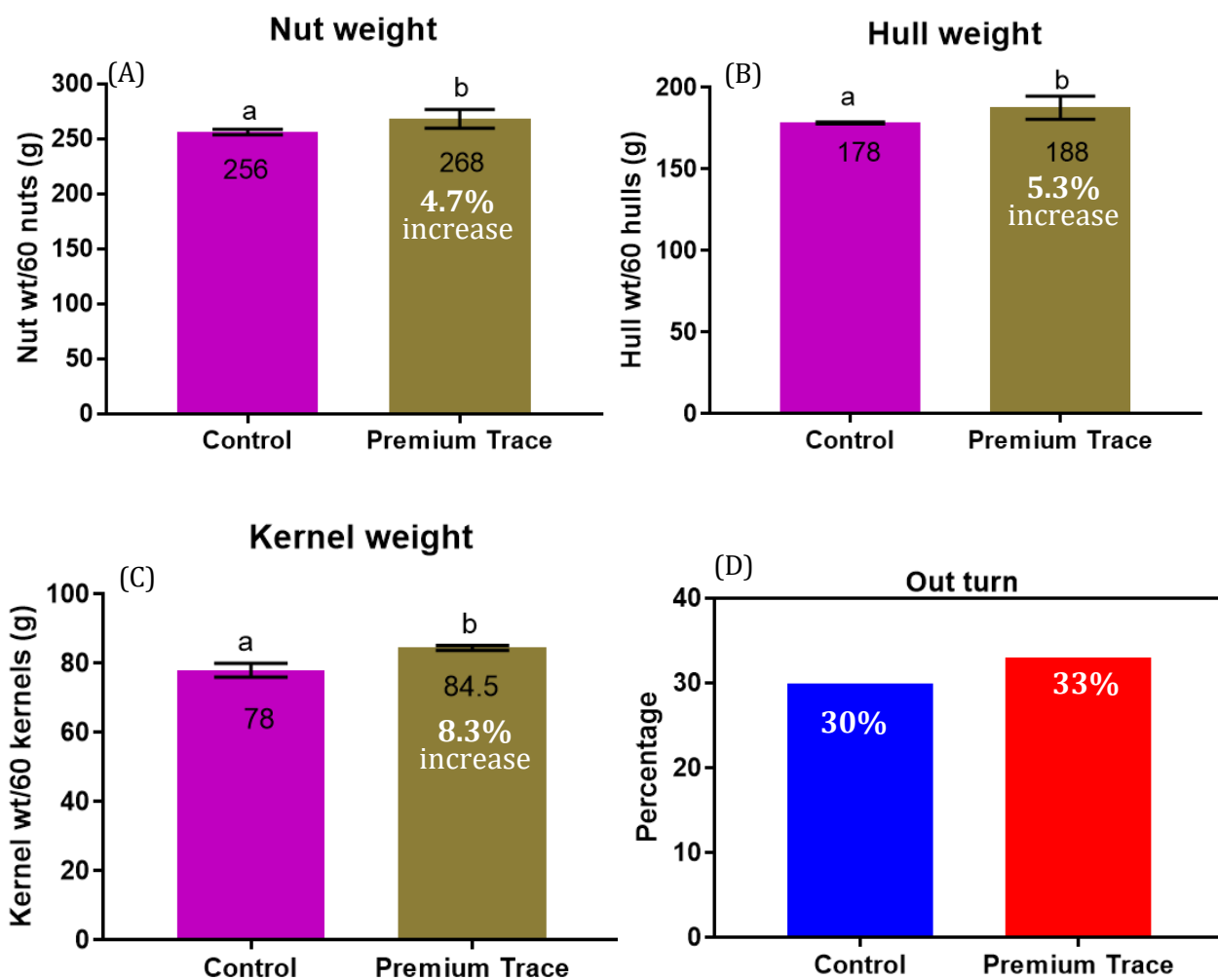


Figure 11 shows that the Premium Trace treatment significantly increased the Nut weight (A), Hull weight (B) and Kernel weight compared to the control. Nut weight, hull weight and kernel weight were increased by 4.7%, 5.3% and 8.3% respectively by the Premium Trace treatment compared to the control (Table 3). Out turn was calculated as the percentage of kernel weight to nut weight. Out turn was increased by 3% by the Premium Trace treatment compared to the control

* **Figure 6 to Figure 11.** Each bar represents mean \pm SE (n=3 replicates). A *t*-test was performed to determine the significant difference between the control Vs treated, different superscripts show significant difference ($P < 0.15$). The *t*-test was performed with Prism 7 (Graph Pad Software).

Table 2. Analysis of different nutrient levels in the leaves and kernels with reference to different Dual Chelated nutrient product treatments.

Parameters	Treatments		P value	Significance	% increase
	Control	Transit Ca			
Leaf Ca %	3.997 ± 0.2028	4.147 ± 0.0318	0.51	ns	-
Kernel Ca %	0.48 ± 0.02646	0.54 ± 0.01	0.1012	yes	12.5%
	Control	Transit Mg			
Leaf Mg %	0.8633 ± 0.02667	0.8267 ± 0.02186	0.35	ns	-
Kernel Mg %	0.39 ± 0.005774	0.4133 ± 0.01202	0.155	yes	6%
	Control	Transit Cu			
Leaf Cu ppm	16.8 ± 1.852	92.4 ± 11.86	0.0033	yes	450%
Kernel Cu ppm	13.27 ± 0.6227	16.8 ± 0.2646	0.0064	yes	21%
	Control	Transit Zn			
Leaf Zn ppm	82 ± 2.517	136.3 ± 5.667	0.0009	yes	66.2%
Kernel Zn ppm	58 ± 0.5774	60.33 ± 4.485	0.633	ns	-
	Control	Transit Fe			
Leaf Fe ppm	135.7 ± 13.59	184.7 ± 22.7	0.1377	yes	36.1%
Kernel Fe ppm	68 ± 4.509	78.33 ± 0.3333	0.0843	yes	15.2%

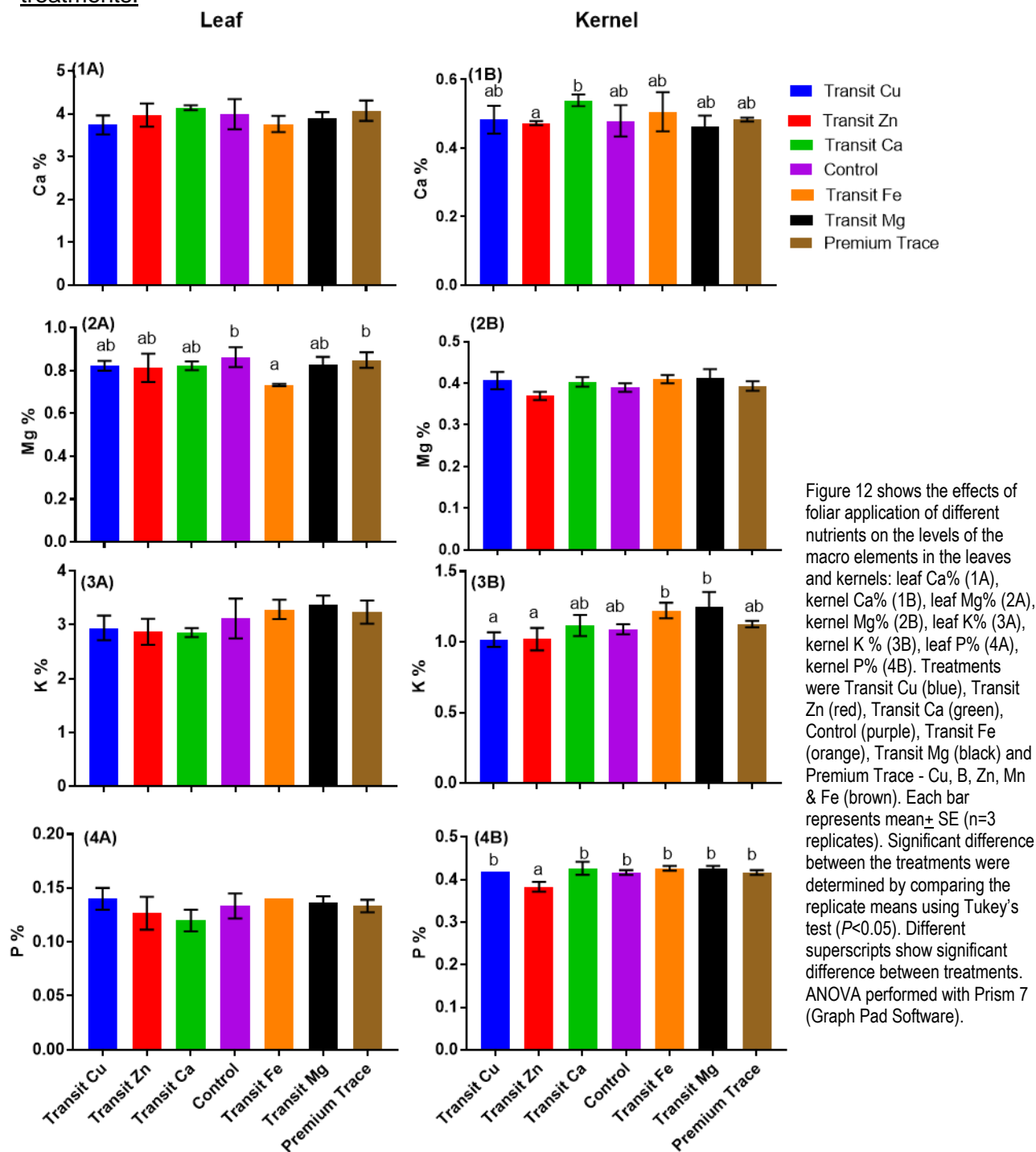
The values given are mean ± standard deviation, n=3. P value <0.15 was considered to be significant.

Table 3. Analysis of yield parameters with reference to Control vs Premium Trace treatment.

Parameters	Treatments		P value	Significance	% increase
	Control	Premium Trace			
Nut weight g (of 60 nuts)	256.3 ± 1.453	268.3 ± 4.91	0.0791	yes	4.68%
Hull wt g (of 60 hulls)	178.3 ± 0.3333	187.7 ± 4.096	0.0856	yes	5.27%
Kernel wt g (of 60 kernels)	78 ± 1.155	84.5 ± 0.5	0.0242	yes	8.33%

The values given are mean ± standard deviation, n=3. P values <0.15 were considered to be significant.

Figure 12. Analysis of macro elements in the leaves and kernels with reference to different nutrient treatments.



The highest levels of kernel Ca were found in the Transit Ca treated plants compared to all the other treatments (Figure 12 - 1A, 1B). There were significantly higher levels of Ca present in the kernels of Transit Ca treated plants compared to the Transit Zn treated plants. Whereas, there was no significant difference between the leaf Ca levels between the treatments. Significantly lower levels of leaf Mg were found in the Transit Fe treated plants compared to the control and the premium trace treated plants, while there was no significant difference between the treatments in the levels of kernel Mg (Figure 12 - 2A, 2B). Kernel K was found to be significantly higher in the Transit Fe and Transit Mg treatments compared to Transit Zn and Transit Cu treatments. There was no significant difference between the treatments in the leaf K levels (Figure 12 - 3A, 3B). Kernel P was significantly lower in the Transit Zn treated plants compared to all the other treatments while there was no significant difference between the treatments in the leaf P levels (Figure 12 - 4A, 4B).

Figure 13. Analysis of micro elements in the leaves and kernels with reference to different nutrient treatments.

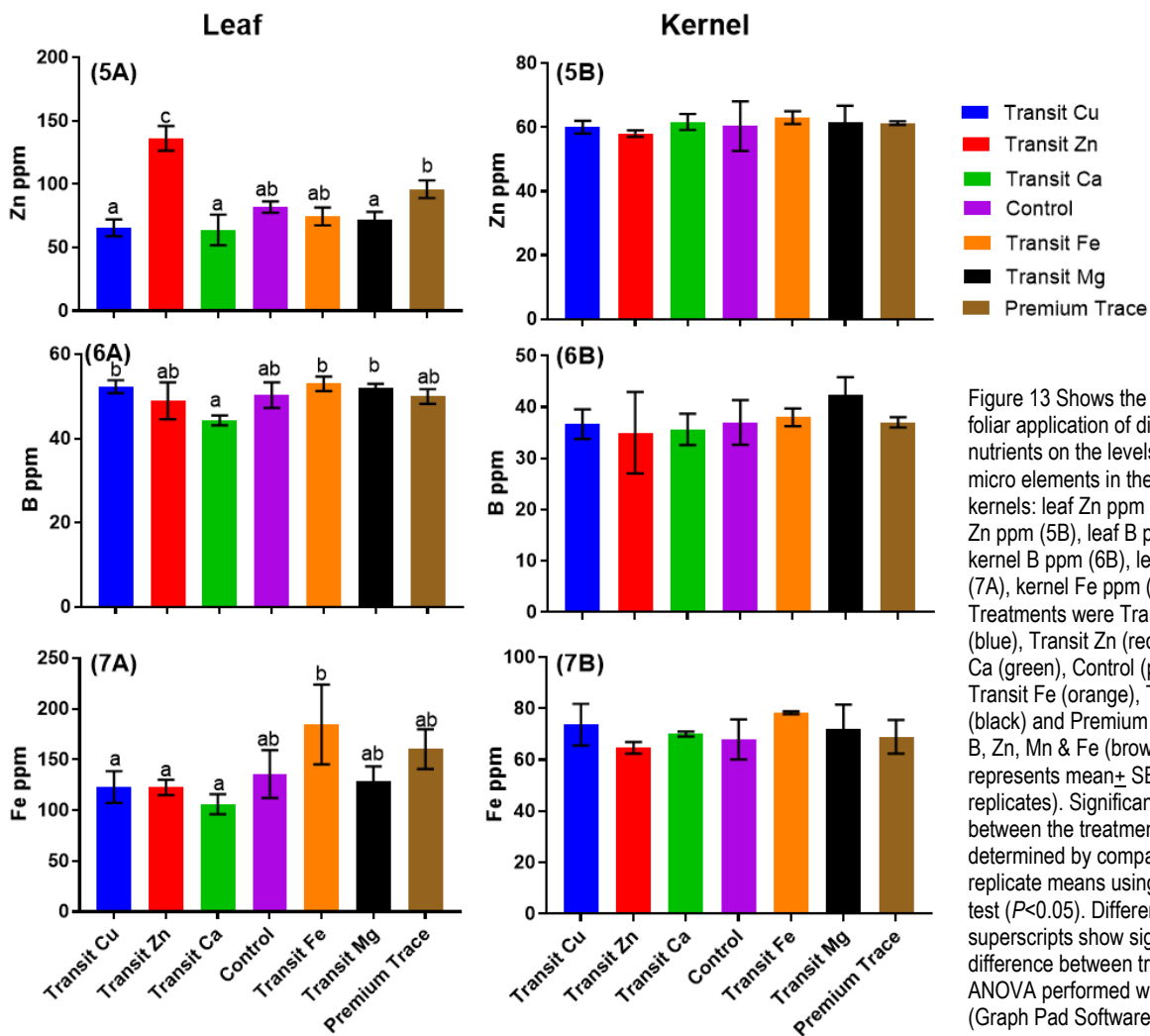


Figure 13 Shows the effects of foliar application of different nutrients on the levels of the micro elements in the leaves and kernels: leaf Zn ppm (5A), kernel Zn ppm (5B), leaf B ppm (6A), kernel B ppm (6B), leaf Fe ppm (7A), kernel Fe ppm (7B). Treatments were Transit Cu (blue), Transit Zn (red), Transit Ca (green), Control (purple), Transit Fe (orange), Transit Mg (black) and Premium Trace - Cu, B, Zn, Mn & Fe (brown). Each bar represents mean \pm SE (n=3 replicates). Significant difference between the treatments were determined by comparing the replicate means using Tukey's test ($P < 0.05$). Different superscripts show significant difference between treatments. ANOVA performed with Prism 7 (Graph Pad Software).

Leaf Zn levels were significantly higher in the Transit Zn and the Premium Trace treatments compared to all the other treatments, while there was no significant difference in the kernel Zn levels between the treatments (Figure 13 - 5A, 5B). Leaf B levels were significantly higher in the Transit, Transit Fe and Transit Mg treatments compared to Transit Ca treatment (Figure 13 - 6A, 6B). Leaf Fe levels were significantly higher in the Transit Fe treatment compared with Transit Cu, Transit Zn and Transit Ca treatments (Figure 13 - 7A, 7B). There was no significant difference between the treatments in the levels of kernel Zn, B and Fe (Figure 13 - 5B, 6B, 7B).

Figure 14. Analysis of micro elements in the leaves and kernels with reference to different nutrient treatments (continued....).

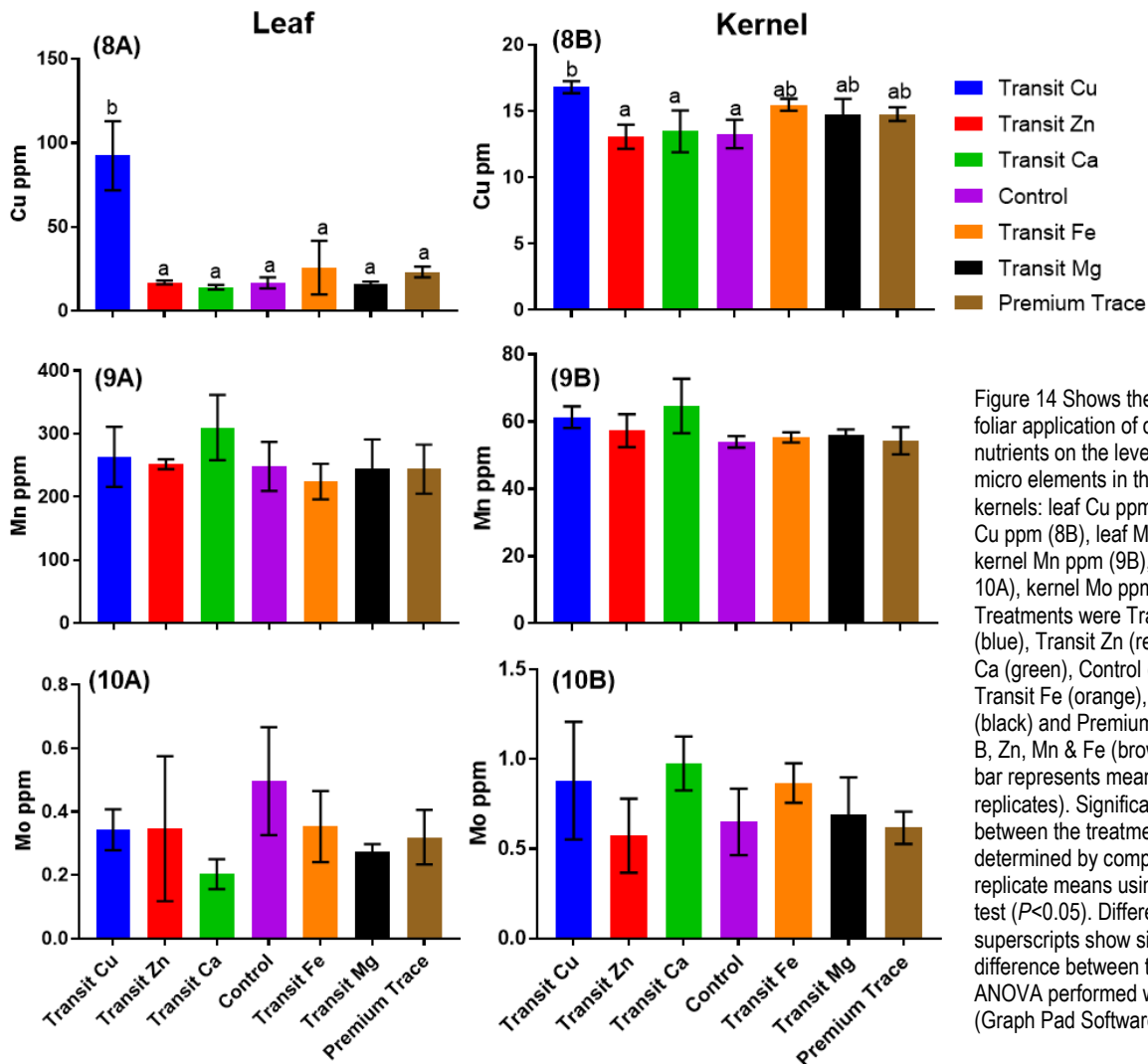


Figure 14 Shows the effects of foliar application of different nutrients on the levels of the micro elements in the leaves and kernels: leaf Cu ppm (8A), kernel Cu ppm (8B), leaf Mn ppm (9A), kernel Mn ppm (9B), leaf Mo ppm (10A), kernel Mo ppm (10B). Treatments were Transit Cu (blue), Transit Zn (red), Transit Ca (green), Control (purple), Transit Fe (orange), Transit Mg (black) and Premium Trace - Cu, B, Zn, Mn & Fe (brown). Each bar represents mean± SE (n=3 replicates). Significant difference between the treatments were determined by comparing the replicate means using Tukey's test ($P < 0.05$). Different superscripts show significant difference between treatments. ANOVA performed with Prism 7 (Graph Pad Software).

Leaf Cu levels were significantly higher in the Transit Cu treatment compared to all the other treatments, whereas the kernel Cu levels were significantly higher in the Transit Cu treatment compared to control, Transit Zn and Transit Ca (Figure 14 - 8A, 8B). There were no significant differences in the levels of Mn and Mo in leaves and kernels between the treatments (Figure 14 - 9A, 9B, 10A, 10B).

There was no significant difference in the levels of N in the leaves and kernel between any of the treatments (data not shown). Significantly higher levels of all the tested nutrients were present in the leaves than the kernels except P and Mo (Figure 12, 13, 14). P% was significantly higher in the kernels compared to the leaves (Figure 12 - 4A, 4B).

Figure 15. Analysis of yield parameters with reference to different nutrient treatments

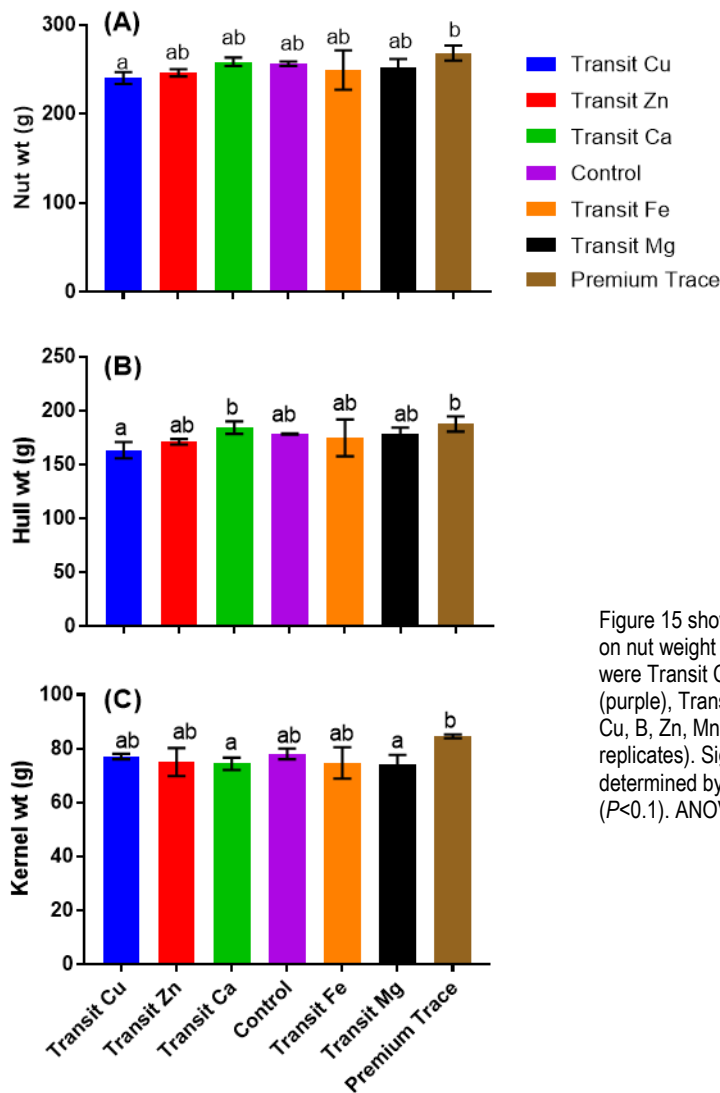


Figure 15 shows the effects of foliar application of different nutrients on nut weight (A), hull weight (B) and kernel weight (C): Treatments were Transit Cu (blue), Transit Zn (red), Transit Ca (green), Control (purple), Transit Fe (orange), Transit Mg (black) and Premium Trace - Cu, B, Zn, Mn & Fe (brown). Each bar represents mean \pm SE (n=3 replicates). Significant difference between the treatments were determined by comparing the replicate means using Tukey's test ($P < 0.1$). ANOVA performed with Prism 7 (Graph Pad Software).

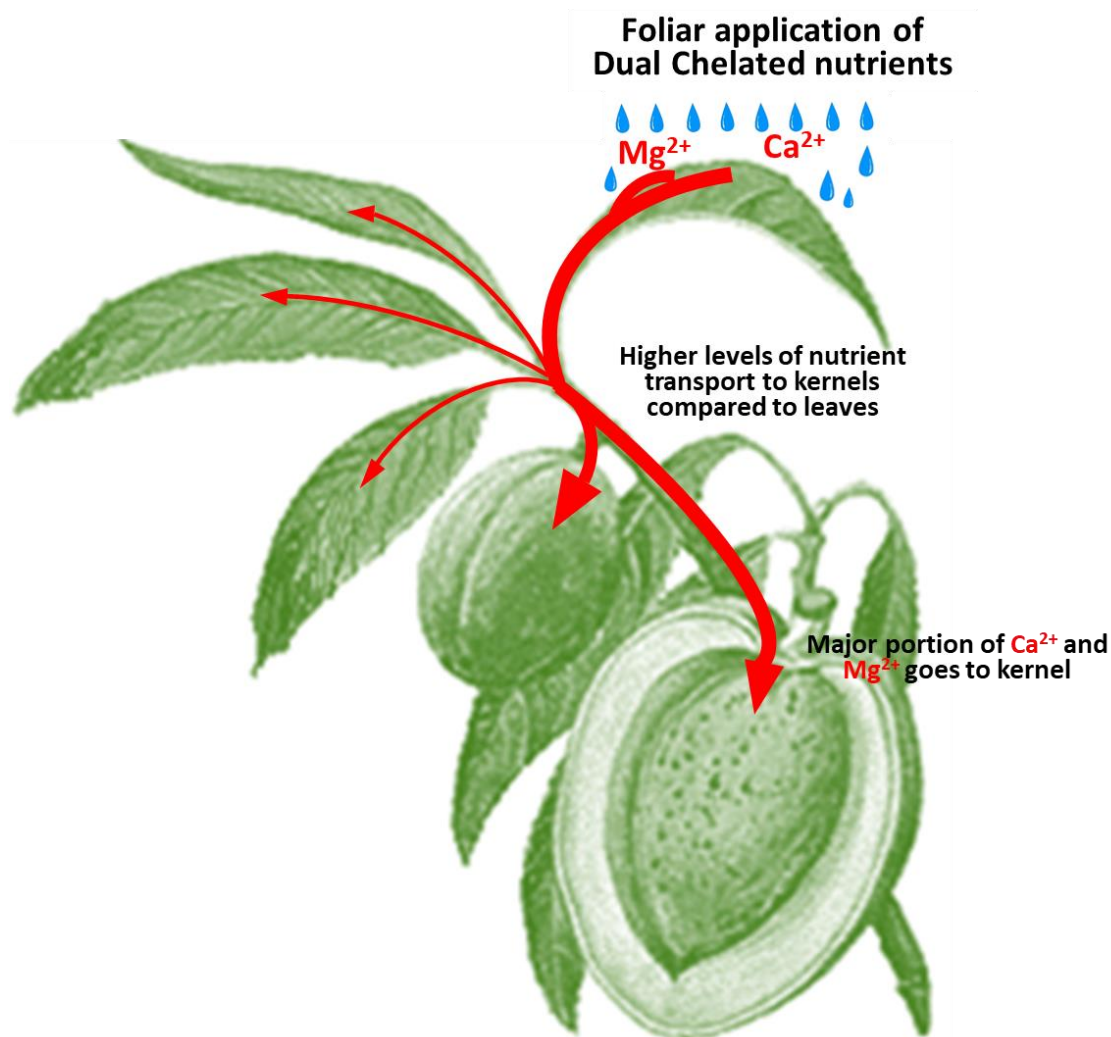
The highest values for average nut weight, hull weight and kernel weight were observed from the Premium Trace treated plants compared to all the other treatments (Figure 15 - A, B, C).

7. CONCLUSION AND FUTURE WORK

Results of this study clearly show the efficiency of Dual Chelation Technology in foliar plant nutrition. More specifically:

- This study showed that the dual chelated foliar nutrients were absorbed via leaves, and translocated to specific sites as per the nutrient requirement of plants at a particular growth stage.
- Also, this study showed that the yield from almonds can be improved by foliar applied dual chelated nutrients.
- Out turn was found to be increased by 3 % by the Premium Trace treatment compared to the control.

A better understanding of the translocation and accumulation of different nutrients to the buds is needed for decision making on post-harvest nutrition, aimed at the next season's yield development. So, along with the leaves and kernel nutrients, bud nutrient levels also need to be assessed.

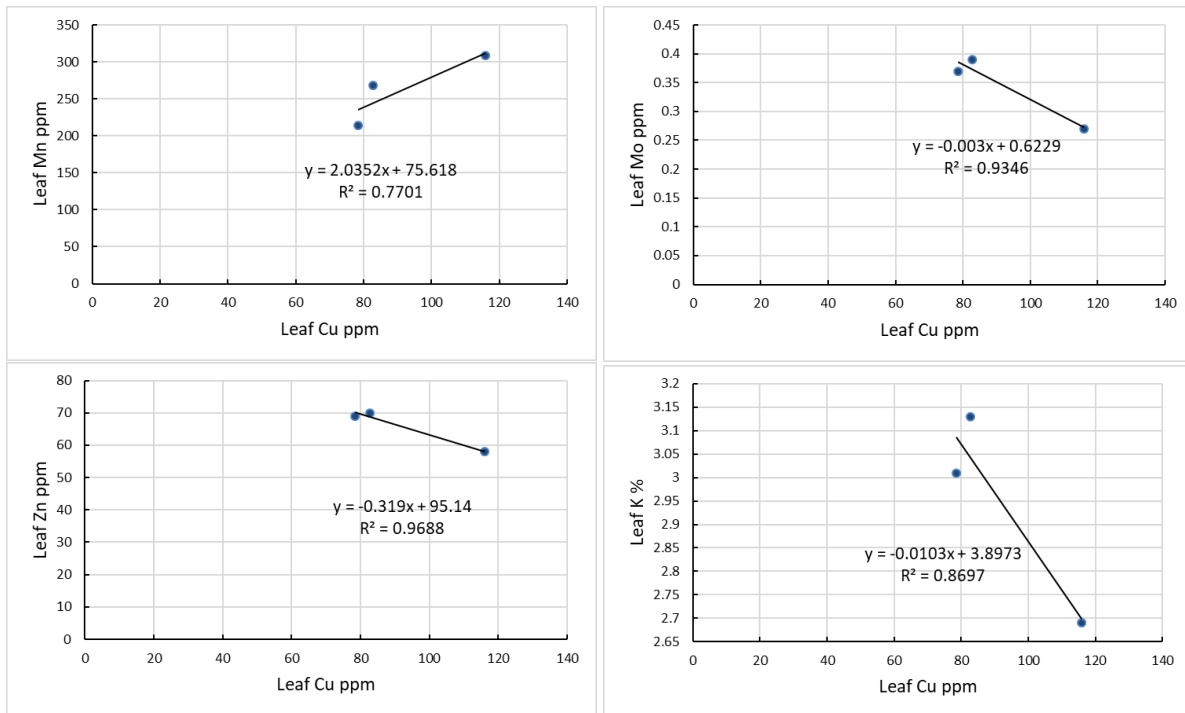


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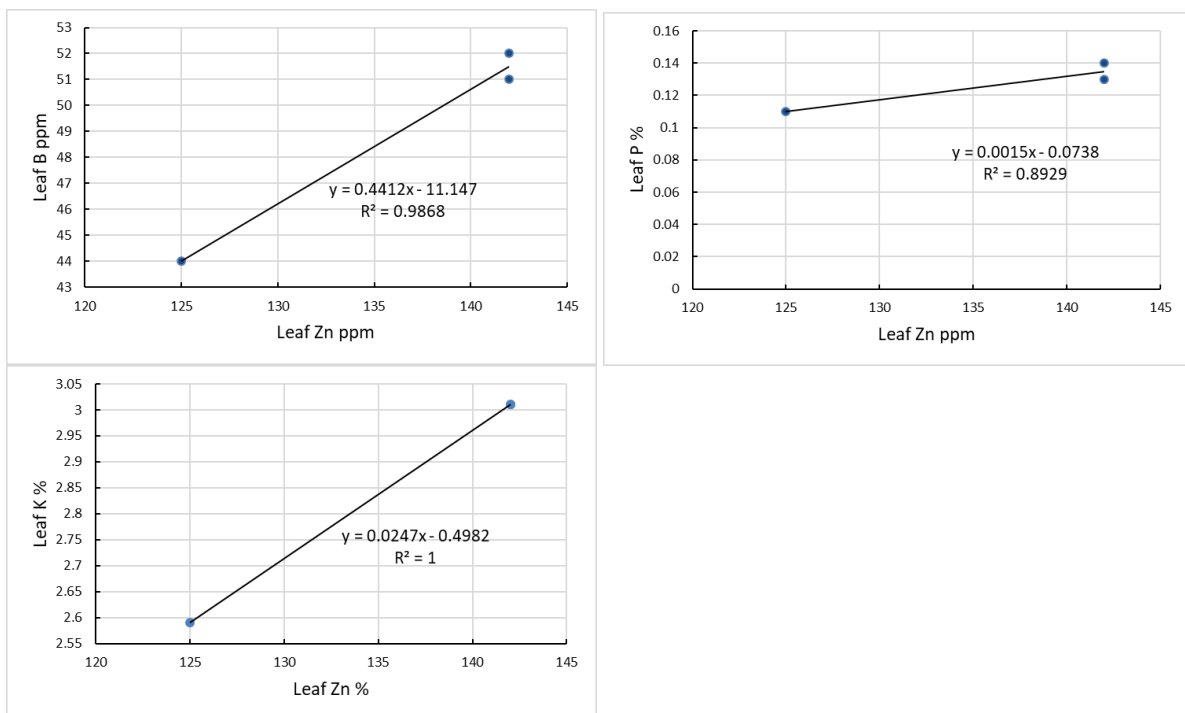
1. Bybordi, A. and Malakouti, M.J. (2006). Effects of foliar applications of nitrogen, boron and zinc on fruit setting and quality of almonds. *Acta Hort.* 726, 351-358.
2. Lovatt, C.J. (2013). Properly Timing Foliar-applied Fertilizers Increases Efficacy: A Review and Update on Timing Foliar Nutrient Applications to Citrus and Avocado. *HortTechnology* 23 (5), 536-541.
3. Dinkeloo, K., Boyd, S. and Pilot, G. (2017) Update on amino acid transporter functions and on possible amino acid sensing mechanisms in plants. *Seminars in Cell & Developmental Biology*. ISSN 1084-9521, <https://doi.org/10.1016/j.semcdb.2017.07.010>.

APPENDICES:

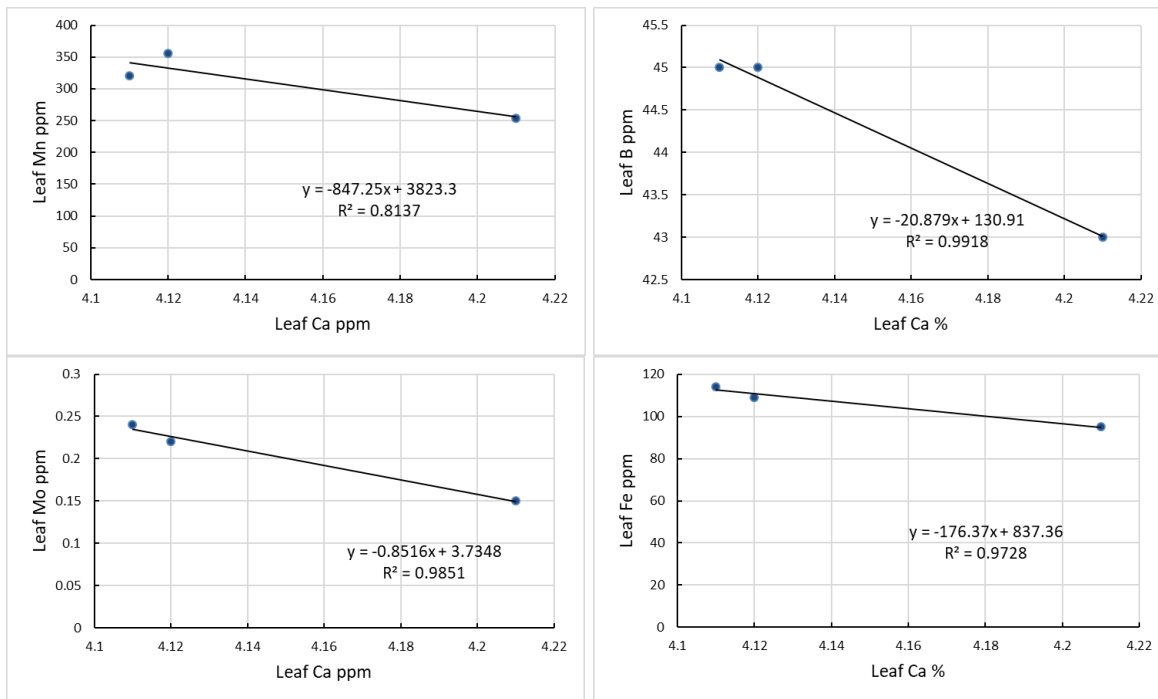
Appendix 1. Correlation between the leaf Cu levels and other mineral concentrations in the leaf tissues of Transit Cu treated plants (only the results that showed high correlation are presented here).



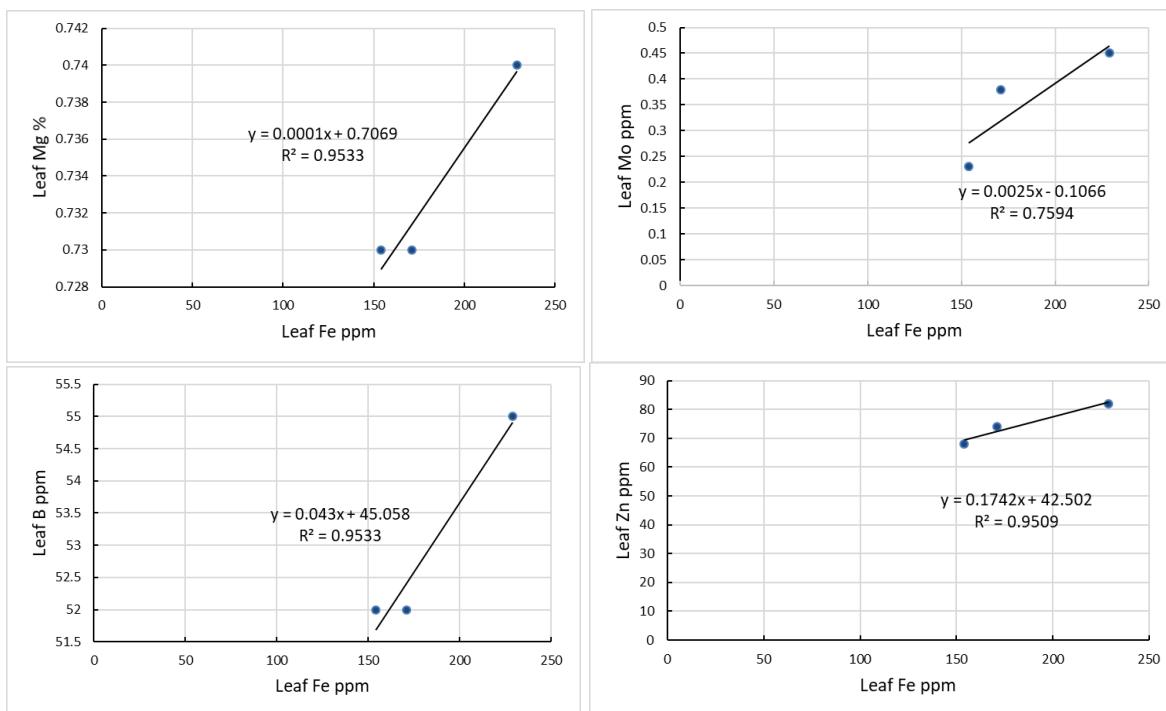
Appendix 2. Correlation between the leaf Zn levels and the other mineral concentrations in the leaf tissues of Transit Zn treated plants (only the results that showed high correlation are presented here).



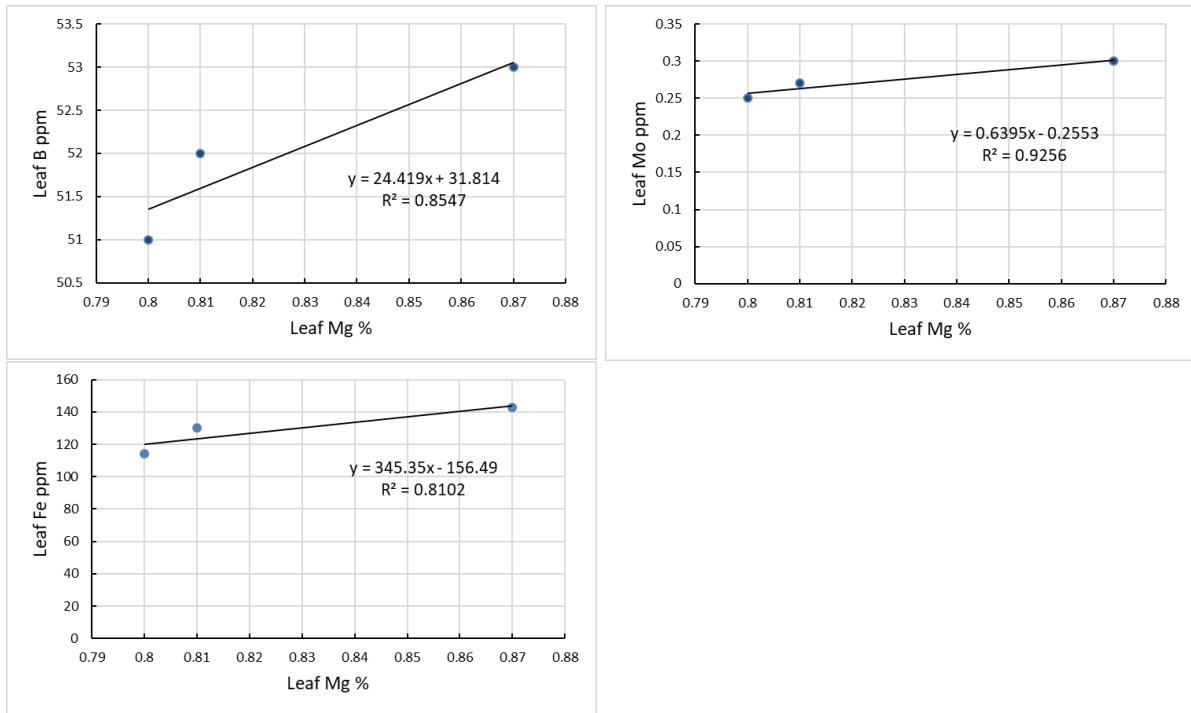
Appendix 3. Correlation between the leaf Ca levels and the other mineral concentration in the leaf tissues of Transit Ca treated plants (only the results that showed high correlation are presented here).



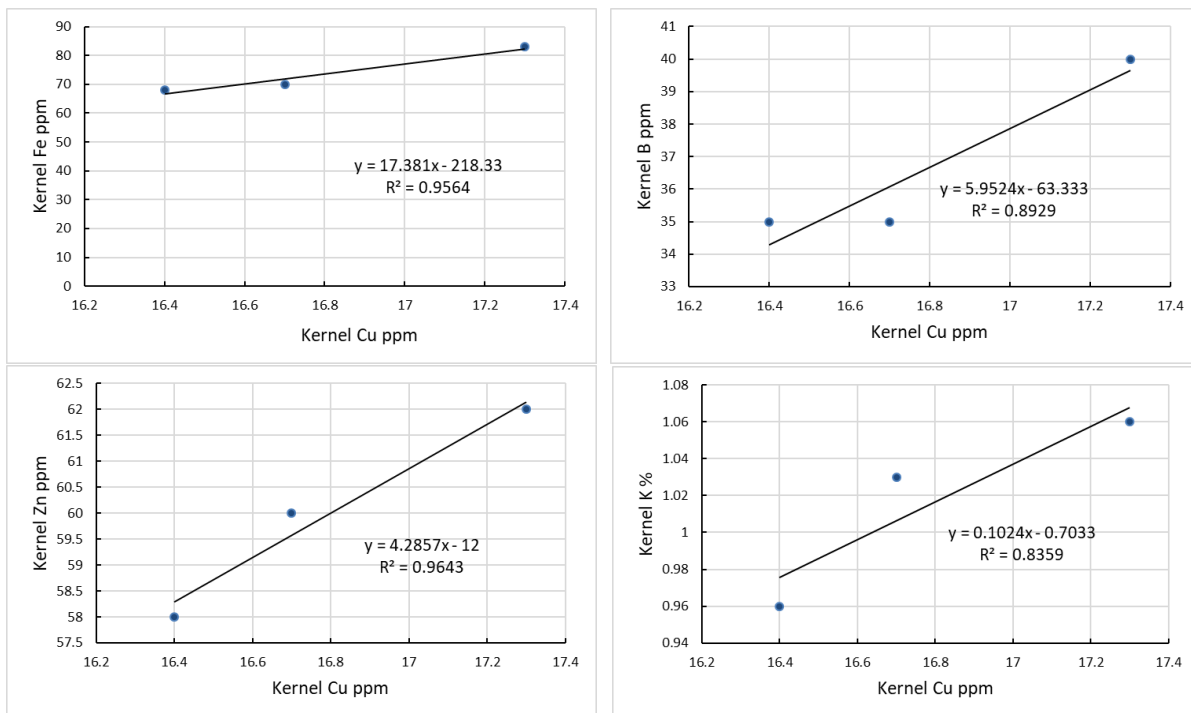
Appendix 4. Correlation between the leaf Fe levels and the other mineral concentration in the leaf tissues of Transit Fe treated plants (only the results that showed high correlation are presented here).

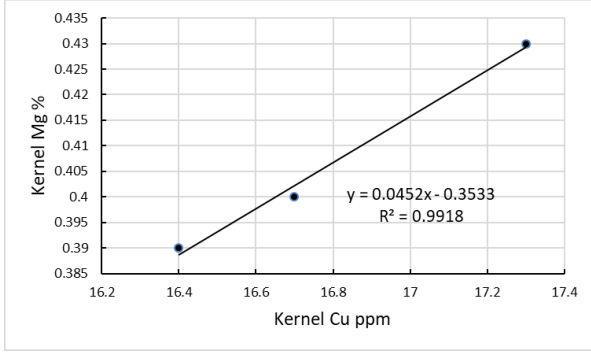
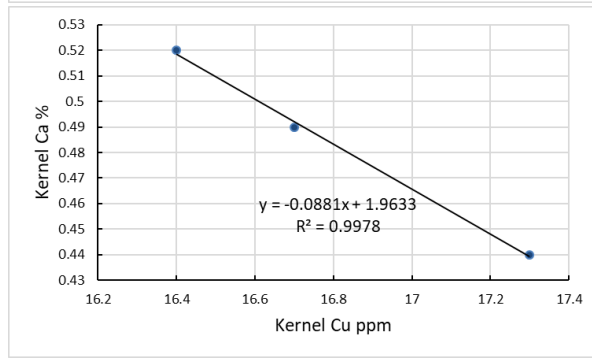
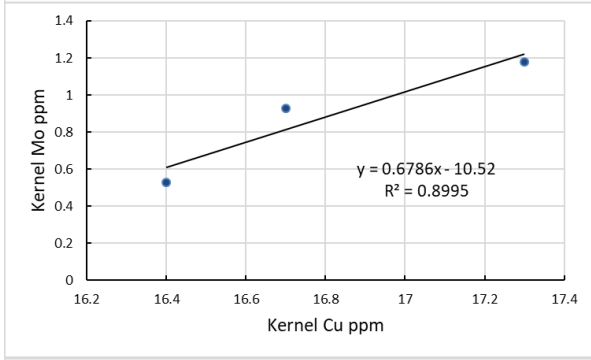
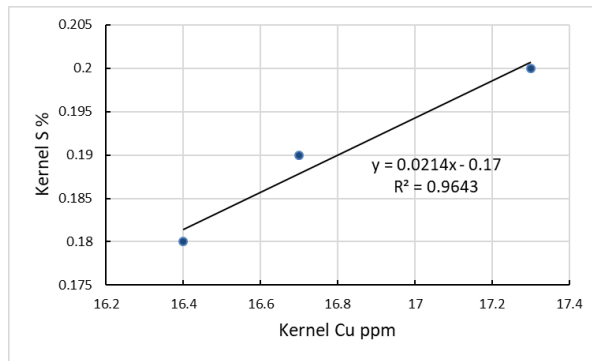
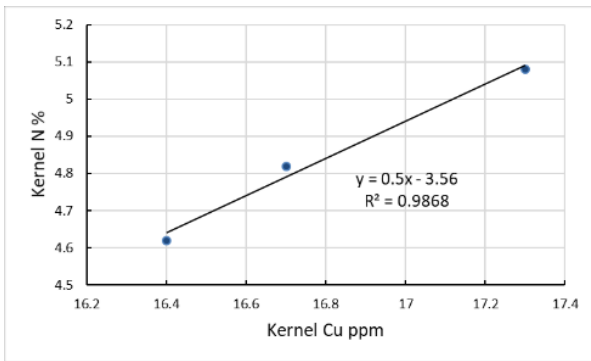


Appendix 5. Correlation between the leaf Mg levels and the other mineral concentration in the leaf tissues of Transit Mg treated plants (only the results that showed high correlation are presented here).

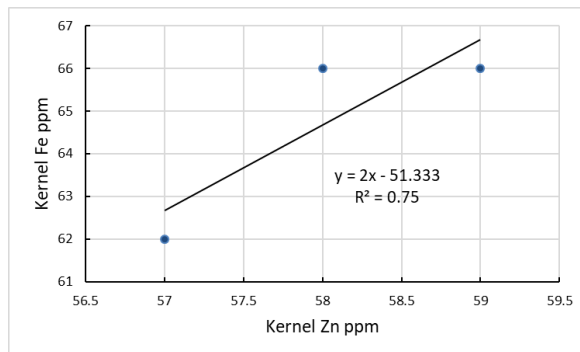
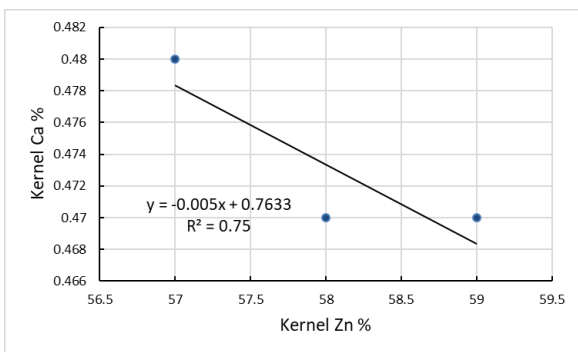


Appendix 6. Correlation between the kernel Cu levels and the other mineral concentration in the kernels of Transit Cu treated plants (only the results that showed high correlation are presented here).

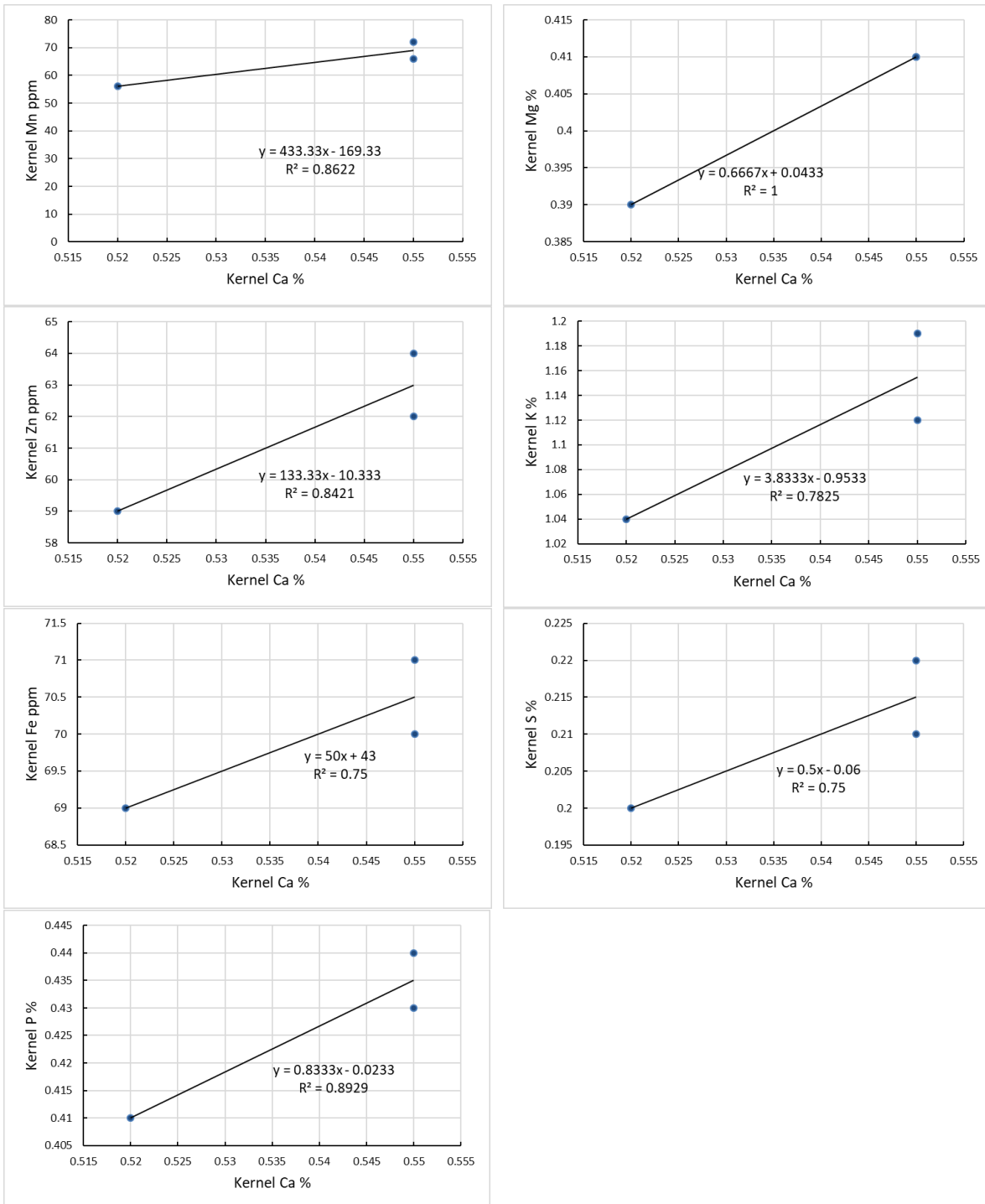




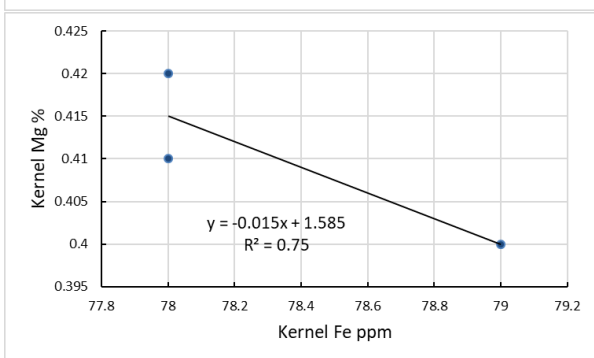
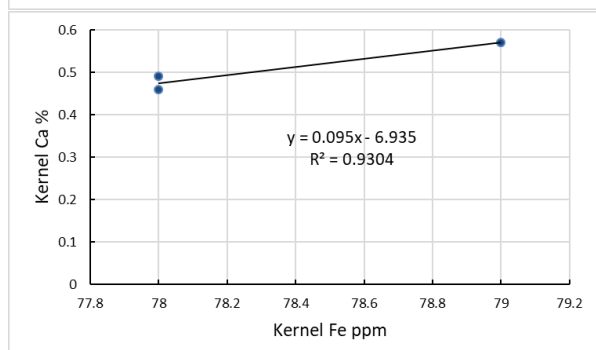
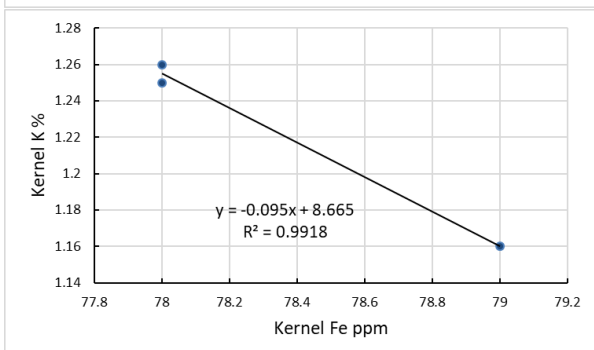
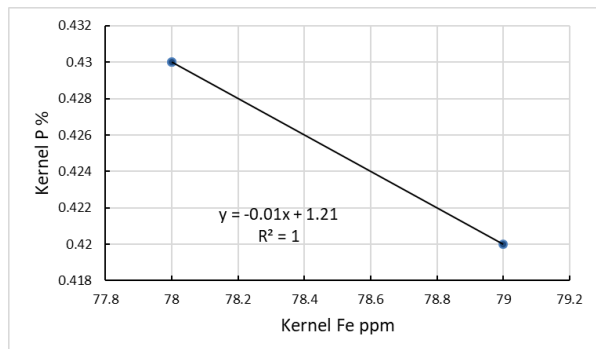
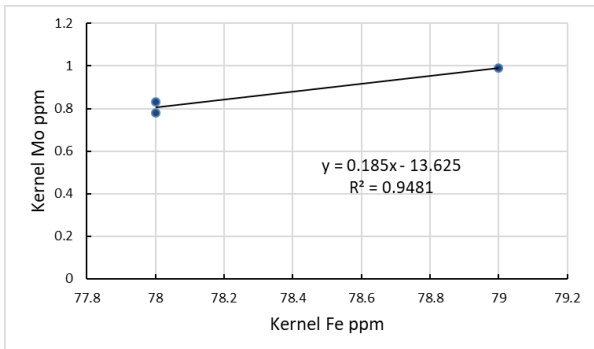
Appendix 7. Correlation between the kernel Zn levels and the other mineral concentration in the kernels of Transit Zn treated plants (only the results that showed high correlation are presented here).



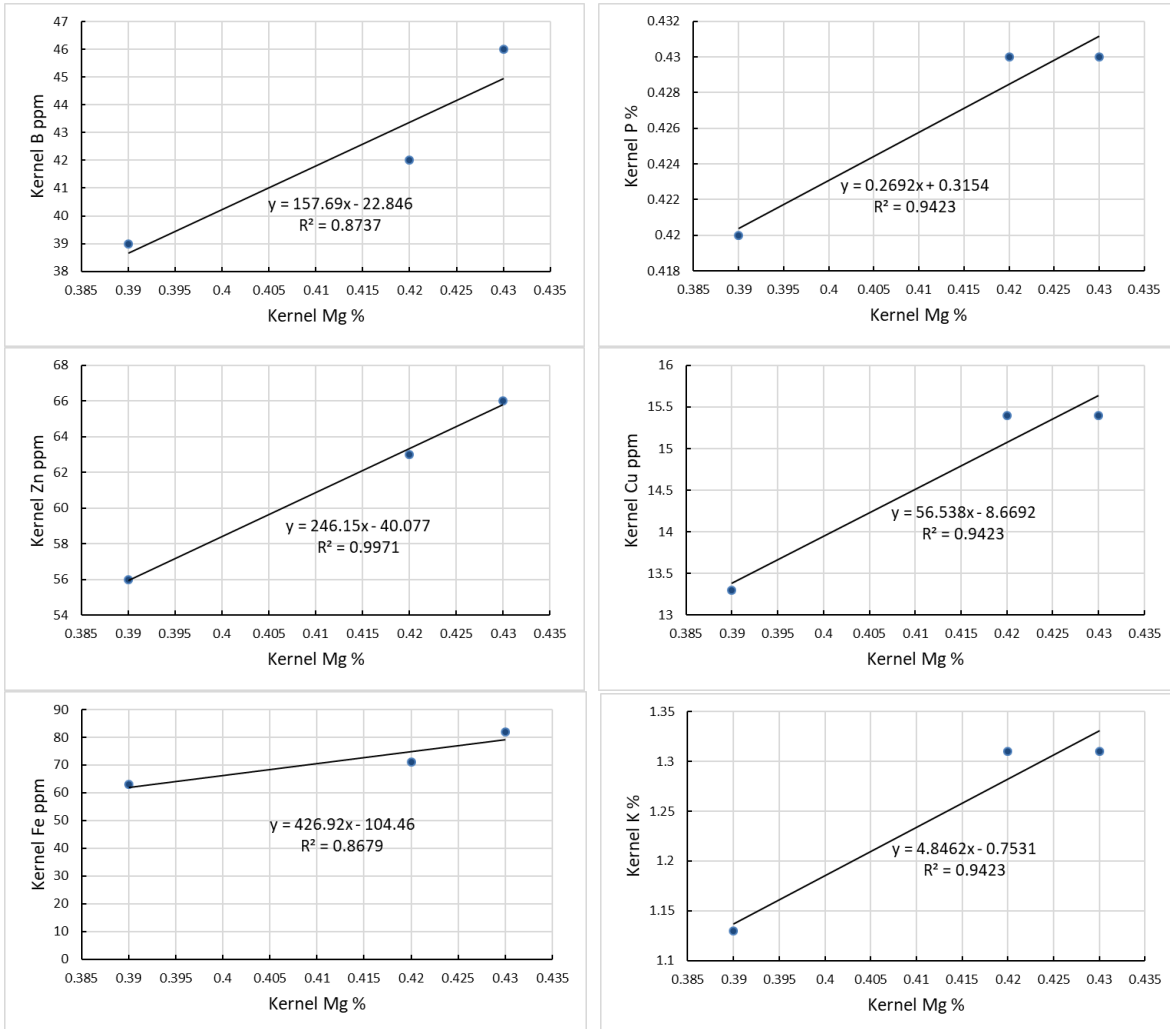
Appendix 8. Correlation between the kernel Ca levels and the other mineral concentration in the kernels of Transit Ca treated plants (only the results that showed high correlation are presented here).



Appendix 9. Correlation between the kernel Fe levels and the other mineral concentration in the kernels of Transit Fe treated plants (only the results that showed high correlation are presented here).



Appendix 10. Correlation between the kernel Mg Ca levels and the other mineral concentration in the kernels of Transit Mg treated plants (only the results that showed high correlation are presented here).



Appendix 11. Statistical analysis of significant results.