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Production and quality of baby spinach (*Spinacia oleracea*. L.) as influenced by NO_3^-: NH_4^+ ratios and cutting management grown in a soilless culture system

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Abstract
The present experiment was conducted on baby spinach variety Knight Walk in the Experimental Centre, University of Torino, Carmagnole, TO, Italy in glass house during the period of January-March, 2011-2012. The objective of this study was to determine the influence of Cutting management during the different harvesting interval and the ratio of $NO3^-$:NH_4^+ on growth yield and the distribution of inorganic elements within the tissues of Spinach grown in soilless culture systems. The spinach was grown in floating systems and nitrogen was supplied at three ratio of $NO3^-$: NH_4 with same total N level of 10mmolL^{-1} and two cutting height were adopted at the time of harvesting. Increasing the rate of NO_3^- and decreasing the rate of $NH4^+$ within the nutrient solution significantly increased the fresh weight production but this effect was most pronounced in the nitrate, phosphate, sulphate and oxalate content in the leaf. Dry matter was higher where ammonium nitrogen concentration was increased and obtained 14.6491 percentage with 20:80 ratio of NO_3^-: NH_4^+. There were no significant effect on number of leaves and chloride content with these treatments. The higher concentration of nitrate and oxalate were found in 60:40 ratio of NO_3^-: NH_4^+ which were within the limitation as allowed by EU regulation for Spinach. Cutting management was not significantly influenced on accumulation of elements in the leaves. However the accumulation of nitrate was found higher when the plants were cut just above the surface of the plants with petioles. It was reverse in case of oxalate content and was noted that higher concentration of oxalate were found when the plants were cut at the top of the plants without petioles.

Keywords: Spinach, soilless culture, floating system, NO_3^-: NH_4 ratio

Introduction

Spinach (*Spinacia oleracea*. L.) is the most important leafy vegetable belonging to the family Chenopodiaceae. Spinach is probably a native of Southwest Asia. It is a traditional potherb, but it is widely used uncooked in salads. It is also processed by canning and freezing. (Rubatzky and Yamaguchi, 1997) [24]. Spinach is greatly responsive to nitrogen fertilization (Cantliffe, 1992; Magnifico *et al.*, 1992) [6, 17] and is one of the highest NO_3^- accumulator plants, due to a very efficient uptake system and inefficient reductive systems. (Maynrd *et al.*, 1976) [18] and it has also been clearly identified as high oxalate containing plant. (Honow and Hesse, 2002) [11]. It is one of the vegetables having inherently high nitrate concentration and petioles have several fold higher concentration than leaf blade. (Hammad, *et al.*, 2007) [12]. Spinach as a dietetic nutrient has long been an object of many investigations. Many authors have described the interdependence of absorbed plant nutrients and their utilization of accumulation in the plant. It is well known that nitrate fed spinach shows enrichment in nitrate and oxalate content which is dependent upon the nutrient level, while with ammonium as the source of nitrogen, spinach has a lower oxalate content. Optimal nutrition is essential for higher yield and good quality of spinach plants. Crop growth mostly depends on water and nutrient in culture media. Of all nutrients, N is the most effective for growth and they are mostly taken up by the ratio of NH_4^+ and NO_3^- in culture media. Immediate and effective modifications in N supply are only possible in hydroponics systems such as NFT, and floating culture, have proven very useful as a tool for nutrient research. (Andresen and Nielsen., 1992) [1].

The effects of different forms of nutrient on spinach production have received little attention. Ammonium and nitrate are the two major sources of inorganic nitrogen taken up by the roots of higher plants. The effects of these two forms on plant growth is dependent on the plant species, but also on $NH4^+$ and $NO_3^–$ ratios and concentration, although most plants can use either or both forms as a source of N (Marschner, 1977) [21]. The major form of N supply in the nutrient solution in the soilless culture is NO_3 based fertilizer, addition of some NH_4 to the nutrient solution seems to be beneficial to the plant and helps to decrease the pH of the solution (Ikeda and Oswa. 1983) [14]. Plant accumulation of $NH4^+$ and $NO_3^–$ is principally determined by the concentration of ion and pH of the growth media, with NH_4^+ uptake favoured by alkalinity and NO_3^- uptake by mild acidity (Haynes and Go., 1978). Lowering the NO_3^- concentration in leafy vegetables is desirable because of possible negative effects (the presence of NO_2^- intoxicant metabolites) of high NO_3^- uptake (Marchner, 1977) [21]. However, NO_3^- will be firstly uptake in spinach. NO_3^- accumulation in leafy vegetables can be of considerable importance for their quality. Many species of vegetables, including spinach can be harvested at various phases of maturity which depends on requirements of the selling in the market and consumers preferences, Therefore, to optimize the optimal cutting height during the harvesting is found to be very important criteria for getting more fresh production and good quality. In general, good plant yields are obtained through a combined supply of both NO_3^+ and NH_4^+, but also strongly depend on the total concentration supplied.

On account of the above remarks, the present research work was carried out on different NO_3^-: NH_4^+ ratios along with cutting management to assess whether fresh production and quality of baby spinach were affected by changing NO_3^-: NH_4^+ ratios in the nutrient solution.

Materials and methods

This study was carried out in the experimental Centre, University of Torino, Carmagnole, TO, Italy ($45^0 22^{'}$N; $7^0 65^{'}$E) in a glass house with automatic environmental temperature control (28 ^{0}C/16 ^{0}C; day/night) during the month of January-March, 2010-2011. The experiment consisted of growing plants in 60-cell Styrofoam trays (44mm top and 25mm lower diameters, respectively), floating continuously in a nutrient solution. Both the pH and the electrical conductivity (EC) of the solution were checked two times during the growing period and kept close to the range of 5.5-6.5 and 2,000µS cm^{-1}, respectively. The trays were filled with a specific peat based horticultural medium. (Neuhaus Huminsubstrat N $17^{'}$; Klasmann-Deilmann, Geeste-Groβ Hesepe, Germany) The seeds of Spinach variety Knight walk were sown on 20th January, 2010 and 2011. After sowing the seeds the seeded trays were incubated for 2 days at 20 ^{0}C in a germination chamber. When the seed started germination, the seed trays were existed from germination chamber and shifted to a glasshouse for further growing and the seeded trays were placed according to a random block statistical design. After proper emergence of seedling, thinning operation was done by keeping the three plants in each hole to achieve a final planting density of 180 plants per tray (approx. 1176.0495 m^{-2}). Then the treatments consisting of three nutrient solutions (Table1), with same total N levels of $10mmolL^{-1}$ with three different $NO3^-$:NH_4^+ ratios (20:80, 40:60 and 60:40) were applied in the respective trays along with two cutting height treatments. The total number of treatments was 3 (NO_3^-:NH_4^+) ratios x 2 (Harvesting interval) x 3 (Blocks) = 24 for the production parameters and 3 ((NO_3^-:NH_4^+) ratios x 2 (Cutting treatments) x 2 (Harvesting interval) x 3 (Blocks) = 48 for the quality parameters. The Statistical design was CRBD with three randomized blocks.

Harvesting was done manually by cutting the plants in different height. Two cutting height was maintained at the time of harvesting. Cutting management was adopted by cutting the leaves at few centimeters just above the substrate with petioles and cutting the leaves at the top of the plant without petioles. After first harvesting of the plant, again fresh nutrient solution of the same amount was given in the respective trays to encourage the luxuriant growth of the plants. During the harvest the observation were taken on fresh weight, number of leaves, LAI, nitrogen in the leaves and samples were taken for determination of nitrate, phosphate, chloride, sulphate and oxalate. Soon after harvesting the weight of 18 plants for each treatment were taken in gram and average of fresh weight of the plant was recorded and the samples were oven dried at 60 ^{0}C for 2 days. Leaf dry weight was measured and dry matter was determined. Nitrogen content in the leaves was measured by taking the reading randomly on 30 number of plant leaves sample and the value was noted by using N-tester.(Konica Minolta Corporation, Yara International ASA, OSLO, Norway). Leaf nitrate contents were measured using ion chromatography Dionex® DX 500, on sample 25g. The oxalate concentration of the plants were determined using the procedure as described by llarslan *et al*., (1997) [15]. 0.01g of dry plant sample was homogenized with 5ml deionized water. Am amount of 5ml EDTA was added and filtered with whatman No.1 filter paper. The oxalate kit purchased from Greiner Diagnostic Gmbh-Unter Gereuth, Germany was used for the determination of oxalate concentrations. For LAI the leaf surface was measured from images captured using a scanner A3 size UNI whose glazed surface scanning were supported leaves, one by one, stretched thoroughly. For each scan a strip of graph paper was placed to get a proper reference of scale. The images were acquired in compressed jpeg format with color depth 24-bit RGB color space and medium-size 3476 x 4708 pixel resolution and 150 × 150 dpi. In order to make more accurate determination of the leaf surface, the background of each scan was aligned and carefully cleaned from imperfections so as to bring on only in the leaves. This intervention was carried out using the software "Gimp" version 2.2.13. Once clean scans, the images changed with "The Gimp" were subjected to determine areas of different leaves using the software "Imagej" with this process it was also possible to obtain the number of leaves analyzed, and thus the number of leaves per plant were noted.

Results and Discussions

The effect of the $NO3^-$: NH_4^+ ratios in the nutrient solution and harvesting on fresh weight was significant, whereas non-significant effect of the interaction of the factors was observed. Spinach plants that were grown under 20:80 (NO_3^-: NH_4^+) ratios did not produce good fresh weight. An increment in the fresh weight of the spinach was observed with the increase in the concentration of ratio of nitrate fertilizer and decrease in the concentration of ratio of ammonium fertilizer in the nutrient solution. Using 40:60 or 60:40 (NO_3^-: NH_4^+) ratios enhanced the growth of the plants in term of fresh weight. There was not a significant differences between 40:60 and 60:40 (NO_3^-: NH_4^+) ratios on fresh weight but there was significant differences obtained on fresh weight within 20:80 (NO_3^-: NH_4^+) ratios amongst with 40:60 and 60:40 (NO_3^-: NH_4^+) ratios. However, lower value obtained by 20:80 (NO_3^-: NH_4^+) ratios on fresh weight may be due to higher amount of ammonium nitrogen which might inhibit the photosynthesis in spinach. Similar results were observed by Kher *et al*., (1991) [16] and Platt and Anther, (1981) [23]. They reported that application of NH_4-N can inhibit the photosynthesis in spinach and also mentioned that the nutrient solution of ammonium salts become extremely acidic as the plants extracted NH_4^+ ions from solution. Increasing acidity was associated with poor utilization of ammonium and can restrict the growth rate of the plant. Harvesting also influenced significantly on fresh weight. The highest fresh weight of 1073.8289 (g/m^2) was obtained from second harvested plants. This might be the fact that after first harvesting the plants might receive the desirable environmental condition and proper balanced nutrients and cutting operation at the time of first harvesting helps to boost the plants to produce luxuriant leaf growth.

Data in table 2 showed that, dry matter percentage of spinach plants did not follow a similar trend as fresh weight with the different (NO_3^-: NH_4^+) ratios. It was noticed that application of 20:80 (NO_3^-:NH_4^+) ratios increased dry matter percentage compared with 40:60 and 60:40 (NO_3^-: NH_4^+) ratios. There were significant variation between 20:80 with 40: 60, and 60:40 (NO_3^-: NH_4^+) ratios, whereas between 40:60 and 60:40 there were no significant differentiation. Blaam, Zandstra *et al*., (1988) [5], observed the similar results that high NO_3^- concentration in two lettuce genotypes were related to a lower dry matter. It might be due to the reason that plants diminish their dry matter as a consequence of a decrease in the demand for N synthesis. A dilution effect occurred throughout plant growth. Older plants required less N, having metabolized N in to proteins throughout their ontogenetic life. Further the present results of this experiment is also inconformity with the findings of Fontana and Nicola, 2008) [8], where they found higher dry matter in garden cress with 25:75 (NO_3^-: NH_4^+) compare to 50:50 and 75:25(NO_3^-: NH_4^+) ratios. Dry matter percentage of spinach demonstrated considerable differences between the harvesting periods. Significantly the highest dry matter appeared in the second harvested plants. This might be due to the influence of cutting during the first harvesting. The results are inconformity with the findings of Nassiri and Elgersma, (1998) [22]. The treatments combination between (NO_3^-: NH_4^+) ratios and harvesting on dry matter was not significant.

Number of leaves per plant was not significant by the influenced significant with different (NO_3^-: NH_4^+) ratios and Harvesting. However, gradual increasing trend was noticed on number of leaves with increasing (NO_3^-: NH_4^+) ratios.

The relative increased of LAI was observed with different (NO_3^-: NH_4^+) ratios. The minimum LAI was observed in 20:80 (NO_3^-: NH_4^+) ratios which were significantly different between the other two ratios. However there were no significant differences between 40:60 and 60:40 (NO_3^-: NH_4^+) ratios. The maximum LAI was obtained by using 40:60 (NO_3^- : NH_4^+) ratios which is at par with 60:40 ratios. This might be due to the fact that possibly the balanced (NO_3^-: NH_4^+) ratios in this regard may be attributed to the provision favorable growing condition and supply of proper nutrients for better growth of the plant which produced good LAI. was found non-significant by harvesting treatment. However the maximum LAI was observed from the first harvested plants. Whereas second harvested plants gradually decreases LAI. This might be the reason that LAI decreases immediately after leaves are cut and removed during the first harvesting.

Application of different (NO_3^-: $NH_4^{+)}$ ratios on the accumulation of nitrate in spinach are presented in table 2. In this experiment the determined nitrate content was significant with different (NO_3^-: $NH_4^{+)}$ ratios. There were significant variation between 20: 80 and 60: 40 (NO_3^-: NH_4^+) ratios. However, there were no significant differences between 40: 60 and 60: 40 (NO_3^-: NH_4^+) ratios. Increasing nitrates in the added nutrient solution ratios gradually increased the accumulation of NO_3 in tissues of spinach plants. It was noticed that maximum accumulation of NO3 was recorded in the ratios of NO_3^-:NH_4^+ at ratios of 60:40. Stated that application of nitrate nitrogen sources enhanced the NO_3^-N accumulation in the plants. They also reported that accumulation of nitrate in the plant tissues is the result of absorption in excess of reduction and assimilation. The lowest nitrate content in this experiment was found with higher ammonium concentration in the nutrient solution of 20:80 ((NO_3^-: NH_4^+) ratios. The results are corroborated with the findings of Blam Zandstra, (1989) [4]. He reported that using NH_4 as a fertilizer can help to reduce the NO_3 content in plant. Minoltti, (1975) [19], Maynard *et al*., (1976) [18], and Gabal (1980) [9] reported that the higher concentration of NO_3 directly increased nitrate ions concentration in plants. Whereas other N fertilizer like NH_4-N could be converted to NO_3 after nitrification, yet it needs some time. Therefore, the lowest values of nitrate in plants supplied with ammonium sulfate may be due to NH_4^+ is used directly in N assimilation in plant tissues.

In this present experiment, in all the different (NO_3^-: $NH_4^{+)}$ ratios the nitrate content was found much lower than 2500mg kg^{-1} FW, which is the maximum content allowed by EU regulation for spinach. Nitrate content was not influenced significantly by cutting treatments. However, nitrate content was significantly influenced by harvesting. The plants when harvested first received the high value of nitrate content compared to second harvested plants.

The chloride content was not influenced significantly by (NO_3^-: $NH_4^{+)}$ ratios, whereas other ions phosphate and sulphate contents were found to be influenced significantly. The significant differences between 20:80 and 60:40 (NO_3^-: $NH_4^{+)}$ ratios were observed in both phosphate and sulphate contents. But the phosphate and sulphate contents were not differ significantly between 40:60 and 60:40 (NO_3^- : $NH_4^{+)}$ ratios. The possible explanation is that high concentration of NH_4^+ is helpful to increase the contents of phosphate and sulphate gradually.

Significant trends were found for oxalate content with different NO_3^-: NH_4^+ ratios, indicating a higher accumulation of oxalate in 60:40 (NO_3^-: $NH_4^{+)}$ ratio. It shows that by using a 60:40 (NO_3^-: NH_4^+) ratio enhanced the oxalate content with consequent higher value. There were significant differences on oxalate content between 20:80 and 60:40 (NO_3^-: NH_4^+) ratios. The oxalate content was observed minimum in the plants that were grown with 20:80 (NO_3^-: NH_4^+) ratios. The results of this study showed that oxalic acid concentration in leaves decreased with increasing ammonium levels in the nutrient solution. This might be the reason that applying nitrogen completely as ammonium reduced the oxalic acid content in plants, ammoniacal N can be directly used by plants in the synthesis of amides and amino acids whereas NO_3^-N has to be reduced by processes that command up to 25% of either photosynthetic or mitochondrial electron transport capacity (Bloom *et al*., 1989) [3]. Apparently when nitrogen is provided in a nitrate form, the nitrate has to be reduced in shoots (nitrate reduction by nitrogen reductase) before the N can be used by the plant. The results are in agreement with the earlier reports on the effects of ammonium nutrition on oxalic acid in New Zealand spinach and Pursulane. (Ahmed and Johnson, 2000; Elia, *et al*., 1999) [2, 7]. Cutting management operation, harvesting and their interaction effect did not found significant. However, it was noted that higher concentration of oxalic acid were found from the second harvesting plants, when the plants were cut at the top of the plants without petioles. Oxalates accumulation proved to be higher in blades than in petioles.

Conclusion

NO_3^-: NH_4^+ ratio had significant effect on production, nitrate, oxalate and other element accumulation in spinach grown under soilless culture systems. The data presented in this paper suggested that application of proper ratio of NO_3^-: NH_4^+ in the nutrient solution is beneficial for better growth and quality. Cutting management practices has to be properly adopted to get subsequent yield from the same plants. Harvesting the plants by cutting in the appropriate height by excluding more petioles will minimize the nitrate contents in the plants. It may be concluded that optimal NO_3^-: NH_4^+ ratio in the nutrient solution might be 40:60.

Table 1: Nutrient Solution Composition. NO_3^-: NH_4^+ ratios with same Total N (10mM/L)

NO_3^-:NH_4^+ 20:80	gL^{-1}	NO_3^-:NH_4^+ 40:60	gL^{-1}	NO_3^-:NH_4^+ 60:40	gL^{-1}
$(NH_4)_2SO_4$	0..37	$(NH_4)_2SO_4$	0.24	$(NH_4)_2SO_4$	0.05
(NH_4) $H_{2PO}4$	0.09	(NH_4) $H_{2PO}4$		(NH_4) $H_{2PO}4$	
K_2SO_4	0.38	K_2SO_4	0.35	K_2SO_4	0.10
$Ca(OH)_2$	0.07	$Ca(OH)_2$	0.07	$Ca(OH)_2$	0.07
KH_2PO_4	0.16	KH_2PO_4	0.27	KH_2PO_4	0.27
$MgSO_4$ $7H_2O$	0.49	$MgSO_4$ $7H_2O$	0.49	$MgSO_4$ $7H_2O$	0.49
NH_4NO_3	0.13	NH_4NO_3	0.19	NH_4NO_3	0.26
KNO_3	0.04	KNO_3	0.16	KNO_3	0.28
Micral	0.30	Micral	0.30	Micral	0.30

Table 2: Fresh Weight, Dry matter, LAI, Number of leaves and Nitrogen content in Spinach as Influenced by NO_3^-: NH_4^+ ratios and harvesting in soilless Culture system.

Treatments NO_3^-: NH_4^+	Fresh weight (g/m^2)	Dry matter (%)	LAI	Number of leaves/plant
20:80	862.5204 b	14.6491 a	3.38883 b	3.892
40:60	1040.9926 a	13.2784 b	4.11011 a	4.242
60:40	1096.9362 a	13.2734 b	4.02411 a	4.192
Significance(P)	*0.007*	*0.016*	*0.002*	0.094
Harvest: 1st	926.4705	11.1960	4.00163	4.289
2nd	1073.8289	16.2714	3.68041	3.928
Significance(P)	*0.017*	*0.000*	*0.059*	0.013
Nitrogen x Harvest				
20:80 x 1st harvest	806.1274	11.8186	3.60026	4.000
20 :80 x 2nd harvest	844.7303	17.4815	3.17739	3.783
40 :60 x 1st harvest	932.9656	10.9444	4.18054	4.317
40 :60 x 2nd harvest	1149.0196	15.6125	4.03968	4.167
60 :40 x 1st harvest	966.1356	10.8267	4.22407	4.550
60 40 x 2ndharvest	1227.7369	15.7202	3.82415	3.833
Significance(P)	0.096	0.599	0.739	0.197

Table 3: Chloride, Nitrate, Phosphate, sulphate and Oxalate content in Spinach leaves as Influenced by NO_3^-: NH_4^+ ratios, cutting height and harvesting.

Treatments NO_3^-: NH_4^+	Chloride (mg kg^{-1})	Nitrate (mg kg^{-1})	Phosphate (mg kg^{-1})	Sulphate (mg kg^{-1})	Oxalate (g/kg)
20:80	174.17	151.50 b	4621.58 a	2821.33a	19.82192c
40:60	139.17	210.75 ab	4267.08ab	2265.25ab	30.92659b
60:40	169.33	329.67a	3588.75b	1397.67b	38.86439a
Significance(P)	0.320	*0.001*	*0.005*	*0.020*	*0.000*
Cutting height:					
1	161.83	239.89	4243.72	2339.67	29.06432
2	159.94	221.39	4074.56	1983.17	30.67762
Significance(P)	0.682	0.244	0.992	0.661	0.984
Harvest:					
1st harvest	165,22	417.50	4489.28	1527.78	29.12457
2nd harvest	156.56	43.78	3829.00	2795.06	30.61737

Significance(P)	0.526	*0.000*	*0.013*	0.003	0.453
NO_3^- : NH_4^+ x Cutting 20:80 x C 1	187.00	179.83	4839.83	3044.33	21.52030
20 :80 x C2	161.33	123.17	4403.33	2598.33	18.12355
40 :60 x C1	145.13	179.25	423.00	2306.25	30.54814
40 :60 x C 2	127.25	273.75	4337.25	2183.25	31.68349
60 :40 x C 1	157.50	451.25	3373.00	1349.50	37.41270
60 :40 x C 2	175.25	268.88	3696.62	1421.75	39.59024
Significance(P)	0.674	*0.002*	0.682	0.801	0.119
NO_3^- : NH_4^+ x Harvest					
20:80 x 1st harvest	175.33	263.50	5026.67	2039.00	21.10223
20:80 x 2nd harvest	173.00	39.50	4216.50	3603.67	18.54162
40:60 x 1st harvest	133.17	382.17	4428.67	1697.00	29.84527
40:60 x 2nd harvest	145.17	39.33	4107.50	2833.50	31.90791
60:40 x 1st harvest	187.17	606.83	4014.50	847.33	36.32620
60:40 x 2nd harvest	151.50	52.50	3163.00	1948.00	41.40259
Significance(P)	0.174	0.107	0.682	0.801	0.319

Table 4: Interaction of cutting x harvesting and NO3$^-$: NH_4^+ ratio x cutting x harvesting on Chloride, Nitrate, Phosphate, sulphate and Oxalate content in Spinach.

Treatments NO_3^-: NH_4^+	**Chloride (mg kg^{-1})**	**Nitrate (mg kg^{-1})**	**Phosphate (mg kg^{-1})**	**Sulphate (mg kg^{-1})**	**Oxalate**
C1 x 1st harvest	172.67	438.56	5265.67	1667.11	27.97217
C1 x 2nd harvesting	151.00	41.22	4414.00	3012.22	30.15646
C2 x 1st harvesting	157.78	396.44	4787.67	1388.44	30.27697
C2 x 2nd harvesting	162.11	46.33	4019.00	2577.89	31.07828
Significance(P)	0.402	0.210	0.838	0.836	0.438
NO_3^-: NH_4^+ ratio x Harvest x Cutting.					
20:80 x C1 x 1st harvest	223.33	304.33	5265.67	2199.00	21.52284
20:80 x C1 x 2nd harvest	150.67	55.33	4414.00	3889.67	21.51776
20:80 x C2 x 1st harvest	127.33	222.67	4787.67	1879.00	20.68162
20:80 x C2 x 2nd harvest	195.33	23.67	4019.00	3317.67	15.56548
40:60 x C1x 1st harvest	129.25	335.75	4355.00	1647.50	28.76038
40:60 x C1x 2nd harvest	161.00	22.75	4109.00	2965.00	32.33589
40:60 x C2x 1st harvest	141.00	475.00	4570.00	1796.00	32.31503
40:60 x C2x 2nd harvest	113.50	72.50	4104.50	2570.50	31.05194
60:40 x C1x 1st harvest	183.50	845.50	3743.50	908.50	36.06973
60:40 x C1x 2nd harvest	131,50	57.00	3002.50	1790.50	38.75566
60:40 x C2x 1st harvest	189.00	487.50	4150.00	816.75	36.45444
60:40 x C2x 2nd harvest	161.50	50.25	3243.25	2026.75	42.72605
Significance(P)	0.157	0.103	0.963	0.893	0.347

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