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Effects of Dolomite Application on Plant Growth, Activities of Polyphenol Oxidase and Internal Quality of Grand Rapids Lettuce

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Abstract: The purpose of this experiment was to investigate the effect of preharvest soil application of dolomite on the growth, activity of Polyphenol oxidase (PPO) and internal characteristics was evaluated on lettuce cv. Grand Rapids under field conditions. A factorial in completely randomized design was arranged with four replications and composed of two factors; application time four levels (25, 40, 55 days after planting, DAP compared with untreated treatment, Control) with four concentration rates (0, 50, 100 and 150 ppm). The results show that dolomite application irrespective of application times or concentration rates had no effect on stem diameter, plant height, degree of leaf browning, fresh weight, biomass, chlorophyll content, leaf colour in terms of a* and b*, the content of phenolic, quinone, Total Soluble Solids (TSS), Titratable Acidity (TA), pH or ascorbic acid content. While maximum response of leaf increment was achieved with treating of 150 ppm dolomite at 25 DAP. Dolomite application irrespective of concentrations at all application times (25, 40 and 55 DAP) reduced the bush size compared with the control. In addition, application of 150 ppm dolomite at 55 DAP had the maximal brightness of leaf colour, L* value. Furthermore, dolomite treatment of 50 ppm at 25 DAP gave the least level of PPO activity at 33 DAP.

Key words: Polyphenol oxidase, dolomite, lettuce, growth, leaf colour

INTRODUCTION

Lettuce (*Lactuca sataiva*) is a popular vegetable and considered as one of the most important all year round crops in Thailand. In 2006-2007, the total area for growing lettuce in Thailand was 1,226 ha with an estimated production of 12,056 tones/year (DOAE, 2007). Generally, fresh lettuce is used for consumption in fast food and prepared salads. In addition, it contains significant amounts of biologically active components that can impart health benefits, including dietary antioxidants, which are known to have a protective effect against various forms of cancer and cardiovascular and cerebrovascular diseases (Lister, 2003; Nicolle *et al*., 2004; Liorach *et al*., 2008; Verlangieri *et al*., 1985). Therefore, it is not surprising to consider lettuce as healthier foods (Ahvenainen, 1996; Dupont *et al*., 2000). By nature, lettuce shows a great sensitivity to enzymatic browning which leads to colour change appearing on the leaf surface (Saltveit, 2000). This discoloration has long been considered as physiological disorder and leads to a major quality problem for growers (Shewfelt, 1994; Peiser *et al*., 1998). Some researchers presumed that this disorder is mainly associated with the enzymatic browning caused from the oxidation of phenolic compounds by enzyme

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Polyphenol oxidase (PPO) to produce quinones that polymerizes and form brown pigments in fresh lettuces (Macheix *et al*., 1990; Kays, 1999; Nicolas *et al*., 1994; Jiang *et al*., 2004). The appearance of this physiological disorders can be observed visually on leaf surfaces during any preharvest period (Kays, 1999; Franck *et al*., 2007). Furthermore, enzymatic browning is a direct consequence of membrane disintegration (Kays, 1991; Felicetti and Schrader, 2009). Therefore, the potential practice affects to maintain the membrane integrity in order to control this disorder during plant growth should be studied to increase the value and quality of harvested lettuce. At present, very little is known about any practical method to control the browning disorder in lettuce planted in the field. There have been reports of several pre-harvest factors, which affect the development of browning disorders in lettuce leaf, linked to calcium deficiency (Martyn *et al*., 2007). Calcium has been shown to play an important role in cell membrane structure to stabilize plant tissues (Marinos, 1962). It was reported that browning incidence in several vegetables could be reduced by calcium application (El-Fattah and Agwah, 1987; Sonneveld and Van Den Ende, 1975; Thibodeau and Minotti, 1969). Dolomite [$CaMg(CO_3)_2$], is a type of compact limestone consisting of a calcium carbonate (contain 22% calcium) and magnesium carbonate (contain 12% Mg) (Lines-Kelly, 1992). In agriculture applications, dolomite is commonly used as soil fertilizer in a range of soils. Cresswell and Weir (1997) reported that application of calcium in the form of dolomite, which is a calcium-releasing compound, could be used to increase calcium in the potting mix. Chen *et al*. (2006) also cited that growth of citrus and vegetable crops was promoted when dolomite fertilizer was applied. In Thailand, dolomite application use in lettuce production has not been documented. Furthermore, there is very little information available on dolomite pertaining to the characteristics of growth, PPO activity and chemical quality in lettuce production. Therefore, research on the investigation of the effect of dolomite to these above attributes in lettuce is warranted. Thus, the purpose of this experiment was to investigate the effect of dolomite by soil application on 'Grand Rapids' lettuce grown under field conditions.

MATERIALS AND METHODS

The experiment was carried out at the experimental field, Division of Agricultural Technology, Faculty of Technology, Mahasarakham University, in the northeast of Thailand between May and July, 2009. Seeds of Grand Rapids lettuce were sown and transplanted at 25 DAP in 2-L pot filled with a sandy loam soil : rice husk : manure ratio 1: 1 : 1 and placed under field conditions. A Factorial in Completely Randomized Design was arranged and composed of two factors: four levels of dolomite application time (25, 40, 55 DAP compared with untreated treatment, control) with four levels of different concentrations (0, 50, 100 and 150 ppm). The crushed dolomite was manually applied by mixing with the soil in each pot. Each treatment was carried out in four replicates, ten plants per replication. Growth measurements in terms of stem diameter, plant height, leaf size in terms of width and length, bush size and level of browning appearance, were recorded at weekly intervals, from 33 DAP through 61 DAP (harvesting date). The level of browning incidence that occurred on the lettuce leafs was scored for an evaluation of the browning as described in González-Aguilar *et al*. (2004). Visual determination used a scale of 1-5, where 1 = none, 2 = slight, 3 = moderate, 4 = severe and 5 = extreme browning. While the activity of Polyphenol oxidase (PPO) were analyzed from lettuce leaf at 33, 47 and 61 DAP and carried out according to the method reported by Jiang and Fu (1998). The attained enzyme extracts were measured by spectrophotometer model V-325-XS, from China. One unit of PPO activity was defined as the amount of enzyme causing a change of 0.01 in absorbance (420 nm) per

60 sec. The PPO activity was analyzed from lettuce leaves at 33, 47 and 61 DAP. Measurements for internal qualities were conducted at the harvesting date (61 DAP) for assessments of (1) Fresh weight (g) (2) Biomass was determined by the method of AOAC (1980) and expressed in percentage. (3) Chlorophyll content was determined using a procedure as described by Whitham *et al.* (1986) and expressed as mg m^{-2}. (4) Leaf colour was measured on the leaf surface with a Hunter Lab Model No. 45/0-L, Serial No. 7092, USA. CIE colour values L* (black = -100 and white = +100), a* (redness) (- = green and + = red) and b* (yellowness) (- = blue and + = yellow) were measured to describe the colour of lettuce's leaf. (5) Phenolic content was performed as described by Ribeiro *et al.* (2008). Content was expressed as absorption at 765 nm/100 g fresh weight of leaf. (6) Quinone content was extracted as described by Pirie and Mullins (1976). Quinone content was expressed as absorbance at 437 nm per g fresh weight. (7) Total soluble solid content (TSS) (juices being squeezed from flesh tissue with the use of distilled water at a ratio between flesh and distilled water of 1:3 was measured by a digital refractometer (Atago-Palette PR 101, Atago Co., Ltd., Itabashi-ku, Tokyo, Japan). (8) Titratable Acidity (TA) evaluation was made by the use of juices described in number 7 with the method of AOAC (1990). (9) The measurement of pH values was carried out with the use of juices described earlier in number 7 and a pH meter ID 100D, from Singapore was used. (10) Ascorbic acid content was measured by the use of juices described in number 7 with the method of AOAC (1990) and were expressed as mg ascorbic acid /100 mL juice. The collected data were statistically analyzed using the SPSS Computer Programme, Version 6 (SPSS, 1999).

RESULTS

The recorded data received from growth measurement, PPO activity and internal quality produced the following results:

Stem Diameter

The results from Table 1 showed that at harvest, there was a similar amount of stem diameter in both dolomite treatments and control samples.

Plant Height

The measured data taken for plant height are shown in Table 2. There was a gradual increase in plant height with an increase in the age of the tested plant. The application of dolomite, regardless of application times or concentration rates, did not show any remarkable significant difference in this parameter compared to control plants.

Leaf Size

Application of 150 ppm dolomite at 25 DAP was sufficient to promote the largest size of lettuce leaves. The results from measuring leaf width from Table 3 show that at harvest, the maximum leaf size was obtained (8.01 cm) when the plants treated with 150 ppm dolomite at 25 DAP . While the maximum leaf length (12.71 cm) of plants also received from plants supplied with 150 ppm dolomite at 25 DAP (Table 4).

Bush Diameter

Significant effect of application times on the size of lettuce bush was observed. The results from Table 5 show that at harvest, plants applied with dolomite at any application time were significantly lower compared to the control treatment.

Table 1: Stem diameter of lettuce after applying different times and concentrations of dolomite

	Stem diameter (cm) at different DAP				
Factors	33	40	47	54	61
Time (DAP)					
25	0.23	0.26	0.29	0.41	0.49
40	0.22	0.25	0.28	0.38	0.46
55	0.23	0.25	0.28	0.39	0.50
Control	0.23	0.26	0.29	0.41	0.52
F-test	ns	ns	ns	ns	ns
CV (%)	9.94	5.12	8.01	2.77	7.11
LSD	0.0052	0.0048	0.0063	0.0120	0.0178
Conc. (ppm)					
50	0.23	0.26	0.28	0.39	0.48
100	0.22	0.26	0.28	0.39	0.48
150	0.22	0.26	0.29	0.40	0.51
Control	0.23	0.26	0.29	0.41	0.52
F-test	ns	ns	ns	ns	ns
CV (%)	9.94	5.12	8.01	2.91	7.19
LSD	0.0052	0.0048	0.0063	0.0121	0.0178
Time × conc.					
25 DAP 50 ppm	0.24	0.26	0.28	0.39	0.48
25 DAP 100 ppm	0.22	0.26	0.30	0.41	0.49
25 DAP 150 ppm	0.22	0.25	0.29	0.42	0.51
40 DAP 50 ppm	0.22	0.26	0.28	0.38	0.44
40 DAP 100 ppm	0.21	0.25	0.27	0.38	0.48
40 DAP 150 ppm	0.23	0.26	0.28	0.38	0.48
55 DAP 50 ppm	0.23	0.25	0.28	0.40	0.52
55 DAP 100 ppm	0.24	0.25	0.27	0.37	0.46
55 DAP 150 ppm	0.22	0.26	0.29	0.40	0.53
Control	0.23	0.26	0.29	0.41	0.52
F-test	ns	ns	ns	ns	ns
CV (%)	9.43	5.12	8.01	2.72	7.04
LSD	0.0074	0.0068	0.0089	0.0170	0.0251

ns: Non significant

Table 2: Plant height of lettuce after applying different times and concentrations of dolomite

	Plant height (cm) at different DAP				
Factors	33	40	47	54	61
Time (DAP)					
25	4.75	8.39 ab	12.75 a	11.61	21.65
40	4.61	7.59 b	10.55 b	10.11	20.19
55	4.66	7.63 b	11.28 ab	12.25	20.52
Control	4.85	8.97 a	12.64 a	11.57	22.00
F-test	ns	*	**	ns	ns
CV (%)	3.09	7.46	7.21	6.77	5.33
LSD	0.1718	0.3736	0.5413	0.9368	0.7076
Conc. (ppm)					
50	4.88	7.91	11.48	11.03	20.83
100	4.51	7.52	11.46	10.56	20.42
150	4.63	8.18	11.63	12.39	21.10
Control	4.85	8.97	12.64	11.57	22.00
F-test	ns	ns	ns	ns	ns
CV (%)	3.96	7.58	7.96	6.88	5.46
LSD	0.1711	0.3747	0.5522	10.75	0.7112
Time × conc.					
25 DAP 50 ppm	4.70	7.91	11.71 bcd	10.75	20.70
25 DAP 100 ppm	4.64	8.23	14.18 a	11.70	21.70
25 DAP 150 ppm	4.91	9.02	12.38 abc	12.39	22.55
40 DAP 50 ppm	4.93	7.81	11.22 bcd	10.50	19.96
40 DAP 100 ppm	4.44	7.30	9.79 d	9.71	20.75
40 DAP 150 ppm	4.46	7.66	10.63 bcd	10.13	19.86
55 DAP 50 ppm	5.00	8.02	11.51 bcd	11.84	21.84
55 DAP 100 ppm	4.44	7.02	10.43 cd	10.27	18.82
55 DAP 150 ppm	4.53	7.86	11.89 bcd	14.64	20.89

Table 2: Continued

	Plant height (cm) at different DAP				
Factors	33	40	47	54	61
Control	4.85	8.97	12.64 ab	11.57	22.00
F-test	ns	ns	**	ns	ns
CV (%)	3.08	7.53	7.01	6.71	5.28
LSD	0.2429	0.5293	0.7614	1.3235	0.9989

Letter(s) within columns indicate least significant differences (LSD) at **p: 0.01, *p: 0.05, ns: Non significant

Table 3: Leaf width of lettuce after applying different times and concentrations of dolomite

	Leaf width (cm) at different DAP				
Factors	33	40	47	54	61
Time (DAP)					
25	2.24	3.91	4.60 a	5.48	7.05ab
40	2.09	3.50	3.99 b	4.93	6.46b
55	2.13	3.56	4.12 b	5.25	7.20ab
Control	2.29	4.13	4.74 a	5.36	7.66a
F-test	ns	ns	**	ns	*
CV (%)	8.52	9.08	3.78	3.31	9.54
LSD	0.0728	0.1810	0.1702	0.2190	0.2756
Conc. (ppm)					
50	2.13	3.57	4.17	5.15	6.61b
100	2.13	3.49	4.10	5.05	6.61b
150	2.21	3.91	4.43	5.46	7.50a
control	2.29	4.13	4.74	5.36	7.66a
F-test	ns	ns	ns	ns	**
CV (%)	8.60	9.08	3.16	3.42	9.30
LSD	0.0730	0.1809	0.1722	0.2197	0.2734
Time × conc.					
25 DAP 50 ppm	2.13	3.56	4.42abc	5.38	6.57cde
25 DAP 100 ppm	2.24	3.93	4.67ab	5.45	6.58cde
25 DAP 150 ppm	2.34	4.23	4.70a	5.61	8.01a
40 DAP 50 ppm	2.05	3.50	4.01bcd	4.87	6.04e
40 DAP 100 ppm	2.08	3.16	3.75d	5.08	6.39de
40 DAP 150 ppm	2.14	3.83	4.21abcd	4.83	6.96abcde
55 DAP 50 ppm	2.20	3.64	4.09abcd	5.20	7.23abcd
55 DAP 100 ppm	2.06	3.38	3.88 cd	4.60	6.85bcde
55 DAP 150 ppm	2.14	3.67	4.38 abcd	5.95	7.53abc
Control	2.29	4.13	4.74 a	5.36	7.66ab
F-test	ns	ns	*	ns	**
CV (%)	8.62	8.98	3.84	3.07	9.13
LSD	0.1033	0.2552	0.2411	0.3073	0.3844

Letter(s) within columns indicate least significant differences (LSD) at **p: 0.01, *p: 0.05, ns: Non significant

Table 4: Leaf length of lettuce after applying different times and concentrations of dolomite

	Leaf length (cm) at different DAP				
Factors	33	40	47	54	61
Time (DAP)					
25	3.69	6.73ab	6.73ab	9.45a	11.64ab
40	3.61	5.93b	5.93b	8.40b	10.68b
55	3.53	5.99b	5.99b	8.91ab	11.45ab
control	3.71	7.10a	7.10a	9.51a	12.00a
F-test	ns	*	*	*	*
CV (%)	3.55	7.51	7.51	8.19	3.28
LSD	0.1303	0.2957	0.2957	0.3383	0.3524
Conc. (ppm)					
50	3.56	6.12	6.12	8.91	10.88b
100	3.48	6.03	6.03	8.62	10.80b
150	3.78	6.50	6.50	9.23	12.09a

Table 4: Continued

	Leaf length (cm) at different DAP				
Factors	33	40	47	54	61
Control	3.71	7.10	7.10	9.51	12.00a
F-test	ns	ns	ns	ns	**
CV (%)	3.41	7.78	7.78	8.43	3.01
LSD	0.1297	0.2978	0.2978	0.3412	0.3483
Time × conc.					
25 DAP 50 ppm	3.45	6.08ab	6.08ab	9.10ab	10.78bcd
25 DAP 100 ppm	3.71	7.14a	7.14a	9.83a	11.42abc
25 DAP 150 ppm	3.90	6.97a	6.97a	9.42ab	12.71a
40 DAP 50 ppm	3.64	6.21ab	6.21ab	8.39bc	10.58cd
40 DAP 100 ppm	3.39	5.36b	5.36b	8.28bc	10.03d
40 DAP 150 ppm	3.80	6.23ab	6.23ab	8.53abc	11.45abc
55 DAP 50 ppm	3.60	6.08ab	6.08ab	9.25ab	11.29bcd
55 DAP 100 ppm	3.33	5.60b	5.60b	7.75c	10.96bcd
55 DAP 150 ppm	3.65	6.30ab	6.30ab	9.73a	12.10ab
Control	3.71	7.10a	7.10a	9.51ab	12.00ab
F-test	ns	*	*	*	**
CV (%)	3.50	7.39	7.39	7.95	2.90
LSD	0.1839	0.4168	0.4168	0.4742	0.4903

Letter(s) within columns indicate least significant differences (LSD) at **p: 0.01, *p: 0.05, ns: Non significant

Table 5: Bush diameter of lettuce after applying different times and concentrations of dolomite

	Bush diameter (cm) at different DAP				
Factors	33	40	47	54	61
Time (DAP)					
25	4.31ab	6.15a	10.45ab	19.22a	18.91b
40	3.85c	4.99b	9.03c	16.91b	18.11b
55	4.04bc	4.95b	9.53bc	18.38ab	19.05b
Control	4.49a	6.06a	10.97a	20.05a	21.26a
F-test	*	**	*	*	*
CV (%)	3.16	4.96	9.41	7.18	6.95
LSD	0.1604	0.2917	0.4825	0.7486	0.6824
Conc. (ppm)					
50	4.08	5.07 b	9.47	17.77	18.43
100	3.90	5.18 b	9.25	17.76	18.45
150	4.21	5.84 ab	10.27	18.98	19.19
Control	4.49	6.06 a	10.97	20.05	21.26
F-test	ns	*	ns	ns	ns
CV (%)	3.32	4.65	9.59	3.78	6.98
LSD	0.1612	0.2964	0.4847	0.7549	0.6829
Time × conc.					
25 DAP 50 ppm	4.08	5.70 abc	10.04	18.33	17.78
25 DAP 100 ppm	4.24	5.91 ab	10.27	19.51	19.79
25 DAP 150 ppm	4.60	6.84 a	11.00	19.82	19.16
40 DAP 50 ppm	3.97	5.22 bcd	8.70	16.33	17.64
40 DAP 100 ppm	3.73	4.68 cd	8.85	16.88	17.70
40 DAP 150 ppm	3.83	5.08 bcd	9.55	17.53	19.01
55 DAP 50 ppm	4.19	4.31 d	9.68	18.63	19.88
55 DAP 100 ppm	3.73	4.94 bcd	8.64	16.89	17.88
55 DAP 150 ppm	4.19	5.59 bc	10.26	19.60	19.38
Control	4.49	6.06 ab	10.97	20.05	21.26
F-test	ns	**	ns	ns	ns
CV (%)	3.15	4.68	9.48	3.57	6.96
LSD	0.2268	0.4098	0.6836	1.0605	0.9653

Letter(s) within columns indicate least significant differences (LSD) at **p: 0.01, *p: 0.05, ns: Non significant

Table 6: Browning incidence of lettuce after applying different times and concentrations of dolomite

	Level of browning at different DAP				
Factors	33	40	47	54	61
Time (DAP)					
25	1.34	1.60	1.76	1.57	1.92
40	1.45	1.63	1.66	1.51	1.79
55	1.41	1.70	1.74	1.60	1.83
Control	1.31	1.66	1.81	1.54	1.89
F-test	ns	ns	ns	ns	ns
CV (%)	5.07	3.69	6.55	3.05	2.32
LSD	0.0575	0.0692	0.0573	0.0667	0.0527
Conc. (ppm)					
50	1.44	1.69	1.71	1.61	1.86
100	1.38	1.59	1.73	1.56	1.82
150	1.38	1.65	1.72	1.51	1.86
Control	1.31	1.66	1.81	1.54	1.89
F-test	ns	ns	ns	ns	ns
CV (%)	5.15	3.70	6.66	3.04	2.48
LSD	0.0576	0.0692	0.0576	0.0667	0.0531
Time × conc.					
25 DAP 50 ppm	1.56 a	1.72	1.84	1.61	2.00
25 DAP 100 ppm	1.28 bc	1.53	1.78	1.61	1.82
25 DAP 150 ppm	1.19 c	1.56	1.66	1.50	1.93
40 DAP 50 ppm	1.50 ab	1.72	1.53	1.57	1.71
40 DAP 100 ppm	1.36 abc	1.50	1.63	1.46	1.89
40 DAP 150 ppm	1.50 ab	1.66	1.81	1.50	1.75
55 DAP 50 ppm	1.28 bc	1.63	1.75	1.64	1.86
55 DAP 100 ppm	1.50 ab	1.75	1.78	1.61	1.75
55 DAP 150 ppm	1.44 ab	1.72	1.69	1.54	1.89
Control	1.31 bc	1.66	1.81	1.54	1.89
F-test	*	ns	ns	ns	ns
CV (%)	4.53	3.71	6.39	3.28	2.23
LSD	0.0801	0.0980	0.0806	0.0950	0.0742

Letter(s) within columns indicate least significant differences (LSD) at *p: 0.05, ns: Non significant

Browning Appearance

Degree of leaf browning showed a similar trend in both dolomite treatments and control samples, excepted at early plant growth of 33 DAP. At this time, the plants treated with 150 ppm dolomite at 25 DAP showed significantly the lowest browning level of 1.19 (Table 6).

PPO Activity

The variation in the PPO activity in lettuce leaf measured at the various developmental stages (33, 47 and 61 DAP) is shown in Table 7-9. Highly significant differences ($p>0.01$) of PPO activities among the dolomite applications were found between treatments only at 33 DAP. For interaction of the application times and various concentration rates of dolomite, the results revealed that PPO from lettuce-treated with 150 ppm dolomite at 25 DAP showed the minimum activity of PPO was observed (Table 7).

Fresh Weight

At harvest, there was no any significant fresh weight of product ranged from 17.65-27.18 g per plant (Table 10).

Biomass

The results from Table 10 showed no significant difference in lettuce biomass among treatments with similar mean values of 9.15-12.54% at harvesting.

Table 7: PPO activities of lettuce at 33 DAP

	PPO activity at different times (sec)					
Factors	0	60	120	180	240	300
Time (DAP)						
25	0.1802	0.2328a	0.2760a	0.3115a	0.3400a	0.3630a
40	0.1444	0.1855ab	0.2210ab	0.2511ab	0.2760ab	0.2966ab
55	0.1326	0.1592b	0.1825b	0.2001b	0.2136b	0.2291b
Control	0.1330	0.1633b	0.1671b	0.1833b	0.2023b	0.2179b
F-test	ns	*	*	**	**	**
CV (%)	6.98	8.05	7.97	7.00	6.41	6.13
LSD	0.0177	0.0228	0.0264	0.0291	0.0312	0.0333
Conc. (ppm)						
50	0.1311b	0.1593b	0.1853b	0.2077b	0.2268b	0.2431b
100	0.1876a	0.2451a	0.2929a	0.3328a	0.3647a	0.3900a
150	0.1385b	0.1730b	0.2013b	0.2223b	0.2382b	0.2556b
Control	0.1330b	0.1633b	0.1671b	0.1833b	0.2023b	0.2179b
F-test	*	**	**	**	**	**
CV (%)	4.04	4.58	6.23	5.23	4.59	4.41
LSD	0.0173	0.0221	0.0255	0.0280	0.0300	0.0320
Time × conc.						
25DAP50 ppm	0.1799ab	0.2246abc	0.2621abc	0.2929abc	0.3179abc	0.3386abc
25DAP100 ppm	0.1126c	0.1386de	0.1613de	0.1809ef	0.1985de	0.2136de
25 DAP150 ppm	0.1009c	0.1146e	0.1324e	0.1494f	0.1639e	0.1771e
40 DAP50 ppm	0.2006a	0.2648ab	0.3159ab	0.3570ab	0.3895ab	0.4145ab
40 DAP100 ppm	0.2136a	0.2794a	0.3350a	0.3819a	0.4188a	0.4481a
40 DAP150 ppm	0.1485abc	0.1912bcde	0.2279bcd	0.2595bcde	0.2858bcd	0.3073bcd
55 DAP50 ppm	0.1600abc	0.2089abcd	0.2501abcd	0.2848abcd	0.3127abc	0.3360abc
55 DAP100 ppm	0.1070c	0.1386de	0.1666de	0.1906cdef	0.2106cde	0.2280cde
55 DAP150 ppm	0.1485abc	0.1716cde	0.1872cde	0.1914cdef	0.1911de	0.2029de
Control	0.1330bc	0.1633cde	0.1671cde	0.1833def	0.2023de	0.2179de
F-test	**	**	**	**	**	**
CV (%)	4.57	4.81	4.24	4.85	4.88	4.73
LSD	0.0233	0.0294	0.0337	0.0366	0.0389	0.0415

Letter(s) within columns indicate least significant differences (LSD) at **p: 0.01, *p: 0.05, ns: Non significant

Table 8: PPO activities of lettuce at 47 DAP

	PPO activity at different times (sec)					
Factors	0	60	120	180	240	300
Time (DAP)						
25	0.2354	0.3141	0.3753	0.4230	0.4615	0.4928
40	0.2258	0.3109	0.3708	0.4209	0.4612	0.4934
55	0.2747	0.3705	0.4404	0.4939	0.5358	0.5678
Control	0.2550	0.3655	0.4458	0.5080	0.5555	0.5925
F-test	ns	ns	ns	ns	ns	ns
CV (%)	4.73	8.66	9.81	5.00	9.59	9.07
LSD	0.0381	0.0577	0.0705	0.0799	0.0864	0.0912
Conc. (ppm)						
50	0.2188	0.3121	0.3792	0.4305	0.4713	0.5035
100	0.2828	0.3824	0.4553	0.5149	0.5623	0.5995
150	0.2343	0.3010	0.3520	0.3924	0.4249	0.4510
Control	0.2550	0.3655	0.4458	0.5080	0.5555	0.5925
F-test	ns	ns	ns	ns	ns	ns
CV (%)	4.16	8.20	9.25	9.26	8.76	8.14
LSD	0.0377	0.0571	0.0697	0.0787	0.0850	0.0894
Time × conc.						
25 DAP50 ppm	0.2050	0.3010	0.3720	0.4253	0.4678	0.5033
25 DAP100 ppm	0.2455	0.3580	0.4358	0.4958	0.5425	0.5785
25 DAP150 ppm	0.2060	0.2773	0.3298	0.3705	0.4038	0.4288
40 DAP50 ppm	0.2775	0.3575	0.4230	0.4743	0.5158	0.5485
40 DAP100 ppm	0.2270	0.2927	0.3368	0.3823	0.4208	0.4530
40 DAP150 ppm	0.3438	0.4970	0.6063	0.6883	0.7503	0.7970
55 DAP50 ppm	0.2238	0.2838	0.3308	0.3695	0.4010	0.4268
55 DAP100 ppm	0.2048	0.2820	0.3400	0.3848	0.4203	0.4488
55 DAP150 ppm	0.2743	0.3373	0.3853	0.4230	0.4535	0.4775

Table 8: Continued

	PPO activity at different times (sec)					
Factors	0	60	120	180	240	300
Control	0.2550	0.3655	0.4458	0.5080	0.5555	0.5925
F-test	ns	ns	ns	ns	ns	ns
CV (%)	4.85	9.65	5.31	5.29	9.76	9.15
LSD	0.0552	0.0832	0.1008	0.1137	0.1227	0.1291

ns: Non significant

Table 9: PPO activities of lettuce at 61 DAP

	PPO activity at different times (sec)					
Factors	0	60	120	180	240	300
Time (DAP)						
25	0.1476	0.1896	0.2218	0.2465	0.2733	0.2932
40	0.1518	0.1905	0.2207	0.2482	0.2699	0.2878
55	0.1785	0.2325	0.2774	0.3137	0.3440	0.3684
Control	0.1455	0.1793	0.2080	0.2328	0.2548	0.2730
F-test	ns	ns	ns	ns	ns	ns
CV (%)	6.87	8.91	9.86	3.33	3.48	3.40
LSD	0.0150	0.0207	0.0249	0.0285	0.0315	0.0335
Conc. (ppm)						
50	0.1456	0.1902	0.2278	0.2587	0.2854	0.3068
100	0.1657	0.2125	0.2492	0.2774	0.3063	0.3274
150	0.1667	0.2099	0.2431	0.2722	0.2955	0.3152
Control	0.1455	0.1793	0.2080	0.2328	0.2548	0.2730
F-test	ns	ns	ns	ns	ns	ns
CV (%)	7.61	3.16	3.60	3.36	3.53	3.52
LSD	0.0154	0.0216	0.0264	0.0305	0.0336	0.0359
Time × conc.						
25 DAP50 ppm	0.1200c	0.1540	0.1828	0.2083	0.2298	0.2482
25 DAP100 ppm	0.1455abc	0.1803	0.2105	0.2358	0.2573	0.2743
25 DAP150 ppm	0.1713abc	0.2363	0.2900	0.3320	0.3692	0.3978
40 DAP50 ppm	0.1950a	0.2525	0.2920	0.3150	0.3515	0.3725
40 DAP100 ppm	0.1240bc	0.1538	0.1808	0.2065	0.2285	0.2480
40 DAP150 ppm	0.1780ab	0.2313	0.2748	0.3107	0.3388	0.3618
55 DAP50 ppm	0.1278bc	0.1623	0.1908	0.2163	0.2385	0.2588
55 DAP100 ppm	0.1860a	0.2375	0.2710	0.3023	0.3240	0.3410
55 DAP150 ppm	0.1862a	0.2300	0.2675	0.2983	0.3240	0.3458
Control	0.1455abc	0.1793	0.2080	0.2328	0.2548	0.2730
F-test	*	ns	ns	ns	ns	ns
CV (%)	3.70	6.24	8.01	9.38	9.70	9.89
LSD	0.0189	0.0265	0.0330	0.0392	0.0434	0.0467

Letter(s) within columns indicate least significant differences (LSD) at *p: 0.05, ns: Non significant

Table 10: Fresh weight, biomass and chlorophyll content of lettuce at harvesting stage

Factors	Fresh weight (g)	Biomass (%)	Chlorophyll (mg m^{-2})
Time (DAP)			
25	22.73	11.18	1002.79
40	21.65	9.77	1012.70
55	20.82	10.28	1045.65
Control	21.55	12.54	1004.36
F-test	ns	ns	ns
CV (%)	6.26	3.66	3.86
LSD	2.7837	1.2268	85.9571
Conc. (ppm)			
50	21.98	9.80	981.45
100	21.49	11.10	945.97
150	21.73	10.33	1133.72
Control	21.55	12.54	1004.36
F-test	ns	ns	ns
CV (%)	6.42	3.73	2.56
LSD	2.7965	1.2296	81.2498

Table 10: Continued

Factors	Fresh weight (g)	Biomass (%)	Chlorophyll (mg m^{-2})
Time × conc.			
25 DAP 50 ppm	27.18	9.89	959.48
25 DAP 100 ppm	18.44	9.68	973.90
25 DAP 150 ppm	20.33	9.84	1010.98
40 DAP 50 ppm	17.65	11.77	835.31
40 DAP 100 ppm	23.33	10.49	1008.72
40 DAP 150 ppm	23.48	11.04	993.89
55 DAP 50 ppm	23.37	11.88	1213.57
55 DAP 100 ppm	23.17	9.15	1055.49
55 DAP 150 ppm	18.66	9.96	1132.08
Control	21.55	12.54	1004.36
F-test	ns	ns	ns
CV (%)	7.01	5.06	3.85
LSD	4.0185	1.8624	121.50732

ns: Non significant

Table 11: Leaf colour of lettuce at harvesting stage

Factors	L*	a*	b*
Time (DAP)			
25	47.93	-9.86	27.96
40	47.92	-9.63	28.70
55	47.68	-9.72	28.43
Control	48.31	-9.11	28.49
F-test	ns	ns	ns
CV (%)	9.56	6.07	10.00
LSD	0.7240	0.2459	0.4486
Conc. (ppm)			
50	48.09	-9.56	28.36
100	47.41	-9.73	28.02
150	48.03	-9.93	28.72
Control	48.31	-9.11	28.49
F-test	ns	ns	ns
CV (%)	9.55	6.03	10.01
LSD	0.7227	0.2452	0.4489
Time × conc.			
25 DAP 50 ppm	49.46 ab	-9.77	29.19
25 DAP 100 ppm	47.72 abcd	-9.87	27.17
25 DAP 150 ppm	46.62 cd	-9.94	27.53
40 DAP 50 ppm	49.06 abc	-9.62	28.28
40 DAP 100 ppm	47.51 bcd	-9.42	28.18
40 DAP 150 ppm	47.19 bcd	-9.87	29.64
55 DAP 50 ppm	45.74 d	-9.30	27.60
55 DAP 100 ppm	46.99 bcd	-9.89	28.71
55 DAP 150 ppm	50.29 a	-9.99	28.99
Control	48.31 abcd	-9.11	28.49
F-test	*	ns	ns
CV (%)	9.30	6.19	9.85
LSD	0.9956	0.3502	0.6248

Letter(s) within columns indicate least significant differences (LSD) at *p: 0.05, ns: Non significant

Chlorophyll Content

The content of chlorophyll analyzed from lettuce leaf were also similar for all treatments (Table 10).

Leaf Colour

Changes in the leaf colour of the tested lettuce were monitored by measuring L*, a* and b* at the harvesting stage. The results showed that no significant different in leaf colour, measured by monitoring in terms of a* and b* values, were observed, except L^* value . From Table 11, plants treated with 150 ppm of dolomite at 55 DAP showed the highest L* value of 50.29.

Phenolic and Quinone Content

At harvesting, the total phenolics and quinone contents of the leaf extracts were shown in Table 12. The results revealed that total phenolics and quinone content in lettuce leaves from both plants-treated with dolomite showed the same trend as control plants, ranging from 2664.31-4284.83 mg per 100 g and 0.0289-0.0433 per g FW, respectively (Table 12).

TSS, TA, pH and Ascorbic Acid

In a comparison of the internal qualities of lettuces at the harvesting stage, data indicated that dolomite application had no significant effect on the internal characteristics of lettuce, including TSS, TA, pH and ascorbic acid content. The mean values of TSS, TA, pH and ascorbic acid content of all treatments ranged from 0.20 -0.35 degree Brix, 0.0280-0.0640%, 6.35-6.70 and 13.81-14.14 mg ascorbic acid/100 mL juice, respectively (Table 13).

Table 12: The content of phenolic and quinone in lettuce at harvesting stage

Factors	Phenolic (mg/100 g)	Quinone (mg FW)
Time (DAP)		
25	3682.96	0.0375
40	3352.36	0.0368
55	2955.83	0.0334
Control	2880.48	0.0289
F-test	ns	ns
CV (%)	3.75	8.41
LSD	380.3906	0.0037
Conc. (ppm)		
50	3816.24	0.0382
100	2863.05	0.0361
150	3311.86	0.0334
Control	2880.48	0.0289
F-test	ns	ns
CV (%)	3.84	8.41
LSD	369.8545	0.0037
Time × conc.		
25 DAP 50 ppm	4154.85	0.0433
25 DAP 100 ppm	4284.83	0.0377
25 DAP 150 ppm	3009.04	0.0337
40 DAP 50 ppm	3098.05	0.0384
40 DAP 100 ppm	2826.79	0.0357
40 DAP 150 ppm	2664.31	0.0341
55 DAP 50 ppm	3795.99	0.0308
55 DAP 100 ppm	2945.47	0.0371
55 DAP 150 ppm	3194.12	0.0325
Control	2880.48	0.0289
F-test	ns	ns
CV (%)	3.18	8.41
LSD	528.6691	0.0055

ns: Non significant

Table 13: Total soluble solids (TSS), titratable acidity (TA), pH and ascorbic acid of lettuce at harvesting stage

Factors	TSS (°Brix)	TA (%)	pH	Ascorbic acid (mg ascorbic acid/100 mL juice)
Time (DAP)				
25	0.27	0.0480	6.41	13.99
40	0.28	0.0427	6.35	13.94
55	0.29	0.0413	6.58	13.99

Table 13: Continued

Factors	TSS (°Brix)	TA (%)	pH	Ascorbic acid (mg ascorbic acid/100 mL juice)
Control	0.20	0.0400	6.53	13.98
F-test	ns	ns	ns	ns
CV (%)	8.19	5.29	5.22	1.47
LSD	0.0272	0.0081	0.1191	0.0727
Conc. (ppm)				
50	0.30	0.0560	6.53	13.94
100	0.26	0.0413	6.41	14.00
150	0.28	0.0347	6.41	13.98
Control	0.20	0.0400	6.53	13.98
F-test	ns	ns	ns	ns
CV (%)	7.71	5.29	5.37	1.47
LSD	0.0267	0.0076	0.1226	0.0726
Time × conc.				
25 DAP 50 ppm	0.28	0.0640	6.53	13.82
25 DAP 100 ppm	0.35	0.0560	6.35	14.02
25 DAP 150 ppm	0.28	0.0480	6.70	13.97
40 DAP 50 ppm	0.25	0.0400	6.35	14.00
40 DAP 100 ppm	0.28	0.0440	6.35	13.98
40 DAP 150 ppm	0.25	0.0400	6.53	14.02
55 DAP 50 ppm	0.28	0.0400	6.35	14.14
55 DAP 100 ppm	0.23	0.0280	6.35	13.81
55 DAP 150 ppm	0.35	0.0360	6.53	13.98
Control	0.20	0.0400	6.53	13.98
F-test	ns	ns	ns	ns
CV (%)	6.72	5.29	5.60	1.44
LSD	0.0362	0.0114	0.1807	0.1002

ns: Non significant

DISCUSSION

The effect of soil dressing of dolomite on the growth, PPO activity and internal quality of grand rapids lettuce was studied. For growth, the results revealed that dolomite application had no remarkable impact on the growth characteristics of the tested lettuce in terms of stem diameter and plant height. However, there was an apparently greater beneficial effect of dolomite on leaf size. Plants treated with 150 ppm dolomite applied at 25 DAP improved the maximal leaf expansion, both width and length. These benefits may possibly be attributed to the fact that the application of the substance was at 25 DAP, in line with the early growth stage of the plants and owing to the fact that the rapidly-growing tissue had high transpiration and could continuously absorb the plant nutrition, especially the calcium and magnesium available from the soil applied with dolomite (De Mello Prado *et al*., 2005). These effects increase the growth of the root system of plants (De Mello Prado *et al*., 2005), with consequent dramatic increase in leaf development. In addition, there is a positive relationship between calcium mobilization via the xylem of the plant and transpiration (White and Brodley, 2003). This forces an improvement of the younger plants ability to absorb the available fertilizer from the dolomite into the xylem better than when the substance is applied to the older plants. Thus, the recommendation for rates of dolomite for activating the leaf expansion in lettuce cv. Grand Rapids should be 150 ppm dolomite at 25 DAP. While De Mello Prado *et al*. (2005) found that calcium application to guava trees cv. Paluma, did not affect fruit growth.

On the other hand, the bush size of plants treated with dolomite, regardless of any application time (25, 40 and 55 DAP), was significantly reduced when compared to the control plants. These results are not in accordance with a report from Fageria *et al*. (2002) that cited

that plant growth of several vegetables can benefit from increased calcium availability in soil. Unfortunately, there was no data available regarding the actual amount of dolomite needed for lettuce crops. In addition, Oyinlola (2007) reported that the amount of dolomite required for crop growth depends on the type of crop, phase of development and efficiency of nutrient uptake (Berry, 2006). In addition, Fageria *et al.* (2002) cited that plant factors such as root and root hair morphology and plant demand have also profound influences on plant ability to absorb and utilize micronutrients from soil.

For the level of browning incidence, browning reactions have generally been assumed to be a direct consequence of PPO action on polyphenols (Martinez and Whitaker, 1995). The results showed that dolomite application did not show any response to control the leaf browning appearance in lettuce, excepted for plants treated with 150 ppm dolomite at 25 DAP gave the lowest score of browning incidence at the early growth stage of 33 DAP. These observations are consistent with the results of other reports that show browning incidence is related to calcium deficiency in rapidly growing tissues. This has been attributed to an increased demand for calcium at times of vigorous growth (Thibodeau and Minotti, 1969; Collier and Tibbitts, 1982). While Berry (2006) reported that the lettuce plant, at final growth, is often limited by the internal translocation rate of calcium supply in the soil. Therefore, lettuce is considered as a sensitive plant to this disorder, especially at older stages (Nhien, 1989). These observations indicated that dolomite application to plants at younger stages could increase resistance to browning disorder. However, the development of browning incidence in lettuce always increases with advancing growth rate (Saure, 1998). Unfortunately, no document has been located that reports application time and concentration rate of dolomite for controlling the browning appearance in lettuce.

For PPO activities, browning reactions have generally been assumed to be a direct consequence of PPO action on polyphenols (Martinez and Whitaker, 1995). In intact tissues, PPO is separated from its substrates due to membrane compartmentation in the normal cells (Mayer, 1987). Upon the loss of membrane integrity, the contact of the enzyme and its substrates initiates the browning reaction (Huang *et al.*, 2005). In this work, it was observed that plants treated with 150 ppm dolomite at 25 DAP dramatically reduced the PPO activity in lettuce plants only at young stage of 33 DAP. This may be attributed to the efficiency of dolomite application to lettuce depended on plant age (Holtschulze, 2005). While Huang *et al.* (2008) found that transportation of calcium absorbed by the root of plant to above ground organs is driven by transpiration (White and Broadley, 2003; Saure, 2005). In such a case, calcium uptake by lettuce is depended upon metabolic activity of the plant and is subjected to the plant's age. Huang *et al.* (2008) also found that calcium was preferentially distributed to and accumulated at the expense of the calcium balance in young shoot tissue (Ho and Adams, 1994). In addition, several authors suggest that membrane stability is potentially a major factor controlling the rate of browning. Calcium in dolomite plays an important role involving processes that preserve the structural and functional integrity of the plant membrane by stabilizing cell wall structures, leading to strengthening of plant tissues and reduction of PPO activity (Rengal, 1992). While De Mello Prado *et al.* (2005) found that since calcium is an element that is immobile in the plant, it should be applied to the plant during the early stage of growth, owing to the fact that calcium uptake by the root is rapid and linear in the early developmental stages thereby enhancing stabilization of the cell wall and membrane integrity. These characteristics decline distinctly, continuing until harvest (Hanson *et al.*, 1991). In addition, Altunkaya and Gökmen (2008) reported that it may be possible that the susceptibility to browning in lettuce increased as plants progressed to the mature stage. This is in accordance with Rowse (1974) who observed that during the last two

weeks before plant maturity, root death of lettuce begins. The death of the root may cause a failure of the plant to uptake nutrition from the dolomite applications to the soil. These results indicated that plant ages have a strong influence on the incidence of leaf browning in lettuce plant (Jiang *et al*., 2004). Thus, dolomite application to lettuce plant at early growth stage should be effective for controlling PPO activity. While late dolomite application to older plants found that the control of PPO activity was lost. At present, the underlying biochemical factors associated with an enzymatic browning disorder of lettuce focused on dolomite application to modulate PPO enzyme activities are poorly understood. Work is in progress for further investigation of the dolomite application to lettuce plant at younger stages to relieve the browning incidence in lettuce.

With regards to the effect of dolomite on fresh weight and biomass, the results showed that both parameters were not influenced by the dolomite treatments, regardless of the time of application or concentration rates. These results indicated that pre-harvest application of dolomite had no remarkable effect on crop yield. These observations are consistent with the results of De Mello Prado *et al*. (2005) who cited that liming did not affect the physical characteristics of the guava fruits such as fruit weight.

For chlorophyll content, the results found that dolomite application at different application times and concentration rates did not show any remarkable differences in the chlorophyll content of lettuce leaves. This may be attributed to leaf tissues among the treatments had the appropriate pH of around 7.0 at harvesting, which can activate chlorophyll degrading enzymes (McFeeters *et al*., 1971; Suzuki *et al*., 2002; Arkus *et al*., 2005) and hence the chlorophyll content of lettuce leaf is decreased (Heaton and Marangoni, 1996). However, these observations are not consistent with the results of Chutichudet *et al*. (2009) who revealed that chlorophyll content in the lettuce leaves was increased significantly by application of gypsum treatment to the soil. While De Mello Prado *et al*. (2005) showed the influence of liming treatments are practical and effective in delaying the chlorophyll pigments on the rind of guava fruit cv. Paluma.

For leaf colour measuring as L*, a* and b* values, the results presented in Table 11 show the influence of dolomite application on the colour of the lettuce leaves, with the plant fertilized with 150 ppm dolomite at 55 DAP presenting the greatest lightness (L* value) of the leaf colour at harvest. These results indicate the dolomite treatments are the most effective in maintaining the brightness of leaf colour. While the colour parameters of a* and b* were unaffected by the application of dolomite.

For the results on the internal qualities, including phenolic, quinone, TSS, TA, pH and ascorbic acid in lettuce at harvesting stage, it was found that all of these chemical parameters in the lettuce leaves were not affected by dolomite application. Therefore, it may be reasonably interpreted that dolomite application had no effect in altering the internal characteristics. These results were similar with the previous researches of De Mello Prado *et al*. (2005) whom reported that liming had no significant affect on some chemical characteristics of guava fruit. A similar result was also obtained by Chutichudet *et al*. (2009) with 'Grand Rapids' lettuce applied with different gypsum concentrations. They found no significant difference in the content of TSS, TA and pH in lettuce after applying gypsum, with the exception of ascorbic acid. They reported that lettuce-treated with gypsum showed lower ascorbic acid content than the control plants. Unfortunately, the lack of information about dolomite application related to chemical quality in lettuce production is scarcely documented. Furthermore, the internal qualities of lettuce are influenced by numerous factors (Cheynier *et al*., 1998; Liu *et al*., 2007).

In conclusion, different dolomite application times of 25, 40 and 55 DAP with four concentration rates of 0, 50, 100 and 150 ppm applied as soil dressing to lettuce cv. Grand Rapids did not produce any significant different effects on plant growth in terms of stem diameter and plant height, except for leaf and bush size. The application of dolomite at 150 ppm at 25 DAP gave the largest leaf size. Treating with dolomite, regardless of application times, had the effect of reducing the bush diameter. In addition, plants treated with 150 ppm dolomite at 25 DAP had the lowest PPO activity at 33 DAP. Lettuce-treated with 150 ppm dolomite at 65 DAP had the highest L* value. Furthermore, treating with dolomite had no effect to improve the following characteristics of browning incidence, fresh weight, biomass, chlorophyll content, the content of phenolic, quinone, TSS, TA, pH and ascorbic acid in lettuce at harvest.

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