THE EFFECT OF TWO LEVELS OF AMMONIUM NITRATE APPLICATION ON THE YIELD OF PLAIN-LEAF, CURLY-LEAF AND TURNIP-ROOTED PARSLEY AND THE QUALITY AND ESSENTIAL OIL COMPOSITION OF THE LEAVES BEFORE AND AFTER STORAGE IN A PARTIALLY DEHYDRATED FORM

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Abstract. Curly-leaf, plain-leaf and turnip-rooted parsley were cultivated under two levels of N (75 and 300 ppm applied in the form of ammonium nitrate) for their foliage, which was stored at 2 or 7^{0}C for 40 days after harvest. During storage leaves lost up to 80% fresh weight due to water loss. Despite this partial dehydration, the leaves of all cultivars retained their green colour (i.e. chlorophyll content), especially at 2^{o}C. Moreover, with the exception of turnip-rooted parsley, the essential oil content of the leaves on a fresh weight basis increased during storage. The higher rate of N application (300 ppm) increased the chlorophyll content of the leaves of all cultivars, but did not affect their essential oil content. The higher N rate also increased the number and weight of leaves per plant. Overall, therefore, it is indicated that high N application increases parsley yield and quality (greenness) without affecting the aroma. Additionally, partial dehydration of parsley leaves during storage does not adversely affect parsley colour or aroma and therefore may be preferable to total drying.

Keywords: nitrogen application; *Petroselinum crispum*; water loss; temperature.

INTRODUCTION

Parsley (*Petroselinum crispum* [Mill.] Nym.) is native to the Mediterranean basin and is widely grown throughout the world for use as a fresh or dry herb. All parts of the plant are aromatic and the foliage is rich in vitamins, minerals, essential oils and antioxidants [3, 5].

Nitrogen (N) removal by leaf parsley is about 2.18 kg per ton of foliage [25] and recommended N application rates for this crop on mineral soil range from 98-134 kg ha^{-1} [7, 25]. For the Mediterranean region, Petropoulos *et al.* [18] recommended irrigation of parsley with water containing 150 mg l^{-1} N in the form of ammonium nitrate. However, differences in response to N application have been noted among parsley cultivars [13]. Moreover, leafy herbs such as parsley tend to accumulate nitrate in the foliage, thereby constituting a possible human health risk [21, 22]. Hence it is important to quantify the optimum amount of N for these crops according to the area of cultivation.

Quality standards for fresh parsley include fresh green coloration of the foliage and the absence of defects, such as wilting, yellowing, disease, insects, foreign matter and the presence of flower stalks [24]. Although it has been suggested that the foliage may be stored for up to 2 months at 0°C and 90-95% relative humidity (R.H.) [20], in practice the duration of storage is normally less than 10 days, primarily due to wilting [11]. Low temperatures are essential for parsley storage [6, 11], whereas at high temperatures and/ or low R.H., the storage life is seriously reduced [14]. Enclosure of parsley in film may assist quality retention during storage at low temperatures [1, 15].

When parsley is dried for long term storage and greater market availability, changes occur in the aromatic and nutritional constituents. This leads to a decrease in product quality. For example, 1,3,8-*p*-menthatriene and β-phellandrene, which are aroma constituents [10, 12], may decrease during processing and storage [8, 16] depending on the genotype, the drying process and the duration of storage after drying [19].

In warm climates, fresh parsley may exhibit visible wilting and shrivelling within a few hours of harvest, followed by yellowing, which rapidly renders the product unmarketable and results in loss [23]. However, disposal of shrivelled parsley is wasteful because although wilting decreases the visual quality of the fresh product, it may not seriously affect its nutritional value. Indeed, partially dehydrated parsley retains a high concentration of essential oils and other valuable constituents [3]. In the present paper, we studied the effect of N application on the yield and quality of fresh parsley and evaluated the effect of partial dehydration on quality during storage.

MATERIALS AND METHODS

Plain-leaf parsley (*Petroselinum crispum* [Mill.] Nyman ex A.W. Hill ssp. *neapolitanum* Danert), curly-leaf parsley (*P. crispum* [Mill.] Nyman ex A.W. Hill ssp. *crispum* L.) and turnip-rooted parsley (*P. crispum* [Mill.] ssp. *tuberosum* [Bernh.] Mart. Crov.) were grown in an unheated, plastic-covered greenhouse using standard cultivation procedures [17]. Seeds were sown in seed trays containing a commercial peat compost on 4 October and the young plants transplanted to the greenhouse at the 2-3-leaf stage on 16 November. The experiment was based on a randomised block design in which there were 3 blocks (one cultivar each) and 2 treatments (75 and 300 mg N l^{-1} in the form of ammonium nitrate), each treatment comprising 60 plants. Fertilizer was applied with the irrigation water throughout cultivation, similar to the method of Petropoulos *et al.* [18], starting 28 days after transplantation. Plants were harvested in the early morning at 147 days (1st harvest) and 180 days (2nd harvest) after sowing (30 plants per cultivar at each harvest) and immediately transferred to the laboratory.

The number of leaves plant^{-1} and the mean weight of leaves plant^{-1} were recorded. Randomly selected leaves from each treatment and cultivar were then weighed and placed in open trays (3 leaves per tray), which were stored in the dark at 2^{0}C or 7^{0}C and 85-90% R.H. for 40 days. Each treatment consisted of 3 replicates.

Prior to and following storage, the chlorophyll content was measured in 1g samples of fresh leaves homogenized in 25 ml acetone (80% v/v), using the method of Arnon [4] and a Perkin-Elmer Lambda 1A spectrophotometer (Perkin-Elmer, Oak Park, Illinois, U.S.A.). Essential oils were extracted from 100g samples of leaves by hydro-distillation for 3 h in a Clevenger apparatus. Analysis of the essential oils was performed using a Hewlett Packard 5890 II GC (Hewlett Packard, Waldbronn, Germany), equipped with a HP-5MS capillary column (30m, 0.25mm id, 0.25μm film thickness) and a mass spectrometer 5972 (Hewlett Packard, Waldbronn, Germany), as described by Petropoulos *et al.* [17]. Due to the relatively low oil yield, especially in samples prior to storage, essential oil analysis was limited to two samples per storage treatment.

The storage experiment used a completely randomized design. Statistical analysis was carried out with the aid of the statistical package Statgraphics Plus 2.1, with the comparison of means by the least significance test or t-test.

RESULTS

The number of leaves per plant at the first harvest ranged from 11 to 14 and was not significantly affected by the rate of N application. Mean leaf weight plant^{-1} at the first harvest varied from 96 to 120 g and was also not affected by N application (Table 1). In contrast, at the second harvest the number of leaves plant^{-1} was significantly higher at the high N level (300 mg l^{-1}) in plain-leaf and turnip-rooted parsley, whereas the mean leaf weight plant^{-1} was higher in all three cultivars at 300 mg N l^{-1} (Table 1).

Storage of the leaves in open trays at 2^{0}C or 7^{0}C resulted in a progressive loss of weight which by the end of the storage period (40 days) was 80% in turnip-rooted parsley (Fig. 1), 72-77% in plain-leaf parsley and 68-71% in curly-leaf parsley, respectively (not shown).

The chlorophyll concentration within the leaves of parsley at the time of the 1st harvest was not affected by N application. During storage, however, the chlorophyll content (chlorophyll a, b, a+b) increased significantly at 2^{o}C, irrespective of N level. When stored at 7^{o}C, an increase in chlorophyll content was observed in some plain-leaf and turnip-rooted parsley, but not in curly-leaf parsley (Table 2). There was no consistent effect of N application on the chlorophyll content of the leaves following storage at either temperature.

The chlorophyll (a, b, a+b) concentration of plain-leaf parsley from the 2nd harvest was higher in the leaves of plants grown under 300 ppm N and this difference was retained during storage at 2^{o}C. Similarly, the chlorophyll concentration of turnip-rooted parsley tended to be higher at 300 ppm N, but to a statistically significant level only in chlorophyll b and total chlorophyll after storage (Table 3). In both plain-leaf and turnip-rooted parsley the chlorophyll content of leaves increased with storage.

The yield of essential oil from the fresh leaves of all three parsley cultivars was very low and not affected by the level of N application (Fig. 2). With the exception of turnip-rooted parsley, the oil yield increased significantly following storage at 2^{o}C for 40 days, irrespective of the N level.

The principal constituents of the essential oil distilled from turnip-rooted parsley were β-myrcene and β-phellandrene, the relative concentrations of which decreased during storage whereas that of 1,3,8-π-menthatriene increased (Table 4). There was no apparent effect of N level on the β-myrcene and β-phellandrene content of turnip-rooted parsley prior to or after storage, but this was compensated for by an increase in 1,3,8-π-menthatriene the concentration of which was higher in stored leaves from the high N level (300 ppm). In both plain-leaf and curly-leaf parsley, the principal constituents of the essential oils were β-phellandrene and myristicin. The relative concentration of myristicin was not affected by either N level or storage in either of these cultivars, but that of β-phellandrene appeared to be lower in leaves of plain-leaf parsley from the 75 ppm N treatment, particularly after storage. In contrast, the relative concentration of apiole, which was higher in plain-leaf parsley than in the other two cultivars, was higher at the 75 ppm N level both prior to and after storage.

DISCUSSION

Parsley foliage is normally marketed as a fresh herb for immediate consumption or as a dried condiment. Dried parsley, with a moisture content of $\leq$5% may be stored for up to 2 years [9], but its organoleptic and aromatic properties are inferior to those of the fresh product [8, 19]. On the other hand, fresh parsley rapidly dehydrates and in so doing loses its market value and is frequently discarded [2, 11]. However, as shown in the present paper, parsley foliage that has lost as much as 80% fresh weight still retains an acceptable aroma and colour and is therefore of potential value. This observation applies equally to plain-leaf and curly-leaf parsley, which are grown typically for their foliage, and also to turnip-rooted parsley the foliage of which may be consumed as well as the root [13].

As shown here, factors that affect the quality of partially dehydrated parsley include the storage temperature and, to a limited extent, the rate of nitrogen application during cultivation. Specifically, a low storage temperature (2^0C) proved better than a moderate temperature (7^0C) with respect to chlorophyll retention (Table 2). Indeed, at 2^oC, chlorophyll (a, b and total) concentration on a fresh weight basis increased during storage due to water loss, whereas at 7^oC in the majority of cases the chlorophyll content decreased. In consequence, the leaves that were stored at 2^oC were greener in colour than those stored at 7 ^{0}C or prior to storage, irrespective of cultivar.

Table 1. The effect of N application on the number of leaves and mean leaf weight plant^{-1} of three parsley cultivars harvested at two stages of development

Cultivar	N concentration (ppm)	Number of leaves plant^{-1}		Leaf weight (g plant^{-1})	
		1st harvest	2nd harvest	1st harvest	2nd harvest
Plain-leaf	75	12.8 a	23.6 b*	106.3 a	136.3 b
	300	14.3 a	30.1 a	115.6 a	197.5 a
Curly-leaf	75	11.4 a	19.7 a	119.5 a	164.5 b
	300	12.2 a	21.3 a	116.6 a	197.7 a
Turnip-rooted	75	11.0 a	17.0 b	98.9 a	108.4 b
	300	10.8 a	21.5 a	96.5 a	162.1 a

* Means within columns for each cultivar separately accompanied by different letters differ significantly at $p \leq 0.05$.

Table 2. The effect of N application on the chlorophyll (a, b and total) content of the leaves of plain-leaf, curly-leaf and turnip-rooted parsley from the 1st harvest before and after storage for 40 days at 2^0C or 7^0C

Cultivar	N concentration (ppm)	Chlorophyll a (mg 100g^{-1} F.W.)			Chlorophyll b (mg 100g^{-1} F.W.)			Chlorophyll a+b (mg 100g^{-1} F.W.)		
		Fresh	2^0C	7^0C	Fresh	2^0C	7^0C	Fresh	2^0C	7^0C
Plain-leaf	75	154.4 b#	307.1 a	237.1 a	66.1 b	265.9 a	110.3 *†a	220.4 b	572.9 a	347.2 *a
	300	140.3 c	302.3 a	193.5 b	56.9 b	336.1 a	80.6 *b	197.1 b	638.2 a	274.0 *b
Curly-leaf	75	144.0 b	299.4 a	144.2 b	59.3 b	235.0 *a	57.5 b	203.3 b	534.2 *a	201.7 b
	300	179.8 b	301.6 a	179.4 b	77.8 b	361.2 *a	71.6 b	257.5 b	662.6 *a	251.0 b
Turnip-rooted	75	166.2 b	300.0 a	277.7 a	76.7 *c	374.5 a	152.0 b	242.5 c	673.2 a	429.0 b
	300	149.3 b	304.3 a	306.4 a	52.0 *b	290.1 a	253.5 a	200.8 b	593.6 a	558.9 a

#Means in rows (separately for each parameter) followed by the same letter do not differ significantly at $p \leq 0.05$.
†Means for each cultivar separately within columns accompanied by an asterisk (*) differ significantly at $p \leq 0.05$.
F.W. = Fresh weight.

Table 3. The effect of N application on the chlorophyll (a, b and total) content of the leaves of plain-leaf, curly-leaf and turnip-rooted parsley from the 2nd harvest before and after storage for 40 days at 2^0C

Cultivar	N concentration (ppm)	Chlorophyll a (mg 100g^{-1} F.W.)		Chlorophyll b (mg 100g^{-1} F.W.)		Chlorophyll a+b (mg 100g^{-1} F.W.)	
		Fresh	2 ^{0}C	Fresh	2 ^{0}C	Fresh	2 ^{0}C
Plain-leaf	75	147.7 *†b#	218.1 *a	58.8 a	99.4 a*	206.5 *b	317.5 *a
	300	211.3 *b	299.8 *a	79.4	166.3 *	290.7 *b	466.0 *a
Curly-leaf	75	172.3 a	221.2 a	75.5 a	102.6 a	247.7 a	323.7 a
	300	188.7 a	240.4 a	87.1 a	114.9 a	275.7 a	355.2 a
Turnip-rooted	75	182.6 b	255.8 a	76.9 a	131.6 *a	259.1 b	386.8 *a
	300	214.7 b	308.7 a	87.9 b	232.5 *a	302.1 b	542.0 *a

Means in rows (separately for each parameter) followed by the same letter do not differ significantly at $p \leq 0.05$.
† Means for each cultivar separately within columns accompanied by an asterisk (*) differ significantly at $p \leq 0.05$.
F.W. = Fresh weight.

Table 4. The effect of N application on the principal constituents of the essential oil distilled from the leaves of plain-leaf, curly-leaf and turnip-rooted parsley before and after storage at 2^0C

Cultivar	N concentration (ppm)	β-myrcene 0991*		β-phellandrene 1030		Terpinolene + π-cymenene 1089		1,3,8-π-menthatriene 1110		Myristicin 1519		Apiole 1678	
		Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored
Plain-leaf	75	3.3	1.5	18.2	11.5	7.4	5.4	4.8	1.4	33.2	20.8	12.0	19.4
	300	4.4	6.1	27.9	23.3	9.9	6.5	4.5	7.9	29.7	18.3	6.5	8.5
Curly-leaf	75	3.1	6.4	23.6	27.7	3.0	4.4	3.8	5.5	35.4	30.9	2.1	1.2
	300	5.7	7.8	26.2	31.9	4.6	4.0	1.7	6.8	37.0	26.7	3.6	0.7
Turnip-rooted	75	24.2	11.3	28.4	17.7	-#	-	0.6	0.7	0.4	1.5	-	-
	300	24.4	15.0	27.2	10.4	-	--	0.5	13.1	0.5	0.7	0.2	-

* Retention index.
# Below the detection limit (0.005 g [100 g]$^{-1}$).

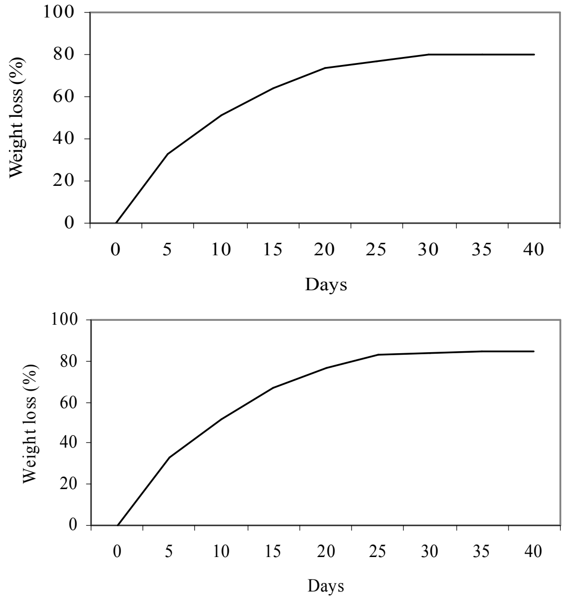

Figure 1. Weight loss from the leaves of turnip-rooted parsley stored at 2°C (upper) or 7°C (lower) for 40 days in open trays

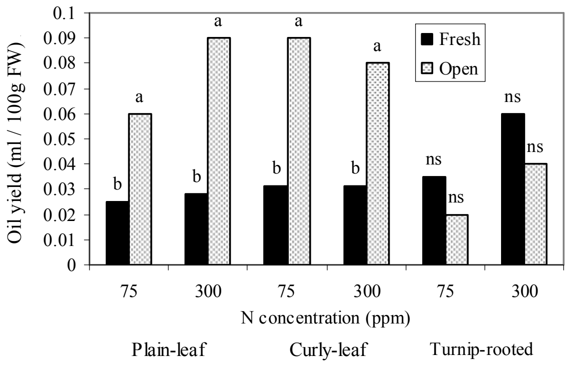

Figure 2. The effect of N application on the yield of essential oil extracted from plain-leaf, curly-leaf and turnip-rooted parsley before (fresh) and after storage (open) for 40 days at 2°C. Different letters accompanying the columns of the same cultivar indicate statistically significant differences; ns =not significant ($p \leq 0.05$)

Similarly, partial dehydration of parsley resulted in an increase in the essential oil yield of plain-leaf and curly-leaf parsley (but not turnip-rooted parsley) on a fresh weight basis (Figure 2). This means that the parsley aroma during partial dehydration was retained, or even improved, in contrast to dried parsley where a significant reduction in essential oil content and product aroma occurs [19]. Moreover, there was little overall change in the relative concentrations of the essential oil constituents during storage. Where a decrease in a particular aroma constituent was observed, e.g. myristicin in plain-leaf and curly-leaf parsley or β-myrcene and β-phellandrene in turnip-rooted parsley leaves, this was compensated for by a relative increase in another constituent, e.g. apiole or 1,3,8-π-menthatriene, as observed in frozen parsley [19]. Differences in the essential oil constituents of leaves harvested from different N levels were few, as observed by Petropoulos *et al.* [18], but shortage of material did not permit adequate replication for statistical analysis. The oil constituents, however, did relate to the cultivar, for example the high apiole content of plain-leaf parsley compared with the low apiole content and absence of terpinolene + π-cymenene in turnip-rooted parsley leaves [19].

In conclusion, the results of the present study clearly show that partially dehydrated parsley leaves retain their green colour and aroma during storage for 40 days at 2°C, irrespective of cultivar and the level of N during cultivation. Consequently, we propose that partially dehydrated leaves constitute a valuable, marketable commodity, with greater quality characteristics (colour and aroma) than the corresponding dried product. Moreover, although high N levels (300 ppm) increased parsley yield (number and weight of leaves per plant) in comparison with a lower N level (75 ppm), the quality characteristics of the foliage studied here (colour and aroma) were mostly unaffected by N level both before and after storage.

REFERENCES

[1] Aharoni, N., Reuveni, A., Dvir, O., (1989): Modified atmospheres in film packages delay senescence and decay of fresh herbs. Acta Horticulturae, 258: 255-260.

[2] Almeida, D.P.F., Valente, C.S., (2005): Storage life and water loss of plain and curled leaf parsley. Acta Horticulturae, 682: 1199-1202.

[3] Antonopoulos, A.I., (2007): Study of the quality characteristics of parsley cultivated under two levels of nitrogenous fertilizer and stored under conditions of modified atmospheres. M.Sc. Thesis, Agricultural University of Athens, pp. 122.

[4] Arnon, D.I., (1949): Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiology, 24: 1-15.

[5] Azeez, S., Parthasarathy, V.A., (2008): Parsley. pp. 376-400. In: Partasarathy, V.A., Chempakam, B., Zachariah, T.J. (eds.). Chemistry of Spices. CABI International, Wallingford, U.K.

[6] Cantwell, M.I., Reid, M.S., (1993): Postharvest physiology and handling of fresh culinary herbs. Journal of Herbs, Spices and Medicinal Plants, 1 (3): 93-127.

[7] Csizinszky, A.A., (1999): Yield response of herbs to nitrogen and potassium in sand in multiple harvests. Journal of Herbs, Spices and Medicinal Plants, 6(4): 11-22.

[8] Díaz-Maroto, M.C., Pérez-Coello, M.S., Cabezudo, M.D., (2002): Effect of different drying methods on the volatile components of parsley (*Petroselinum crispum* L.). European Food Research and Technology, 215: 227-230.

[9] Fisher, L., Medeiros, L., (2010). Pantry food storage. Family and Consumer Sciences Fact Sheet HYG-5401-94-R10, Ohio State University, pp. 6.

[10] Freeman, G.G., Whenham, R.J., Self, R., Eagles, J., (1975): Volatile flavour components of parsley leaves (*Petroselinum crispum* [Mill.] Nyman). Journal of the Science of Food and Agriculture, 26: 465-470.

[11] Hruschka, H.W., Wang, C.Y., (1979): Storage and shelf-life of packaged watercress, parsley and mint. Market Research Report No. 1101, U.S.D.A., Washington D.C., pp. 19.

[12] Kasting, R., Anderson, J., von Sydow, E., (1972): Volatile constituents in leaves of parsley. Phytochemistry, 11: 2277-2282.
[13] Kmiecik, W., Lisiewska, Z., (1999): Comparison of leaf yields and chemical composition of the Hamburg and leafy types of parsley. I. Leaf yields and their structure. Folia Horticulturae, 11: 53-63.
[14] Lisiewska, Z., Kmiecik, W., Budnik, A., (1997): Effect of conditions and time of storage on technological quality changes of parsley leaves. Folia Horticulturae, 9: 21-29.
[15] Manzano, M., Citterio, B., Maifreni, M., Paganessi, M., Comi, G., (1995): Microbial and sensory quality of vegetables for soup packaged in different atmospheres. Journal of the Science of Food and Agriculture, 67: 521-529.
[16] Nitz, S., Kollmannsberger, H., Spraul, M.H., Drawert, F., (1989): Oxygenated derivatives of menthatriene in parsley leaves. Phytochemistry, 28: 3051-3054.
[17] Petropoulos, S.A., Daferera, D., Akoumianakis, C.A., Passam, H.C., Polissiou, M.G., (2004): The effect of sowing date and growth stage on the essential oil composition of three types of parsley (*Petroselinum crispum*). Journal of the Science of Food and Agriculture, 84: 1606-1610.
[18] Petropoulos, S.A., Daferera, D., Polissiou, M.G., Passam, H.C. (2009): Effect of nitrogen-application rate on the biomass, concentration, and composition of essential oils in the leaves and roots of three types of parsley. Journal of Plant Nutrition and Soil Science, 172: 210-215.
[19] Petropoulos, S.A., Daferera, D., Polissiou, M.G., Passam, H.C., (2010): Effect of freezing, drying and the duration of storage on the composition of essential oils of plain-leafed parsley (*Petroselinum crispum* [Mill.] Nym. ssp. *neapolitanum* Danert) and turnip-rooted parsley (*Petroselinum crispum* [Mill.] Nym. ssp. *tuberosum* [Bernh.] Crov.). Flavour and Fragrance Journal, 25: 28-34.
[20] Ryall, A.L., Lipton, J.W., (1979): Handling, transportation and storage of fruits and vegetables, Volume 1, Second edition - Vegetables and melons. AVI publishing Company Inc., Westport, Connecticut, pp. 587.
[21] Sanchez-Echaniz, J., Benito-Fernandez, J., Mintegui-Raso, S., (2001): Methemoglobinemia and consumption of vegetables in infants. Pediatrics, 107: 1024-1028.
[22] Santamaria, P., (2006): Nitrate in vegetables: toxicity, content, intake and EC regulation. Journal of the Science of Food and Agriculture, 86: 10-17.
[23] Snowdon, A.L., (1991): Post-harvest diseases and disorders of fruits and vegetables, Vol. 2. Vegetables. Wolfe Scientific, London, pp. 416.
[24] USDA, (2007): United States standards for grades of parsley, p. 1.
[25] Warncke, D., Dahl, J., Zandstra, B., (2004): Nutrient recommendations for vegetable crops in Michigan. Extension Bulletin E2934, Michigan State University Extension Service, pp. 31.

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