

Research Article

Phytochemical characterization of organically grown lettuce varieties for antioxidant status

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Abstract

Phytochemical properties of two varieties of Lettuce grand rapid and ice-berg were studied in this experiment. The Complete Randomized Design was followed in the laboratory of Horticulture Department, PMAS Arid Agriculture University Rawalpindi. Highest activity of the superoxide dismutase (SOD) enzyme (159.64 U g⁻¹ protein) was observed in V₂. Minimum activity of SOD enzyme (141.14 U g⁻¹ protein) was calculated in V₁, lettuce grown in T₀ (Control) treatment. Highest activity of the Poly Phenol Oxidase enzyme (32.59 U g⁻¹ protein) was observed in V₂ grown in T₀ Treatment (Control), Minimum activity of PPO enzyme (28.14 U g⁻¹ protein) was calculated in V₁ lettuce grown in T₃ (Peat moss+ chicken+ compost) treatment. Highest activity of the Peroxidase enzyme (28.43 U g⁻¹ protein) was observed in V₂ grown in T₃ Treatment (Peat moss+ chicken+ compost). Minimum activity of POD enzyme (27.08 U g⁻¹ protein) was calculated in V₁ lettuce grown in T₀ (Control) treatment. Highest activity of the CAT enzyme (24.17 U g⁻¹ protein) was observed in V₂ grown in T₃ Treatment (Peat moss+ chicken+ compost). Minimum activity of CAT enzyme (20.13 U g⁻¹ protein) was calculated in V₁ lettuce grown in T₀ (Control) treatment. All analytical attributes were highly significant among treatments as well as between two varieties of lettuce. From this study it was concluded that hybrid variety ice-berg is better than grand rapid phytochemically. In case of treatments, T₃ (Peat moss+ chicken+ compost) give best results.

Keywords: Antioxidant status; Lettuce; Organic fertilizer; Phytochemical characterization; Varieties

Introduction

Lettuce, scientifically known as *Lactuca sativa* L. is generally found in mixture of sandwiches and salad as well as avital

component in nutrition and diet of west countries. Lettuces were fresh vegetables consumed in the United States the 2nd largest at 28.0 pounds/capita in 2008, after 36.7

pounds of potatoes [1]. Lettuce belongs to the family of Asteraceae, the tribe of more than 100 species *LactucaCichoriaceae*, only three that are (*Lactucavivosa* L., *Lactucaserriola* L., and *Lactucasaligna* L.) can pass to the crossbar spleen by traditional breeding methods and thus represent the most essential breeding group. They are all diploids with $2n = 2x = 18$ chromosomes [2]. There are five most important types of lettuce plant which are romaine lettuce, crisphead lettuce also known as iceberg, butter head lettuce, stem lettuce and leaf lettuce in which Stem lettuce is large shaped and produced in China. In the year of 2010, up to 58% of the production was generated in the United States was head type lettuce, up to 29% was romaine lettuce and 13% was leaf types [3]. Lettuce also includes a broad collection of wild plant species and has caught the interest of growers, that's why become increasingly admired as a vegetable in groups of salads. In most urban areas the popularity of lettuce has also led a road to production increase as well as increase in consumption [4]. Lettuce is generally utilized as rich and raw source of iron, high calcium and vitamin A, also having high nutrient value [5]. In the United States it is reported as the most key vegetable crop produced for fresh market in terms of production, acreage and market value [6]. Lettuce exhibit many healthy and vigorous properties due to antioxidant compounds such as polyphenols and vitamin C [7]. It was also reported that lettuce salad utilization low down the total cholesterol level as well as improves the status of antioxidant due to antioxidants richness [8]. That's why; normal eating of lettuce leaves could also contribute in the treatment of cardiovascular diseases [9]. In one of a new report it was also studied that lettuce has therapeutic effect in mice's over carbon tetrachloride induced oxidative injury [10]. Lettuce production quantity of Pakistan was 278 tons in 2011, 395 tons in 2012 while 414 tons in 2013. Pakistan has a

world share in lettuce production and ranked at 85th in the lettuce production, whereas India produced 1,080,000 tons' lettuce in 2013 [11].

Grand Rapids lettuce is bright green, with wavy, frilled leaves, if left to mature it forms a head of loose leaves that reach 7 - 8" tall. Whereas ice-berg lettuce is a heading type but with a very thick, bulky and crunchy leaves with flabellate leaf venation, this lettuce is also mostly grown in the United States [12]. Not only in providing of nutrients, organic manure and compost also enhance root development, augments the level of soil in organic matter, increases the water retention and holding power of soil as well as also recover seeds germination rate [13]. The antioxidant influence is highly relies on different factors such as generation site, antioxidant concentration, unambiguous chemical reactivity near radicals, reactivity of radical, result product of antioxidant derived radical, stability degree and its interactive capability with some other antioxidants [14]. Some oxidative enzymes such as (Polyphenoloxidases (PPO), Superoxide dismutase (SOD), Catalases (CAT) and peroxidases) in organic fruit and vegetable products produce browning or cause some color changes [15].

Keeping in view the significance of lettuce in terms its importance and the present study was planned with the following objectives.

- i) To characterize organically grown different varieties of lettuce for Phytochemicals.
- ii) To evaluate total antioxidant levels in hybrid and local lettuce.

Materials and methods

The research trial was carried out in the field area of Horticulture Department, Pir Mehr Ali Shah-University of Arid Agriculture Rawalpindi in the year 2015-2016. Healthy and disease free seeds of two varieties of lettuce grand rapid and ice-berg (local and hybrid) were taken and sown in pots contains soil, silt, and farm yard manure in the ratio of

1:1:1 in month of October 2015 as shown in (Table 1). Soil was thoroughly prepared by adding well rotten farm yard manure one month before bed preparation. Land was prepared by ploughing the field twice followed by planking and seedlings were transplanted. After 40 days, healthy seedling

were transplanted on well prepared raised beds according to recommended spacing for both hybrid and local lettuce, Plant x Plant distance was 15-20 cm and Bed x Bed distance was 50 cm, All treatments were replicated three times with following treatments.

Table 1. List of four organic fertilizers with specific concentration and ratios

Treatments	Code	Concentrations	Ratio
T ₀	Control	Soil	
T ₁	Chicken manure + compost	5kg/bed	1:1
T ₂	Compost + peat moss	5kg/bed	1:1
T ₃	Peat moss+ chicken manure + compost	5kg/bed	1:1:1

The data on following traits was recorded at post-harvest

Super Oxide Dismutase (SOD)

Super oxide dismutase activity was analyzed according to its capability to inhibit and occupy photochemical reduction of nitrobluetetrazolium (NBT) according to the method of Stagner and Poporic [16]. Two sets of 5 cuvettes (3 milliliter) each of them were holding 0, 100, 200, 300 or 400 μ L of enzyme extract. Then 50 milli Molar KPO₄ buffer having pH 7.8, 2 micro Molar Riboflavin as a substrate, 0.1 micro Molar EDTA, 13 micro Molar methionine, and 75 micro Molar nitrobluetetrazolium to each reaction mixture were added carefully.

Cuvettes were then inverted several times to mix the reaction well. Then one set was kept in dark serving as a control while the other five reaction cuvettes set was located for 10 minutes under fluorescence. The SOD of fluorescence cuvettes against dark incubated cuvettes served a blank was read at 560 nm using spectrophotometer (Optima[®] 3000 plus). One unit of SOD activity is the enzyme quantity that holds back 50% photochemical reduction of nitro bluetetrazolium (NBT) under assay. The enzyme unit was expressed as U g⁻¹ protein.

Poly Phenol Oxidase (PPO)

Poly phenol oxidase activity in both varieties of lettuce were determined by using the method of Waite [17]. The test mixture for

poly phenol oxidase was consisting of newly prepared 1.45 milliliter of 100 millimeter potassium citrate buffer with 6.8 pH and 0.5 milliliter of 100 millimeter 4-methylcatechol (4mc). The absorbances were recorded at 412nm on ELISA plate reader using (Model ELX 800). Poly phenol oxidase (PPO) activity were find out as enzyme units (U mg protein) characterize as the enzyme quality required to produced 1 μ mol min⁻¹ products.

Peroxidase (POD)

Peroxidase activity in both varieties of lettuce were determined according to the method as mentioned by Hassan *et al.* [18] with few exceptions. The test mixture was consisting of NaKPO₄ with exact amount of 15 milli Molar buffer with pH 6.0 and enzyme extract with exact amount of 200 micro liters. Two substrates consisted of 1 milli Molar of 100 micro liters H₂O₂ and 0.1 milli Molar of 100 micro liters guaiacol was added which started the reaction. Peroxidase activity were than recorded as a change in the OD at 470 nm more than 3 minutes time and described as U g⁻¹ protein.

Catalase (CAT)

Catalase activity in both varieties of lettuce were assayed according to the technique as described by Abbasi *et al.* [19]. Two buffers solution were used for test reaction. One solution comprised of 50 milli Molar KPO₄

buffer with pH 7.0, while the other was consisting of 12.5 milli Molar H₂O₂ in 50 milli Molar KPO₄ buffer with pH 7.0. 300 micro liter extract of enzyme was then also added to both cuvettes and placed in darkness. The absorbance was then recorded at 240 nm at 45 sec and 60 sec by spectrophotometer (Optima® 3000 plus). The difference in OD (45 and 60 sec reading) was noted to workout CAT activity. One unit CAT activity was expressed as enzyme U g⁻¹ protein.

The data was subjected to Analysis of Variance (ANOVA) and least significant difference (LSD) test was used to compare differences between treatments at 5 % level of significance.

Results and discussion

In the present study two varieties of Lettuce, grand rapid and ice-berg (local and hybrid) were grown in four different treatments of organic fertilizers. The data of parameters was collected and statistically analyzed which are following,

Super Oxide Dismutase (SOD) Analysis

Activity of superoxide dismutase (SOD) was calculated significantly higher in all the treatments than control during current research. Highest activity of the enzyme (159.64 U g⁻¹ protein) was observed in V₂ (ice-berg lettuce) grown in T₃ Treatment (Peat moss + chicken + compost) followed by T₂ (Compost + peat moss) (158.01 U g⁻¹ protein) and T₁ (Chicken manure + compost) (155.47 U g⁻¹ protein) treatments. Minimum activity of SOD enzyme (141.14 U g⁻¹ protein) was calculated in V₁ (grand rapid) lettuce grown in T₀ (Control) treatment. Data regarding in the current study, depicted that highest activity of SOD enzyme was given by T₃ Treatment (154.95 U g⁻¹ protein) followed by T₂ (152.74 U g⁻¹ protein) and T₁ (149.84 U g⁻¹ protein) treatments. Least values of SOD (146.82 U g⁻¹ protein) were calculated in control as shown in (Table 2&Fig. 1) respectively.

The results of present study are in line with the findings of Hunter *et al.* [20], who reported that plants grown under organic agricultural conditions are reported to have higher antioxidant activity. A factorial experiment was also conducted by Arough *et al.* [21] which showed that both application of biofertilizer and organic fertilizers increases the activities of Catalase (CAT), Peroxidase (POD) Polyphenol Oxidase (PPO) enzymes. Presented results showed that treatments of organic fertilizers slightly affect the activity of superoxide dismutase. The SOD enzyme's activity is suggested to be highly controlled by gene expression within plants as well as the environmental and developmental stimuli [22].

Peat moss + chicken manure + compost are common soil amendments. When added to soils combine, each will improve them in different ways. These fertilizers in the soil hold nutrients by increasing what is called the CEC or "cation exchange capacity [23].

Poly Phenol Oxidase (PPO) Analysis

Activity of Poly Phenol Oxidase (PPO) was calculated in the current research. T₀ (Control) was highly significant than all other treatments in both varieties of lettuce i. e. grand rapid and ice-berg as shown in (Table 3&Fig. 2) respectively. Highest activity of the Poly Phenol Oxidase enzyme (32.59 U g⁻¹ protein) was observed in V₂ (ice-berg lettuce) grown in T₀ Treatment (Control) followed by T₁ (Chicken manure + compost) (31.23 U g⁻¹ protein) treatment. Minimum activity of PPO enzyme (28.14 U g⁻¹ protein) was calculated in V₁ (grand rapid) lettuce grown in T₃ (Peat moss+ chicken+ compost) treatment. Data regarding in the current study, depicted that highest activity of PPO enzyme was given by T₀ Treatment (30.96 U g⁻¹ protein) followed by T₁ (30.05 U g⁻¹ protein) and T₂ (29.36 U g⁻¹ protein) treatments. Least values of PPO (29.14 U g⁻¹ protein) were calculated in T₃ (Peat moss+ chicken+ compost) as shown in (Table

3&Fig. 2) respectively. In comparative study of two varieties, V2 (ice-berg) lettuce had maximum value of PPO (31.08 U g⁻¹ protein) as compared to V1 (grand rapid) lettuce (28.68 U g⁻¹ protein). This could be attributed to differences in the varietal characteristic of the two lettuce varieties.

The results of present study are in line with the findings of Arough *et al.* [21] who resulted that both application of biofertilizer and organic fertilizers highly increases the activity of Polyphenol Oxidase (PPO) enzymes as well as other antioxidant activities. PPO oxidizes phenolics in the presence of oxygen on the cut surface of fruits and vegetables, producing quinines, which produce to form brown-colored pigments [24]. Peatmoss + chicken manure + compost are common soil amendments. When added to soils combine, each will improve them in different ways. These fertilizers in the soil hold nutrients by increasing what is called the CEC or "cation exchange capacity [23].

Peroxidase (POD) analysis

Activity of Peroxidase (POD) show slight significant difference in all the treatments as well as in both varieties of lettuce i.e. grand rapid and ice-berg during this experiment as shown in (Table 4&Fig. 3) respectively. Highest activity of the Peroxidase enzyme (28.43 U g⁻¹ protein) was observed in V2 (ice-berg lettuce) grown in T₃ Treatment (Peat moss+ chicken+ compost) followed by T₂ (Compost + peat moss) (28.31 U g⁻¹ protein) treatment. Minimum activity of POD enzyme (27.08 U g⁻¹ protein) was calculated in V₁ (grand rapid) lettuce grown in T₀ (Control) treatment. Data regarding in the current study, depicted that highest activity of POD enzyme was given by T₃ Treatment (27.94 U g⁻¹ protein) followed by T₂ (27.81 U g⁻¹ protein). Least values of POD (27.57 U g⁻¹ protein) were calculated in T₀ (Control) as shown in (Table 1). In comparative study of two varieties, V2 (ice-berg) lettuce had

maximum value of POD (28.24 U g⁻¹ protein) as compared to V₁ (grand rapid) lettuce (27.26 U g⁻¹ protein). This could be attributed to differences in the varietal characteristic of the two lettuce varieties.

Findings of the present study are in line with previous researchers that organic fertilizers enhanced peroxidase activity as well as other antioxidant activities like PPO and SOD in several plants [21]. This increase is probably due to the availability of various major and minor elements in organic fertilizer contrary to mineral fertilizer which has only three major elements, nitrogen, phosphorus, and potassium.

Catalase (CAT) analysis

Activity of CAT enzyme was found significantly higher in all the treatments as well as in both varieties of lettuce i.e. grand rapid and ice-berg during this experiment as shown in Table 5&Fig. 4) respectively. Highest activity of the CAT enzyme (24.17 U g⁻¹ protein) was observed in V2 (ice-berg lettuce) grown in T₃ Treatment (Peat moss+ chicken+ compost) followed by T₂ (Compost + peat moss) (23.21 U g⁻¹ protein) treatment. Minimum activity of CAT enzyme (20.13 U g⁻¹ protein) was calculated in V₁ (grand rapid) lettuce grown in T₀ (Control) treatment. Data regarding in the current study, depicted that highest activity of CAT enzyme was given by T₃ Treatment (23.13 U g⁻¹ protein) followed by T₂ (22.22U g⁻¹ protein). Least values of CAT enzyme (21.18 U g⁻¹ proteins) were calculated in T₀ (Control) as shown in (Table 5&Fig. 4) respectively. In comparative study of two varieties, V2 (ice-berg) lettuce had maximum value of CAT enzyme (23.18 U g⁻¹ protein) as compared to V₁ (grand rapid) lettuce (21.14 U g⁻¹ protein).

This could be attributed to differences in the varietal characteristic of the two lettuce varieties. Present study concluded that different treatments of organic fertilizers also increase higher activities of catalase.

Findings of the present study are in line with previous researchers that organic fertilizers enhanced catalase activity as well as other antioxidant activities like PPO and SOD in several plants [21]. CAT protects cells against ROS because it catalyzes the decomposition of hydrogen peroxide to form oxygen and water [25]. Thus, the increase of CAT activity in treatments indicates that

hydrogen peroxide may be removed, and therefore, the formation of hydroxyl radicals and chilling-induced damage was mitigated. This increase is probably due to the availability of various major and minor elements in organic fertilizer contrary to mineral fertilizer which has only three major elements, nitrogen, phosphorus, and potassium.

Table 2. Super Oxide Dismutase activity (U g⁻¹ protein) in lettuce varieties grown with different manure treatments

Treatments	grand rapid	ice-berg	Mean
Control	141.14d	152.51c	146.82d
Chicken manure + compost	144.22d	155.47b	149.84c
Compost + peat moss	147.48d	158.01a	152.74c
Peat moss+ chicken manure + compost	150.27c	159.64a	154.95b
Mean	145.77d	156.40b	

Means in the same column or row followed by a common letter (s) are not significantly different at 5% level by LSD

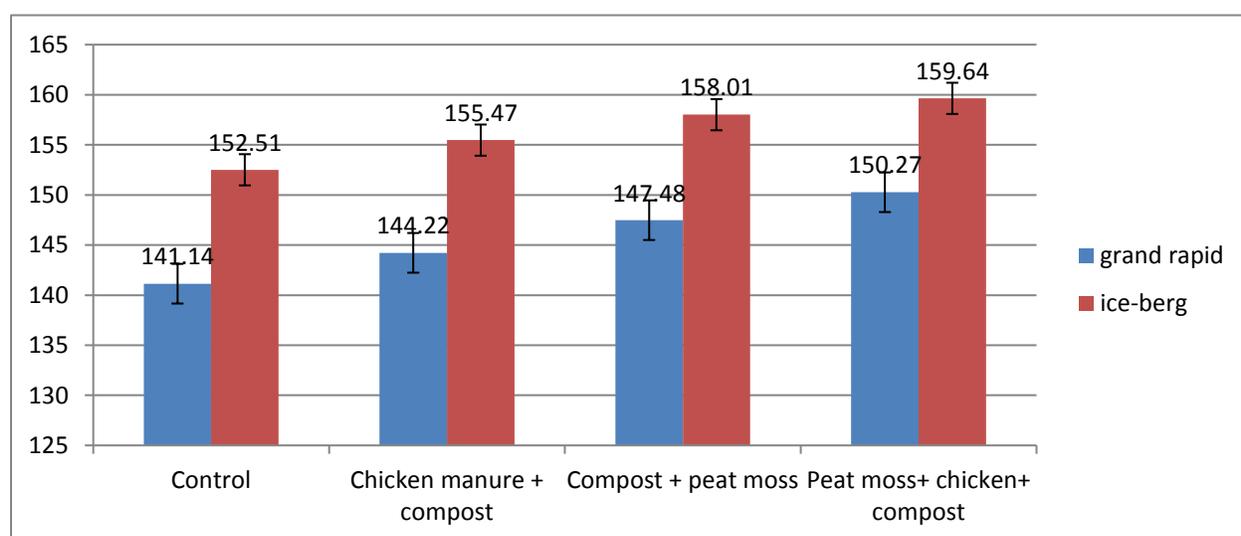


Figure 1. Super Oxide Dismutase activity (U g⁻¹ protein) in lettuce varieties grown with different manure treatments

Table 3. Polyphenol Oxidase (PPO) activity (U g⁻¹ protein) in lettuce varieties grown with different manure treatments

Treatments	grand rapid	ice-berg	Mean
Control	29.34b	32.59a	30.96b
Chicken manure + compost	28.88c	31.23a	30.05b
Compost + peat moss	28.36d	30.36b	29.36b
Peat moss+ chicken manure + compost	28.14c	30.14b	29.14c
Mean	28.68c	31.08a	

Means in the same column or row followed by a common letter (s) are not significantly different at 5% level by LSD

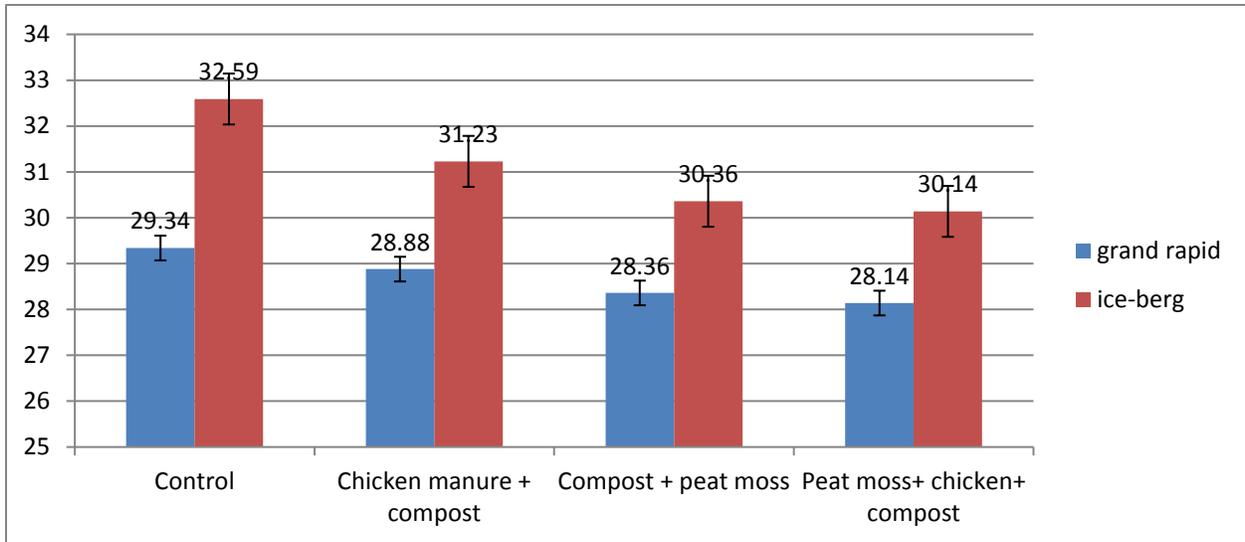


Figure 2. Polyphenol Oxidase (PPO) activity (U g-1 protein) in lettuce varieties grown with different manure treatments

Table 4. Peroxidase activity (U g-1 protein) in lettuce varieties grown with different manure treatments

Treatments	grand rapid	ice-berg	Mean
Control	27.08d	28.06b	27.57c
Chicken manure + compost	27.20d	28.19b	27.69c
Compost + peat moss	27.32c	28.31a	27.81c
Peat moss+ chicken manure + compost	27.45c	28.43a	27.94c
Mean	27.26d	28.24a	

Means in the same column or row followed by a common letter (s) are not significantly different at 5% level by LSD

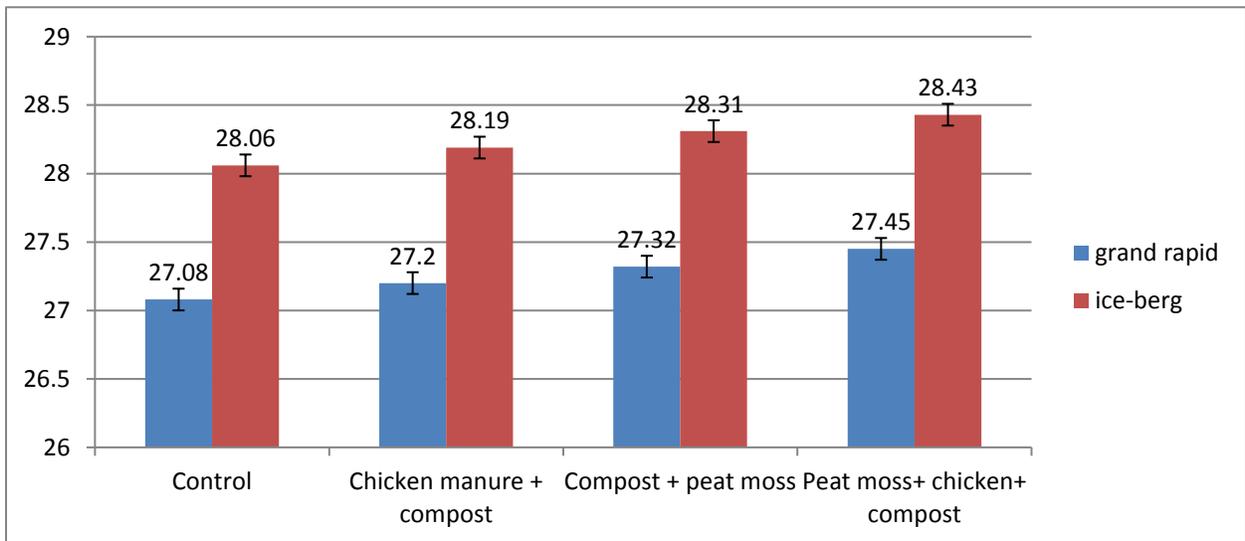
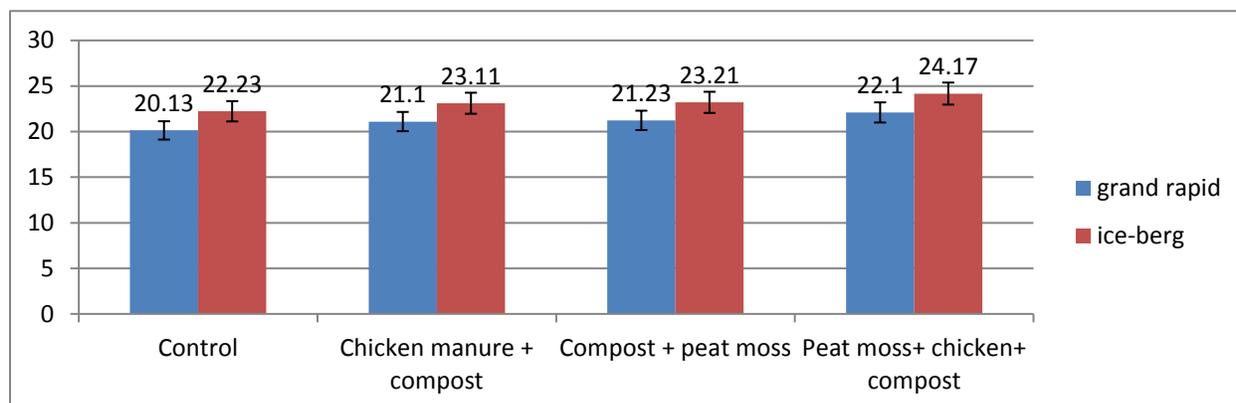


Figure 3. Peroxidase activity (U g-1 protein) in lettuce varieties grown with different manure treatments

Table 5. Catalase activity (U g⁻¹ protein) in lettuce varieties grown with different manure treatments

Treatments	grand rapid	ice-berg	Mean
Control	20.13d	22.23b	21.18c
Chicken manure + compost	21.10c	23.11a	22.10b
Compost + peat moss	21.23c	23.21a	22.22b
Peat moss+ chicken manure + compost	22.10b	24.17a	23.13a
Mean	21.14c	23.18a	

Means in the same column or row followed by a common letter (s) are not significantly different at 5% level by LSD

**Figure 4. Catalase activity (U g⁻¹ protein) in lettuce varieties grown with different manure treatments**

Conclusion

All the analytical attributes were significant among treatments as well as between varieties of lettuce. From this study it was concluded that hybrid Variety ice-berg was much better than grand rapid lettuce as phytochemically. In case of treatments, T₃ (Peat moss+ chicken+ compost) give best results as compared to other manure treatments.

Author's contributions

Conceived and designed the experiments: N Sajjad & FM Bangulzai, Performed the experiments: N Sajjad & FM Bangulzai, Analyzed the data: N Ahmed, Contributed reagents/materials/analysis tools: Z Bibi, M Ashraf & RA Baloch, Wrote the paper: FM Bangulzai & N Sajjad.

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