Net Journal of Agricultural Science
Vol. 2(4), pp. 124-130, November 2014
ISSN: 2315-9766
Full Length Research Paper

The effect of mulch and fertilizers on broccoli (*Brassica oleracea* L. *Var. Italica*) oxidants and antioxidants

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Accepted 21 October, 2014

ABSTRACT

Factorial field experiments was conducted during 2012/2013 growth season to study the effect of soil mulch (mulch and without mulch) and some fertilizers (control, sulfur, organic and high potash fertilizer) on H_2O_2 content and the activity of catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH) oxidative enzymes of broccoli leaves and flowers. The results showed that using mulch, fertilizer treatment and the interaction between mulch and high potash treatment led to a significant increase in CAT and SOD activity. Furthermore, high potash and sulfur supplementation caused a significant increase in broccoli antioxidants both in leaves and flowers respectively. We conclude that mulch and high potash treatment led to increase plant tolerance to salt stress by increasing antioxidant mechanisms.

Keywords: Mulch, broccoli, oxidants, antioxidants.

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INTRODUCTION

Vegetables are a great source of antioxidants, because almost all contain one or more of the following: vitamin C, vitamin E, selenium and beta-carotene, all hailed for having high antioxidant properties. Although vegetables are the best sources of antioxidants, the problem with them is that they are produced by the use of excessive chemical herbicides, pesticides, and different types of fertilizers. Studies on vegetables revealed that those organically grown have high antioxidants concentration than those that were produced inorganically. Taie et al. (2010) confirmed that application of organic fertilizer using different compost levels with biofertilizer to soybean plants resulted to the highest values of total phenolics and flavonoids as compared to chemically grown counterpart. Organic fertilization is apparently becoming increasingly important particularly in terms of health benefits. A number of research studies showed that organic farming ensures better yields at low cost and fetches more income (Mitchell et al., 2007). Few studies have been conducted on the evaluation of the influence of the application of the various soil organic amendments on the antioxidant components in broccoli.

Broccoli (*Brassica oleracea var* Italica) is a nutritionally important crop grown all over the world and it is a floral vegetable with an important nutritional value due to its content of vitamins, antioxidants, glucosinolates and anti-carcinogenic compounds (Parente et al., 2013; Lemoine et al., 2010; Lemoine et al., 2009; Chuanphongpanich et al., 2006).

Plant nutrients in fertilizers are classified as major nutrients and micronutrients. The most important major nutrients are nitrogen (N), phosphorus (P) and potassium (K). Plants require these nutrients in relatively large amounts, and these are the nutrients most likely to be deficient for plant growth. The other major nutrients, also called secondary nutrients, are calcium (Ca), magnesium (Mg) and sulfur (S) (Vitosh et al., 1994). So, foliar feeding has been used as a means of supplying supplemental doses of minor and major nutrients, plant hormones, stimulants, and other beneficial substances. Observed effects of foliar fertilization have included yield increases, resistance to diseases and insect pests, improved drought tolerance, and enhanced crop quality. Plant response is dependent on species, fertilizer form, concentration, and frequency of application, as well as the stage of plant growth (Kuepper, 2003).

Potassium (K) is an essential nutrient that affects most of the biochemical and physiological processes that influence plant growth and metabolism (Wang et al., 2013). The importance of K fertilizer for the formation of crop production and its quality is known. As a consequence, potash consumption has increased dramatically in most regions of the world (Pettigrew, 2008). K plays essential roles in enzyme activation, protein synthesis, photosynthesis, osmoregulation, stomatal movement, energy transfer, phloem transport, cation-anion balance and stress resistance (Marschner, 2012). So it is vital to many plant processes. While it does not become a part of the chemical structure of plants, but it plays many important regulatory roles in development for example enzyme activation which serves as catalysts for chemical reactions, being utilized but not consumed in the process. They bring together other molecules in such a way that the chemical reaction can take place. Potassium "activates" at least 60 different enzymes involved in plant growth. The amount of K present in the cell determines how many of the enzymes can be activated and the rates at which chemical reactions can proceed. Thus, the rate of a given reaction is controlled by the rate at which K enters the cell (Potash and Phosphate Institute, 1998).

The availability of potassium to the plant is highly variable, due to complex soil dynamics, which are strongly influenced by root–soil interactions. The molecules that signal low K status in plants include reactive oxygen species and phytohormones, such as auxin, ethylene and jasmonic acid potassium deprivation triggers developmental responses in roots. All these acclimation strategies enable plants to survive and compete for nutrients in a dynamic environment with a variable availability of potassium (Ashley et al., 2006). Increasing evidence suggests that improvement of potassium (K)-nutritional status of plants can greatly lower the Reactive Oxygen Species (ROS) production by reducing activity of NAD(P)H oxidases and maintaining photosynthetic electron transport causing enhanced activity of enzymes involved in detoxification of H_2O_2 and utilization of H_2O_2 in oxidative process (Cakmak, 2005).

Inorganic nitrogen fertilization plays an essential role in increasing broccoli yield and quality (Yildirim et al., 2007). And, it is found that organic tomatoes presented higher content of antioxidants (Borguini et al., 2013).

Sulfur is a constituent of certain amino acids (cystine, cysteine, glutathione and methionine) and the proteins that contain these amino acids. It is found in vitamins, enzymes and co-enzymes. Plants take up sulfur primarily as sulfate ions (SO_4^-). In the plant, it is reduced and assembled into organic compounds. Sulfur is present in glycosides, which give the characteristic odors and flavors to mustard, onion and garlic plants. It is also required for nodulation and nitrogen fixation of legumes. As the sulfate ion, it may be responsible for activating some enzymes (Vitosh et al., 1994). The protective effects of sulfur compounds are commonly attributed to radical scavenging and enzymatic decomposition of oxygen metabolites (Battin and Brumaghim, 2009).

Mulching and fertilization influence the water and nutrient supply to the plant and can affect the nutritional composition of the harvested fruit. Mulching consistently resulted in higher flavonoids, anthocyanin and total phenolic content compared to the traditional matted row culture system (Wang, 2006). In addition, plastic mulch increased minimum temperature of soil, accelerated plant height, early growth, early yield, and bring satisfactory weed control without any application of herbicides (Najafabadi et al., 2012). Mulching system also caused an increase in fruit soluble solids content, total sugar, fructose, glucose, ascorbic acid, and citric acid contents, flavonoid contents and antioxidant capacities (Wang et al., 2002).

MATERIALS AND METHODS

Field experiment was conducted in Babylon province, Agriculture college field during the growing season 2012–2013, to study the effect of soil mulch (with black polyethylene) and its interactions with fertilizers treatment to elevate the injury of soil salt stress of broccoli. The experimental soil was sandy loam with pH 7.8 and EC 8.1 dS/m^{-1}. Broccoli seeds were germinated at 1/10/2012, after 35 days seedlings were planted on ridges 75 cm apart and 30 cm between plants. DAP (di-ammonium phosphate at the rate of 200 kg/ha was added as soil dressing down the plant line 10 cm.

Factorial experiment within (R.C.B.D.) was adopted. Two levels of mulch with black polyethylene (mulching and without mulching) were added. Four levels of treatments with: 1- control (without treatments), 2- sulfur at the rate (100 k/ha), 3- foliar treatment with organic fertilizer (polyexal) as poly hydro carboxylic was applied, that is 2 mg/L at 4 and 6 leaf stage, and 4- high potash complete fertilizer (13-10-15, + TE) was applied, that is 3 mg/L as foliar application at 4 and 6 leaf stage. Each treatment was with 3 replicates. The data represented in tables and figures were the average of three replicates. The experimental unit included 3 ridges (3 m long). At flowering stage: leaf area was calculated, chlorophyll content was measured by chlorophyll meter (spad).

Enzymatic assays

To determine catalase activity and superoxide dismutase activity, 0.1 g of frozen leaves and roots were homogenized in 1 ml of phosphate buffer 0.5 M, pH 7 and 0.03 g of poly venial pyrollidone (PVP) with a warring blender. The homogenate were centrifuged at 6000 rpm for 10 min at 4°C. The clear supernatant was used for measurement of enzyme activities with three replicates done for each assay.

SOD activity was assayed according to Marclund and Marclund (1974). This method monitored the ability of SOD to inhibit photochemical reduction of pyrogallol at 420 nm.

One unit of SOD activity was defined as the amount of enzyme that caused 50% inhibition of pyrogallol reduction per min.

The activity of CAT was assayed using the method of Whitaker et al. (2003) for chemicals preparation and Aebi (1984) for CAT assay.

The equations used for activity determination were:

$$\text{SOD activity (units)} = \frac{\%\,inhibition/50\% \times reaction volume}{total\,test\ period}$$

$$\text{CAT activity (units)} = \frac{\Delta\,Abs/\min \times reaction\,volume}{0.001}$$

Table 1. Effect the interactions on oxidants and antioxidants in leaves.

Parameters	Treatments	H_2O_2 (%)	SOD (unit)	CAT (unit)	GSH (µg/g F.W)
Without mulch	Without treatment (control)	4.3345	2.4051	53.1429	39.0278
	Sulfur	7.4319	2.3143	118.400	50.0000
	High potash	5.5230	2.6796	68.6118	49.4281
	Organic fertilizer	6.0091	2.6798	43.2000	49.8611
With mulch	Without treatment (control)	4.0901	2.7079	37.9200	74.7222
	Sulfur	6.4519	2.6343	110.400	55.0000
	High potash	3.7725	3.1228	68.8000	5.0926
	Organic fertilizer	4.5922	3.1382	55.2000	9.1667
LSD $_{(0.05)}$		1.488	0.27	10.48	7.2

Glutathione content (GSH) was determined by the procedure of Owens and Belcher (1965) with slight modifications. Freshly collected sample was ground in pestle and mortar and then freed from proteins by mixing with an equal volume of 3% (w/v) metaphosphoric acid and 30% (w/v) NaCl solution. Deproteinized sample was centrifuged at 3,500 g for 10 min at 4°C. This spectrophotometric procedure measures the change in absorbance at 412 nm occurring when glutathione reduces 5,5-dithio-bis-(2-nitrobenzoic acid).

The hydrogen peroxide content in the leaves was determined according to the method of (Velikova et al., 2000). Evaluation, samples (100 mg) were extracted with 5.0 ml of TCA (0.1%, w v-1) in an ice bath, and the homogenate was centrifuged at 12.000 g for 15 min then 0.5 ml of phosphate buffer (pH 7.0) and 1.0 ml of potassium iodide (1 M) were added to 0.5 ml of the supernatant and the absorbance of the mixture was measured at 390 nm.

Absorbance values were calibrated to a standard curve generated with known concentrations of H_2O_2.

Means of treatments were compared by using Least Significant Difference test (LSD) at 0.05 probability level (Steel and Torrie, 1980).

RESULTS

Table 1 demonstrated that the plants grown with mulch showed a significant increase in SOD activity and GSH content of the leaves (2.7079 units and 74.7222 µg/g F.W) compared with no mulching plants (2.4051 units and 39.0278 µg/g F.W) respectively. In addition, we observed that mulching plants showed no significant effect in H_2O_2 content (4.0901%) compared with untreated plants (4.3345%). But, sulfur, high potash and organic fertilizer supplementation cause a significant increase of antioxidants both in mulching and no mulching plants.

Table 2 showed the interaction between oxidants and antioxidants in the flowers of broccoli plants. It demonstrated that mulching plants showed an increase in SOD activity and GSH content (3.5947 units and 16.3333 µg/g F.W) compared with no mulching plants (2.7754 units and 2.6984 µg/g. F.W) respectively. Both sulfur and high potash had no significant effect in H_2O_2 content of no mulching plants compared with control plants (3.3242%). But, mulching plants supplemented with high potash and organic fertilizer caused an increase of H_2O_2 content compared with control plants (2.7809%). SOD and CAT increased significantly of no mulching plants supplemented with high potash and organic fertilizer compared with control plants (2.7754 and 58.6286 units, respectively). Whereas, GSH increased significantly in mulching plants supplemented with organic fertilizer (29.8611 µg/g F.W) compared with control plants (16.3333 µg/g F.W).

Figure 1 showed that plant mulching and sulfur supplementation increased H_2O_2 content of broccoli leaves, while there were no significant effect in H_2O_2 content in plants treated with high potash and organic fertilizer.

In the flowers, mulching plants and plants treated with sulfur (Figure 2) showed no significant effect in H_2O_2 content compared with control plants.

Figure 3 showed that mulching plants caused a decrease in SOD activity in broccoli leaves. In another side, plants supplemented with high potash and organic fertilizer showed a significant increase in enzyme activity.

An opposite effect was observed in broccoli flowers (Figure 4). That is, there were a significant increase in SOD activity of mulching plants and a significant decrease in its activity in plants treated with high potash.

GSH content (Figure 5) increased significantly in plants covered with mulch, while there were a significant decrease in GSH content in plants treated with high potash and sulfur.

Figure 6 showed a significant decrease in GSH content of the flowers of the plants covered with mulch, while sulfur and organic fertilizer supplementation caused a significant increase in GSH content.

Figure 7 demonstrated no significant differences in CAT activity between mulching and no mulching plants, while sulfur and high potash caused significant increase in CAT activity.

Figure 8 showed a significant increase in CAT activity

Table 2. Effect the interactions on oxidants and antioxidants in flowers.

Parameters	Treatments	H_2O_2 (%)	SOD (unit)	CAT (unit)	GSH (µg/g F.W)
Without mulch	Without treatment (control)	3.3242	2.7754	58.6286	2.6984
	Sulfur	3.9515	2.6374	43.7333	25.7716
	High potash	4.5095	3.6711	92.6118	5.5556
	Organic fertilizer	5.1058	4.3260	98.6667	5.5556
With mulch	Without treatment (control)	2.7809	3.5947	64.3200	16.3333
	Sulfur	2.6513	3.1877	49.7684	18.8889
	High potash	4.6485	1.5395	78.4000	12.2222
	Organic fertilizer	9.5580	1.6316	48.0000	29.8611
LSD $_{(0.05}$		1.78	0.5	10.36	7.5

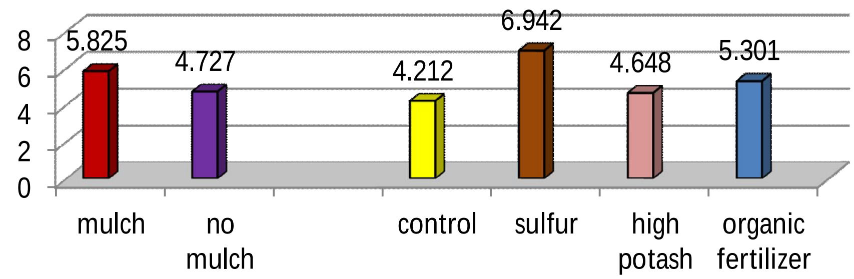

Figure 1. Effect of much and fertilizers on H_2O_2 content (%) in leaves. LSD for mulch$_{(0.05)}$ = 0.7, LSD for treat$_{(0.05)}$ = 1.1.

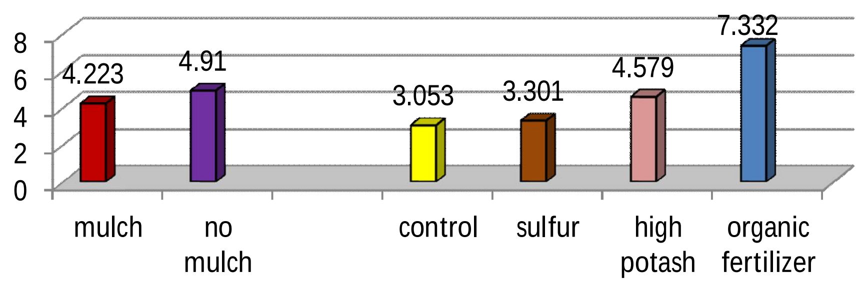

Figure 2. Effect of much and fertilizers on H_2O_2 content (%) in flowers. LSD for mulch$_{(0.05)}$ = 0.8, LSD for treat$_{(0.05)}$ = 1.2.

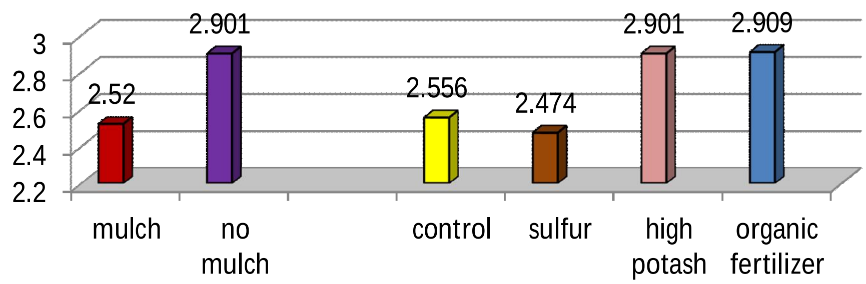

Figure 3. Effect of much and fertilizers on SOD activity (U) in leaves. LSD for mulch$_{(0.05)}$ = 0.1, LSD for treat$_{(0.05)}$ = 0.2.

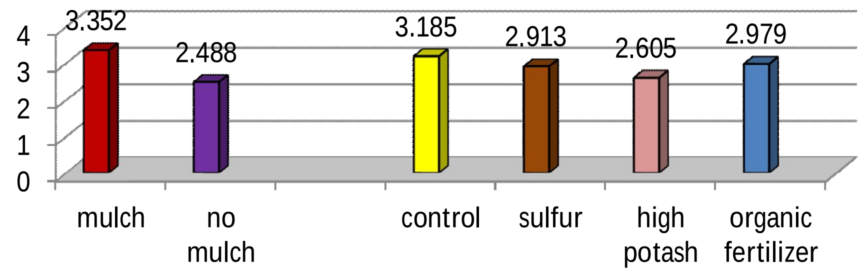

Figure 4. Effect of much and fertilizers on SOD activity (U) in flowers. LSD for mulch$_{(0.05)}$ = 0.2, LSD for treat$_{(0.05)}$ = 0.3.

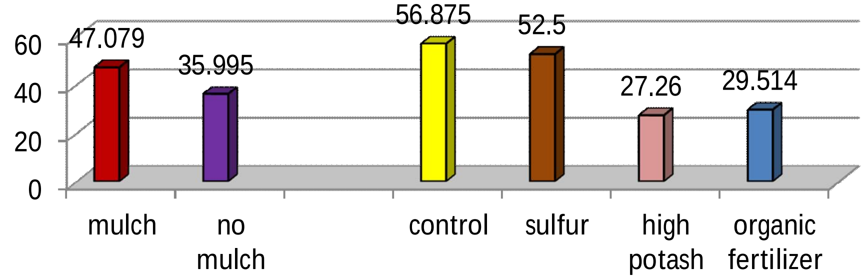

Figure 5. Effect of much and fertilizers on GSH activity (U) in leaves. LSD for mulch$_{(0.05)}$ = 3.6, LSD for treat$_{(0.05)}$ = 5.1.

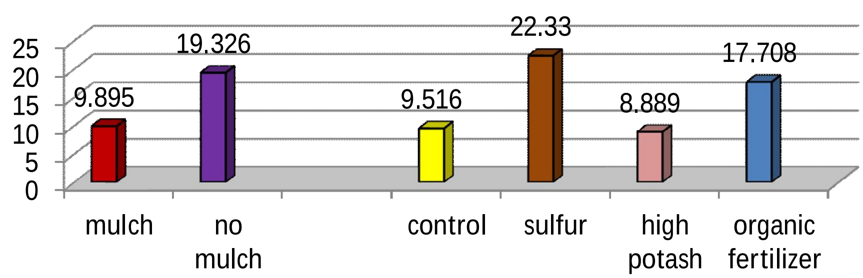

Figure 6. Effect of much and fertilizers on GSH activity (U) in flowers. LSD for mulch$_{(0.05)}$ = 3.8, LSD for treat$_{(0.05)}$ = 5.3.

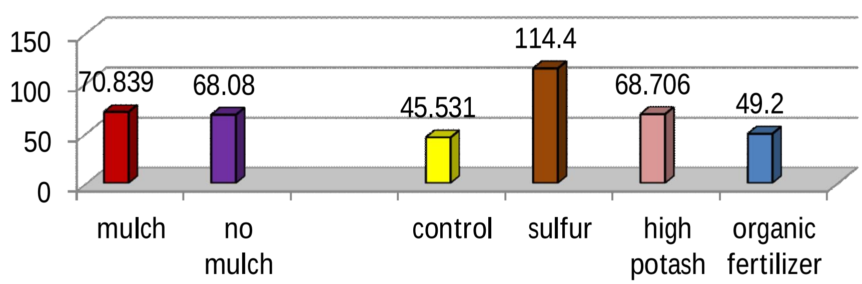

Figure 7. Effect of much and fertilizers on CAT activity (U) in leaves. LSD for mulch$_{(0.05)}$ = 5.2, LSD for treat$_{(0.05)}$ = 7.4.

of the flowers of mulching plants. Supplement plants with high potash and organic fertilizer caused a significant increase in CAT activity.

DISCUSSION

Broccoli is one of the main sources of vitamins and

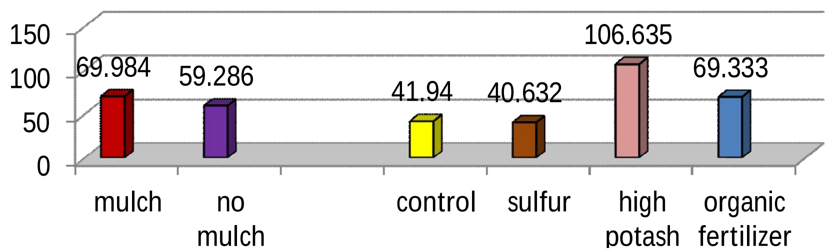

Figure 8. Effect of much and fertilizers on CAT activity (U) in flowers. LSD for mulch$_{(0.05)}$ = 5.2, LSD for treat$_{(0.05)}$ = 7.3.

antioxidants. Cover crop mulches positively affect pest management by suppressing weeds and other pests and reducing runoff or groundwater infiltration of pesticides and nutrients (Harrison et al., 2004). Mulch covering (with pine bark) in blueberry cultivation influences soil physical properties primarily, especially soil temperature and evaporation (Krewer et al., 1997). Furthermore, it enriches the soil organic matter, and therefore, helps conserving soil moisture (Pliszka et al., 1997). On the other hand, soil water availability affects the mineral nutrition of plants. All these factors might have an effect on the biosynthesis of bioactive compounds in fruits especially antioxidants in flowers (Figures 4 and 8). This finding is compatible with Wang et al. (2002) observation; while, Eichholz et al. (2010) observed an opposite effect of mulch on antioxidants of blueberries (*Vaccinium corymbosum* L.) fruits. In the leaves of broccoli, high potash caused a significant increase of antioxidant activity both in leaves and flowers (Tables 1 and 2). It may be related to the role of K in survival mechanism of the plant. Devi et al. (2012) shows clearly the specific effect of potassium on the antioxidant level which is increase at 0.05 mM KNO_3 or because its role in enzymes activation, protein synthesis, photosynthesis, osmoregulation, stomatal movement, energy transfer, phloem transport, cation-anion balance and stress resistance (Marschner, 2012). Soleimanzadeh et al. (2010) pointed out that low potassium level provokes antioxidant enzyme responses while high potassium levels decreased the activity of antioxidant enzymes and malondialdehyde (MDA) content may be by elimination of free radicals. But, our results were supported by the findings of Siddiqui et al. (2012) who demonstrated that the application of Ca_2^+ and/or K^+ was highly effective against the toxicity of Cd by improving activity of antioxidant enzymes and solute that led to the enhanced plant growth of faba bean plants and the finding of Heidari and Jamshidi (2011) who demonstrated that potassium treatment increased antioxidant activity in millet plants. In contrast, sulfur application was the best in improving antioxidant enzymes especially GSH in flowers of broccoli plants (Figure 6). This is may be related to the role of S in plant which is found in amino acids (Cys/Met), oligopeptides (glutathione [GSH] and phytochelatins), vitamins and cofactors (biotin, thiamine, CoA and S-adenosyl-Met), and a variety of secondary products (glucosinolates in Cruciferae and allylCys sulfoxides) (Saito, 2004). These results were compatible with the findings of Schonhof et al. (2007) who demonstrated that broccoli crop yield, product appearance as well as glucosinolate concentration were influenced by S supply which is a crucial importance in terms of crop management. Furthermore, sulfur application in the field of broccoli were essential to produce higher yield with good quality curds (Elwan and El-Hamed, 2011) because sculpture-containing compounds can provide a highly effective antioxidant system (Al-Malaika, 1984).

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Citation: Jasim AH, Al-Timmen WMA, (2014). The effect of mulch and fertilizers on broccoli (*Brassica oleracea* L. *Var. Italica*) oxidants and antioxidants. Net J Agric Sci, 2(4): 124-130.