

Full Length Research Paper

# Assessment of compost for suppression of *Fusarium oxysporum* and improving *Zea mays* and *Hibiscus sabdarriffa* resistance to wilt diseases

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Accepted 26 June, 2012

The present research was conducted to evaluate the compost effectiveness on *Zea mays* and *Hibiscus sabdarriffa* under *Fusarium* wilt disease. Compost physical, chemical and biological characters were monitored weekly during the ripening process. Both coliform and nematode were tested. Finally, the effect of compost on pathogenic *Fusarium* was examined within *Z. mays* and *H. sabdarriffa* in soil amended with 10% compost. Biochemical tests and antioxidant enzyme activities were determined for all the treated plants. The values of organic carbon recorded a percentage of 3.8%, while nitrogen (N), phosphorus (P) and potassium (K) values recorded 1.7, 4.6 and 5.6% higher than the commercial compost. The concentrations of nickel (Ni), copper (Cu), zinc (Zn), manganese (Mn), cobalt (Co), cadmium (Cd) and lead (Pb) in compost were below the threshold standard values. The compost is free of nematodes and coliform bacteria at maturity stage. Microbial population densities were usually high for *Bacillus sp.* compared to other microorganisms. In infected *Z. mays* with compost, shoot height, fresh and dry weight increased significantly (62, 248 and 130%) and *H. sabdarriffa* also recorded increase in the same plant criteria. In infected *Z. mays*, a significant increase in catalase (CAT) and ascorbate peroxidase (APX) activities was recorded (162 and 150%). For *H. sabdarriffa* there was a significant decrease in APX activity (35%) with compost, while no significant differences in peroxidases (PODs) activity for both plants. In case of infection, a significant decrease was observed for both *Z. mays* and *H. sabdarriffa* compared to infected plants without compost. The observed disease suppression in compost-amended soil was associated with the reduction in soil pathogen population and increase in microbial activity of composts. Moreover, diversification of different organic materials in compost enhanced the activation of the microbial population in soil that eventually increases disease suppressiveness and effectively controlling *Fusarium* wilt.

**Key words:** Antioxidant enzymes, compost, *Hibiscus sabdarriffa*, *Zea mays*.

## INTRODUCTION

Composts are teaming of microorganisms that manage the fermentation correctly, and pathogens are killed during the heat period (Engeli et al., 1993). At the same time, antagonists develop during maturation of the

compost. Therefore, composts can reduce the incidence of various plant diseases (Fuchs, 2002). Composts are known to suppress plant diseases through a combination of physiochemical and biological characteristics. Physical or chemical aspects of composts that reduce disease severity directly or indirectly affecting the pathogen or host growth (Jeanine et al., 2002), examples of these aspects include nutrient levels, organic matter, moisture, pH, and other factors (Whipps, 1997).

Biological characteristics including compost inhabiting microbial populations in competition for nutrients with pathogens, antibiotic production, lytic and other extra-

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**Abbreviations:** APX, Ascorbate peroxidase; CAT, catalase; MDA, Malondialdehyde; PODs, peroxidases.

cellular enzymes production, parasitism and predation, induction of host-mediated resistance in plants, and other interactions could decrease the disease development (Boulter et al., 2000). *Fusarium oxysporum* is difficult to control as it survives in field soil for several years. Several reports suggested various methods for the control of soil-borne diseases induced by *Fusarium* spp. (Boulter et al., 2002). Disease suppressive conditions have been induced in soils after the addition of certain composts in greenhouse systems and under field conditions (Keener et al., 2000).

Numerous reports show that soil amendments with composts control several important soil-borne pathogens including *Fusarium* wilts (Reuveni et al., 2002). These composts suppress plant diseases through physiochemical factors that reduce disease severity (Garbeva et al., 2004). However, there are very little information regarding the kinds of compost that suppress soil-borne pathogens and the possible mechanism of suppression (Boulter et al., 2002). Thus, the main objective of this work was to study the ripening and some microbiological and chemical aspects of compost combinations from natural resources in Qena, Egypt. Another target was to test the biological, chemical and physical characters of these composts, for better plant growth. Finally, we evaluated the compost efficacy to improve *Zea mays* and *Hibiscus sabdarriffa* resistance to wilt diseases.

## MATERIALS AND METHODS

### Composting site, sampling and processing

Natural organic waste materials were sampled at August 2008 from inside the South Valley University campus at Qena city, Egypt. The compost composition was as follows (v/v): plant wood 20; tree leaves 2; phosphate 0.3; and  $\text{NH}_3$  1.5. The compost piles were processed in open windrows of about 1:2 m length, 3 m width and a height of 2 m. The windrows were turned four times per month with a compost turner. Piles were moisturized daily then every two days for 12 weeks. Samples from each pile replicate were collected every two weeks for analysis until the end of maturation. Samples were taken at a depth of 20 cm below the surface of the pile. From each pile, three samples were taken at different positions. Each of these samples composed of three sub-samples that were bulked after sieving (<8mm mesh). Samples were transported to laboratory rapidly and all the analyses were made in the same day.

### Physical and chemical parameters

Temperature of the piles was monitored daily during the process using composting thermometer inserted at different heights in the piles. A solid-liquid extraction was carried out then; pH was determined by using AS-501 pH Analyzer (STEM Corporation, England). Conductivity, total dissolved salts (TDS) and dissolved oxygen (DO) were measured by using a 'Checkmate 90' meter equipped with conductivity/ TDS, and DO sensors (Corning®, USA). For analysis of metals, filtrates of the samples collected after 12 weeks were digested in a "TURBOTHERM®" digestion system attached to "TURBOSOG®" scrubbing unit according to Page et al. (1982) and then analyzed for the content of iron (Fe), manganese

(Mn), zinc (Zn), magnesium (Mg), copper (Cu), nickel (Ni), potassium (K), lead (Pb), cobalt (Co), cadmium (Cd) and phosphorus (P). Analysis was carried out using Buck Scientific INC 210- atomic absorption spectrophotometer (AAS). Organic carbon and organic matter (OM) were determined using the Walkley-Black wet combustion method (Tan, 1996).

Finally, compost total nitrogen content was determined using the micro-Kjeldahl technique (Nelson and Sommers, 1973).

### Test of nematodes and coliform bacteria

The compost was tested for the presence of nematodes by using the Baermann funnel (Briones, 2006). Coliforms were detected and enumerated by the multiple-tube technique using the most probable number (MPN) method. The number of tubes showing acid and gas is then counted and the figure compared to statistical tables (Case and Johnson, 1984).

### Isolation of compost microbial populations

Mature compost (5 g) was diluted with 45 ml of sterilized distilled water. Samples were shaken vigorously to form uniform solution of  $10^{-1}$  concentrations. The decimal serial dilutions ( $10^{-1}$  to  $10^{-7}$ ) were prepared for the isolation of both fungi and bacteria using plate count method.

### Compost for suppression of *F. oxysporum*

#### Pathogen growth and inocula preparation

Corn seeds were soaked in distilled water for 3 h, and then distributed equally into 250 ml Erlenmeyer flasks (100 g each). Flasks were then sterilized in an autoclave; flasks were then inoculated with *F. oxysporum* (SVUML 71) and incubated at 28°C for 10 days for better growth.

### Plant treatments

Seeds of *Z. mays* and *H. sabdarriffa* L. (cv. deep red sepals) were obtained from the breeding program of agriculture research, dokky, Giza, Egypt. These seeds were surface-sterilized by immersing in 95% ethanol for 30 s and then in 0.2% solution of  $\text{Hg}_2\text{Cl}_2$  for 2 min, followed by several rinses with sterilized water to remove disinfectant (Russell et al., 1982). A pot experiment was conducted in the green house as follows: Seeds of *Z. mays* and *H. sabdarriffa* (7 and 15 seeds, respectively) were sown in each pot that contained 1 kg soil amended with 10% compost and 50 g of the pathogen inoculum. The following treatments were also set up as reference controls: control (soil only without compost); soil + 10% Compost; soil + pathogen inoculums. The pots were daily irrigated with water until the appearance of seedlings in 21 days. By the end of the experiment, plants were harvested, washed carefully under tap water and dried on a paper towel. The plant fresh weight, shoot height and root length were recorded. Plants were then dried in an oven at 80°C for 48 h, then plant shoot and root dry weights were determined. Finally, protein content, amino acids, soluble carbohydrates, proline and malondialdehyde (MDA), catalase (CAT), ascorbate peroxidase (APX) and peroxidases (PODs) were also determined.

### Determination of soluble carbohydrates

Plant water soluble carbohydrates were colourimetrically

**Table 1.** Physical and chemical characteristics of the prepared compost compared to commercial one.

Parameter	Data							
<b>Compost physical parameters</b>								
Time (weeks)	2	4	6	8	10	12		
Temperature (°C)	45	45	45	41	42	ND		
Humidity (%)	54	53	51	56	54	ND		
pH	8.10	7.83	7.26	7.26	7.66	7.35		
DO* (µs)	1.2	44	24	24	25	21		
TDS <sup>§</sup> (mg/l)	6.7	1.44	2.37	2.37	0.5	23.6		
Conductivity (S)	13.5	3.33	4.35	4.35	ND	25.9		
<b>Compost chemical composition/mg Kg<sup>-1</sup></b>								
Compost chemical composition	N	P	K	Organic C	OM			
Prepared compost	0.139	0.571	0.39	36.57	63.05			
Commercial compost	0.079	0.122	0.07	9.75	16.81			
<b>Metal contents/mg Kg<sup>-1</sup></b>								
Metals	Pb	Co	Ni	Zn	Mg	Mn	Cd	Cu
Compost standards <sup>^</sup>	150	NA	50	400	NA	NA	1.5	200
Prepared compost	0	0	1.17	5.8	3.466	4.09	0.03	7.81

\*DO = Dissolved oxygen, §TDS = total dissolved solids, ND = not determined, OM = organic matter. ^The compost maximum limits that were identified by the composting association, U.K. (2000). NA = not available.

determined by using a "Spectronic® Genesys™ 2PC" Spectrophotometer, Spectronic Instruments, USA, according to Fales (1951) and Schlegel (1956).

#### Determination of total protein and proline

Total protein was calculated by summing the soluble and insoluble protein fractions of the same sample according to the method of Lowery et al. (1951). Free proline was determined according to the method of Bates et al. (1973).

#### Determination of total free amino acids

Free amino acids were extracted from plant tissues and determined according to the method of Moore and Stein (1948).

#### Determination of antioxidant enzyme activities

Catalase (CAT) activity (EC 1.11.1.6) was assayed in a 3 ml reaction solution composed of 50 mM phosphate buffer (pH 7.0), 30% (w/v) H<sub>2</sub>O<sub>2</sub> and 0.5 ml of plant extract (Aebi, 1984). The activity of catalase was estimated by the decrease of absorbency at 240 nm as a consequence of H<sub>2</sub>O<sub>2</sub> consumption compared to free enzyme extract sample (blank) (Havir and Mellate, 1987). Peroxidases (PODs) activity (EC 1.11.1.7) was determined using guaiacol, according to Maehly and Chance (1954). The increase in absorbance due to formation of tetraguaiacol was monitored at 470 nm. Ascorbate peroxidase (APX) (EC 1.11.1.11) activity was determined according to the method of Asada and Chen (1992).

#### Determination of lipid peroxidation

The level of lipid peroxidation was measured as the amount of

malondialdehyde (MDA) produced due to peroxidation of unsaturated fatty acids (Heath and Packer, 1968).

#### Statistical analysis

The data of all experiments were subjected to analysis by the least significant differences test (LSD) with two-way analysis of variance (SAS) using the software package JMP version 7.0 (SAS Institute, Cary, North Carolina, USA) to compare the means of every treatment against the control and simultaneously establish their significance ( $P < 0.05$ ). All data were presented as mean  $\pm$  standard deviation (mean  $\pm$  S.D.). All experiments were performed independently three times.

## RESULTS

### Physical and chemical parameters of compost

Figure 1 shows compost after complete maturity. The compost structure is homogeneous with normal particle size (1/8 inch approximately) with no foul odour, no other residues or large pieces of the starting materials. Table 1 shows values of the physical and chemical parameters measured during the composting process. Data illustrate that pH is varied between neutral to slightly alkaline (7.3 to 8.1) and the temperature values ranged from 41 to 45°C. Considering the chemical composition of compost, the values of organic carbon recorded a percentage of 3.8%, while N, P and K values recorded higher percentage of 1.7, 4.6 and 5.6% compared to the commercial compost.

The metal constituents of different compost

**Table 2.** Biological characteristics and percentage of the isolated microorganisms of the prepared compost.

Biological characteristics					
Coliform bacteria (-ve)			Nematodes (-ve)		
Percentage of the isolated microorganisms					
<b>Bacteria</b>	<i>Bacillus</i> sp. (94%)	<i>Micrococcus</i> sp. (1%)	<i>Azotobacter</i> sp. (1.5%)	<i>Streptomyces</i> sp. (3.5%)	<i>Actinomyces</i> sp. (3.5%)
<b>Fungi</b>	<i>Acremonium</i> sp. (65%)	<i>Alternaria</i> sp. (3%)	<i>Aspergilli</i> sp. (5.5%)	<i>Cladosporium</i> sp. (4%)	Other fungi genera (22.5%)

combinations compared to the standards of the composting association (UK) are shown in Table 1. The concentrations of Ni, Cu, Zn, Mn, Co, Cd and Pb in the collected compost were below the threshold values of these standards.

#### Test for nematodes and coliform

At maturity stage, the analyses of compost are found to be free of nematodes and coliform bacteria.

#### Isolation of microbial populations

The following microorganisms were identified in the mature compost combinations: Gram-negative aerobes of the genus *Azotobacter* 1.5% or, Gram-positive cocci of the genus *Micrococcus* 1%, Gram-positive spore forming bacteria of the genus *Bacillus* 94%, Gram-positive actinomycetes of the genera *Streptomyces* and *Actinomyces* 3.5%. The dominant fungi were *Alternaria*, *Acremonium*, *Cladosporium*, and *Aspergilli*. Density of *Bacillus* sp population was the highest among the other isolated microorganisms (Table 2).

#### Suppression of *F. oxysporum* by compost

##### Plant growth characteristic

Inoculation of *Z. mays* and *H. sabdarriffa* with *Fusarium* inhibited shoot growth and reduced plant total dry weight (Figures 2 and 3). Soil amendment with compost enhanced plant growth in the presence of pathogenic fungus.

Thus, in *Z. mays*, shoot height, fresh and dry weights of the plant were enhanced by 62, 248 and 130% more than those of the control (soil without compost). In *H. sabdarriffa*, shoot height, fresh and dry weights also recorded higher values (7, 19, and 11%) than those of the infected plants without compost. Although, infected plants with compost did not give the same growth as non-infected plants, but obviously they appeared better than those without compost.

#### Determination of protein, amino acids, carbohydrates and proline

Figure 4 (A, B) shows the values of total protein, free amino acids, carbohydrates and proline content of plants infected with *F. oxysporium* in the presence or absence of compost. Values of both protein and carbohydrates in either plant showed slightly increase in absence (T2) and or presence (T3) of fungus. There were no significant differences between T2 and T3 for the same tests.

On the other hand, for *Z. mays*, there was an increase in both amino acids and proline (320 and 165% respectively) in case of fungus alone (T2) compared to the control plants (Figure 4A). When infected plants were amended with compost (T3), a significant decrease was recorded for both amino acids and proline content (74 and 47%, respectively) as compared to infected plants without compost. For *H. sabdarriffa*, no significant differences were recorded in amino acids for infected plants, with and without compost, as compared to control plant (Figure 4B). Also, when infected plants were amended with compost (T3), a significant decrease was recorded in proline content (31%) as compared to infected plants without compost.

#### Antioxidant enzyme activities (CAT, APX and PODs)

##### CAT activity

There was a significant decrease in CAT activity for both *Z. mays* and *H. sabdarriffa* (52 and 56%) when amended with compost (T1) as compared to the control (Figure 5). In infected *Z. mays* without compost (T2), a significant increase in CAT activity (162%) was recorded (Figure 5). The same plant with compost (T3) showed a significant decrease in CAT activity (76%) as compared to T2 (Figure 5A). In case of *H. sabdarriffa*, the same results were obtained for both T2 and T3 treatments (Figure 5B).

##### APX activity

An increase in APX activity for *Z. mays* was recorded (150%) when amended with compost (T1) followed by a

little reduction when infected (T2) than increased again with compost (T3). In case of *H. sabdarriffa* there was a decrease in APX activity (35%) when amended with compost (T1) as compared to the control. The same plants when amended with compost (T3), showed a significant decrease (150%) as compared to infected plant without compost (T2, Figure 5B).

### PODs activity

There were no significant differences for PODs activity in both plants when amended with compost (T1) as compared to the control. In case of *H. sabdarriffa* there was a significant decrease in PODs activity (77%) when plants were amended with compost (T3) as compared to infected plant without compost (T2, Figure 5).

### Lipid peroxidation

Figure 6 shows that plant inoculation with *F. oxysporium* (T2) stimulated the accumulation of MDA content in both *Z. mays* and *H. sabdarriffa* as compared to control plants (43 and 16%, respectively). Both plants showed the same results for MDA content in case of T1 treatment compared to the control. On the other hand, when infected plants were amended with compost (T3) a decrease in MDA was recorded in both *Z. mays* (19%) and *H. sabdarriffa* (14%) as compared to infected plants T2 treatment.

## DISCUSSION

In the current study, stabilization of the end-product was assessed by characterizing compost's physico-chemical properties and comparing them to those of the commercial compost and the international standards (Table 1). The prepared compost exhibited an adequate quality with high content of organic matter, organic C and low C/N ratio reflecting a stabilization and maturity of the compost (Figure 1, Table 1). To get the EU eco-label, composted material should contain not less than 20% organic matter in the end product (Lasaridi et al., 2006). Our results showed that organic matter values were from 35 to 70%.

The pH of the compost was monitored and was found to increase as the composting process progressed. This is caused by humification and consumption of organic acids during the composting process (Fogarty and Tuovinen, 1991). Moreover, increasing compost pH to 8.1 can also cause increasing the release of  $\text{NH}_4$  especially at the first two weeks during the composting process that is known to suppress different pathogens (Steven and Walter, 2004). According to the study of Lasaridi et al. (2006), compost should have a pH value within the range

of 7.3 to 8.1 to ensure compatibility with most plants. Accordingly, our compost was within the same range (Table 1). The compost had higher levels of organic matter, N, P, K and organic carbon than the commercial compost (Table 1). On the other hand, low levels of metals were recorded; therefore toxicity is not a possibility in case of using it without dilution (Table 1) (Arvanitoyannis and Kassaveti, 2007). It also means that these compost combinations have more available nutrient for plant growth in poor soils.

The compost temperature followed approximately a similar profile that is fluctuating between the lower (41) to the higher degrees (45), as the decomposition proceeds. According to the study of Levi-Minzi et al. (1990), the extent of organic matter decomposition is related to the temperature at which composting takes place and to the chemical composition of the organic substrate undergoing composting. Accordingly our compost recorded a highest temperature relatively and this may illustrate the highest organic carbon and organic matter (Table 1).

Heavy metals are toxic, undegradable in the environment and are dangerous pollutants (Helfrich et al., 1998). Heavy metal concentrations indicated the extent of contamination in the prepared compost. Indeed, when applied as a soil amendment or fertilizer, the final product must be free of hazardous pollutants such as heavy metals (Pinamonti et al., 1997). After composting maturity, the total metal concentrations in the final composts showed wide variations (Table 1). All the values were below the eco-label standards for Cu, Ni, Zn and Pb and in agreement with those of Pinamonti et al. (1997). Compost can influence the mobility, reactivity and availability for vegetation absorption (Walker et al., 2003). Although composting is a microbiological process, little is known about microorganisms involved and their activities during specific phases of the composting process (Rebollido et al., 2008). Microorganisms, mainly bacteria and fungi, play crucial roles in making nutrients available to plants, many microbial processes are essential for the long-term sustainability of agricultural systems (Wardle and Ghani, 1999). Changes in a microbial community can thus be used to indicate alterations in soil quality (Breure, 2005). In this study, different bacterial and fungal species were isolated from our compost. Bacterial isolation from composting agricultural substrates suggests that a form of fermentation had taken place during the composting process. This is because (Kolawole and Okonkwo, 1985) these organisms and fermentation of various agricultural substrates are linked.

In the current study, isolation of *Bacillus* spp. from fermented compost was also carried out previously (Ojey, 1981). Prescott et al. (1999) also suggested that some *Bacillus* species inhabit high temperature habitats. These bacteria produce spores that are heat resistant thus making them survive in an extremely high temperature of the compost (Jones, 1993). Accordingly, the prepared



**Figure 1.** Mature compost showing homogeneity and normal particle size.

compost showed the highest percentage (96%) of *Bacillus* species (Table 2). The common species of fungi was *Acremonium* and different species of *Aspergillus* (Table 2). These results are in agreement with previous ones in the fact that the most of common species of compost belong to the *Acremonium* and *Aspergillus* (Antonella et al., 2005).

There are number of reports about isolation of *Salmonella*, *Yersinia*, and *Escherichia coli* from organic compost (Goldstein et al., 1988). These results indicate that organic compost may be a potential harbour of food-borne pathogens in vegetables. The occurrence of some pathogenic microbial organisms in raw vegetables and their implications in causing human health, resulting in diarrhea/dysentery or serious diseases like yersiniosis and listeriosis have been documented (Summer and Peters, 1997). Considering quality of our compost, negative results were recorded for both nematodes and coliform bacteria, and these results were in agreement with the previous mentioned ones (Table 2).

In conclusion, compost quality meets the required standards for organic compost as previously discussed. This compost is natural, uniform, biodegradable by soil microorganisms, non-toxic, free from both nematodes and coliform bacteria. It also contains large uniform bacterial and fungal populations, providing the slow release of organic matter for long periods, causes no ecological pollution, and its biological characteristics can be effectively controlled by manipulating its chemical features. *Fusarium* wilts, caused by the fungus *F. oxysporum*, are important diseases of horticultural and agricultural crops and lead to significant yield losses. The pathogen infects the roots and colonizes the vascular tissue, leading to wilting and finally death of the plant (Heremans et al., 2005). Compost, the final product of aerobic biodegradation of organic matter, exhibits marked disease suppression (Hoitink et al., 1993).

The improvement effects of compost fertilization

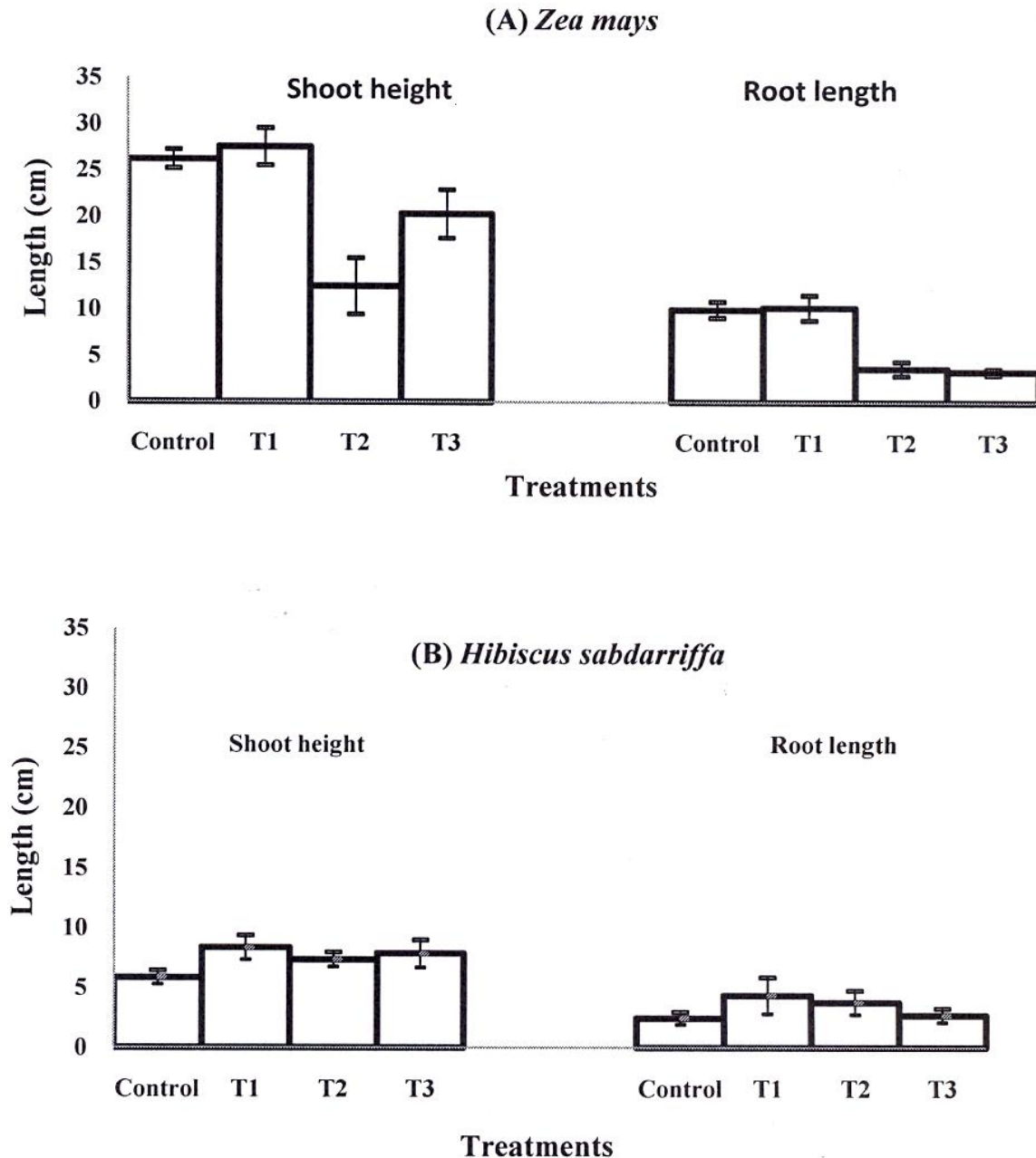
treatments on vegetative growth characters can be rendered to the important role of compost on soil properties, moisture retention, better nutrient availability which resulting in good plant growth (Rauiv, 1998). The present study showed that *F. oxysporum* treatment induced a marked reduction of growth characteristics for both plants grown without compost (T2 treatment). The infected plants when grown with compost (T3) showed an increase in growth characteristics (Figures 2 and 3). Similar results were found in compost application that resulted in linear increases of root elongation and quadratic increases of the germination in soybean and wheat seeds (Ademir and Regina, 2005).

Different mechanisms have been postulated to control plant diseases by compost application such as competition for nutrients, antibiotic production by beneficial microorganisms or activation of disease-resistance genes in plants (Hoitink and Boehm, 1999). Compost obtained from heterogeneous vegetable wastes shows important suppressive effects against diseases caused by several plant pathogens such as *Fusarium* spp. (Suarez-Estrella et al., 2001). In this sense, *Bacillus* spp., *Streptomyces* spp. and other bacterial genera, as well as *Penicillium* spp., *Aspergillus* spp., *Trichoderma* spp., *Gliocladium virens*, and other fungi have been identified as bio-control agents in compost-amended substrates (Hoitink et al., 1997).

In the current study, most of the previous antagonistic bacteria and fungi were isolated from our compost. Suppression of pathogens and/or diseases is largely induced during compost curing, because most bio-control agents recolonize composts after peak heating (Hoitink et al., 1997). Content of soluble protein showed slightly increase in *Z. mays* and *H. sabdarriffa* plants infected with fungus (T2) and infected plants with compost (T3) compared to the control plants (Figure 4). This increase in protein content was recorded by other authors under the same conditions through the synthesis of several protective compounds such as pathogenesis-related protein (Shebany, 2005).

In the present study, the microorganisms present in compost may give biological defense strategies to plants. This is supported by the fact that if the fermentation is correctly managed, pathogens are killed during the heat period and antagonists develop during maturation of the compost (Engeli, et al., 1993). Therefore, composts can reduce the incidence of various plant diseases (Fuchs, 2002). The content of soluble carbohydrates slightly increased when infected plants were amended with compost. These results are in agreement with those obtained by Bishop et al. (2002) who reporting that infection of wheat sheaths by *F. proliferatum* resulted in increased cellular levels of arabinose, glucose, fructose, melibiose and sucrose compared to control sheaths.

In case of fungus alone, both amino acid and proline were increased (320 and 165%, respectively) in *Z. mays* compared to the control plants (Figure 4A). These results



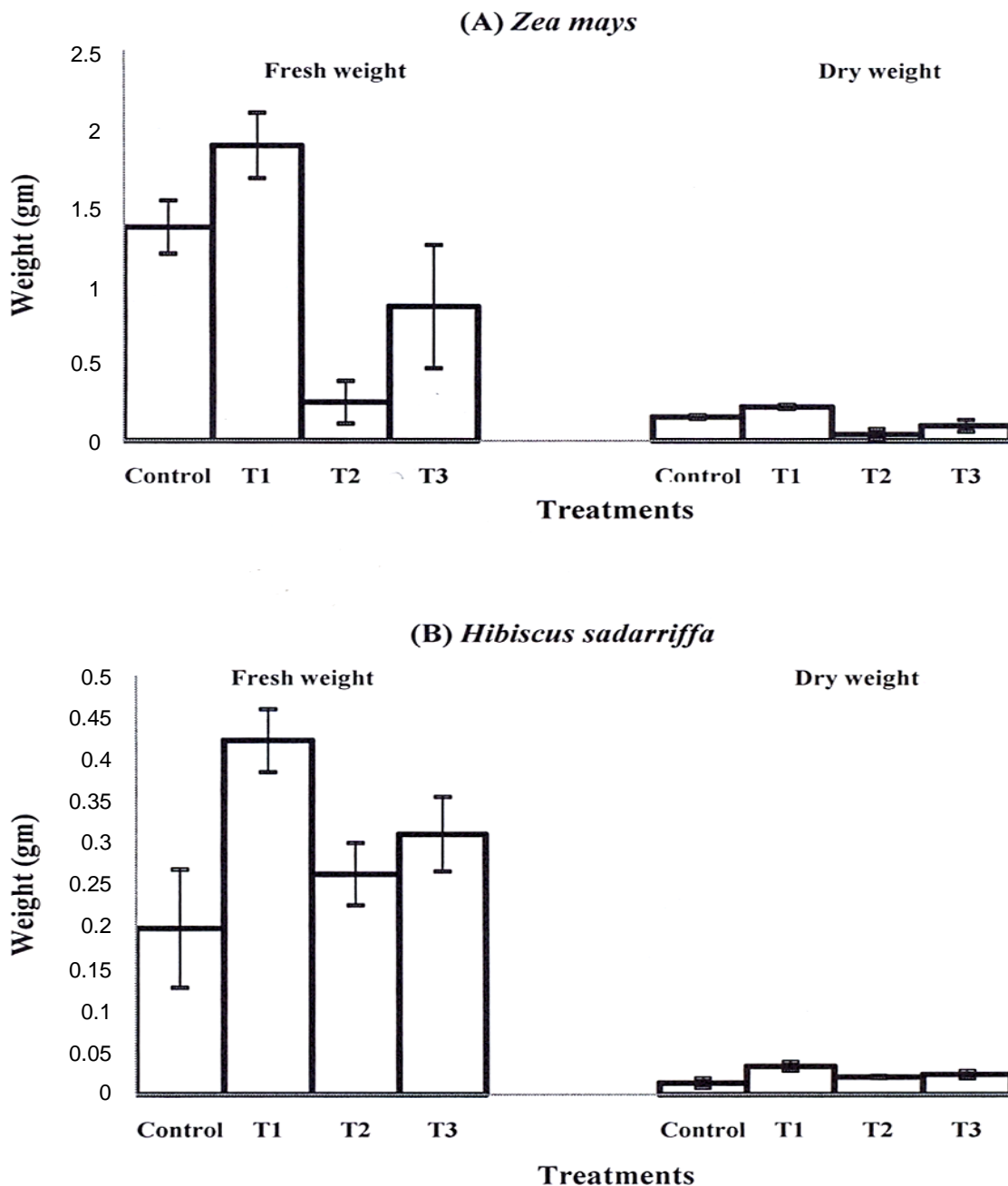
**Figure 2.** Shoot height and root length of *Z. mays* and *H. sabdarriffa* cultivated in soil amended with 10% compost. T1 = Plant + compost T2 = Plant + fungus, T3 = plant + fungus + compost.

are in agreement with other findings of increased proline as a cell defense reaction (Shebany, 2005).

Moreover, diversification of different organic materials in compost enhanced the activation of the microbial population in soil in which disease suppressiveness of *Fusarium* wilt is activated in response to increasing proline content. Total free amino acids content increase in infected *Z. mays* and *H. sabdarriffa* (T2). These results are in agreement with the results obtained by Starratt and Lazarovits (1996). Amino acids might act indirectly by changing the metabolism of plant leading to the

production of antifungal substances directly from amino acids, or by the activation of other synthetic pathways (Van Andel, 1966). Compost may contain antifungal and antibacterial components able to suppress *Fusarium* infection in soils.

Response of plant to biotic and abiotic stresses leads to the generation of active oxygen species (AOS) such as the superoxide anion  $O_2^-$  and hydrogen peroxide ( $H_2O_2$ ). Thus, AOS accumulation causes oxidative damage to plants. Plants are able to defend against phytopathogenic agents by producing a wide spectrum of

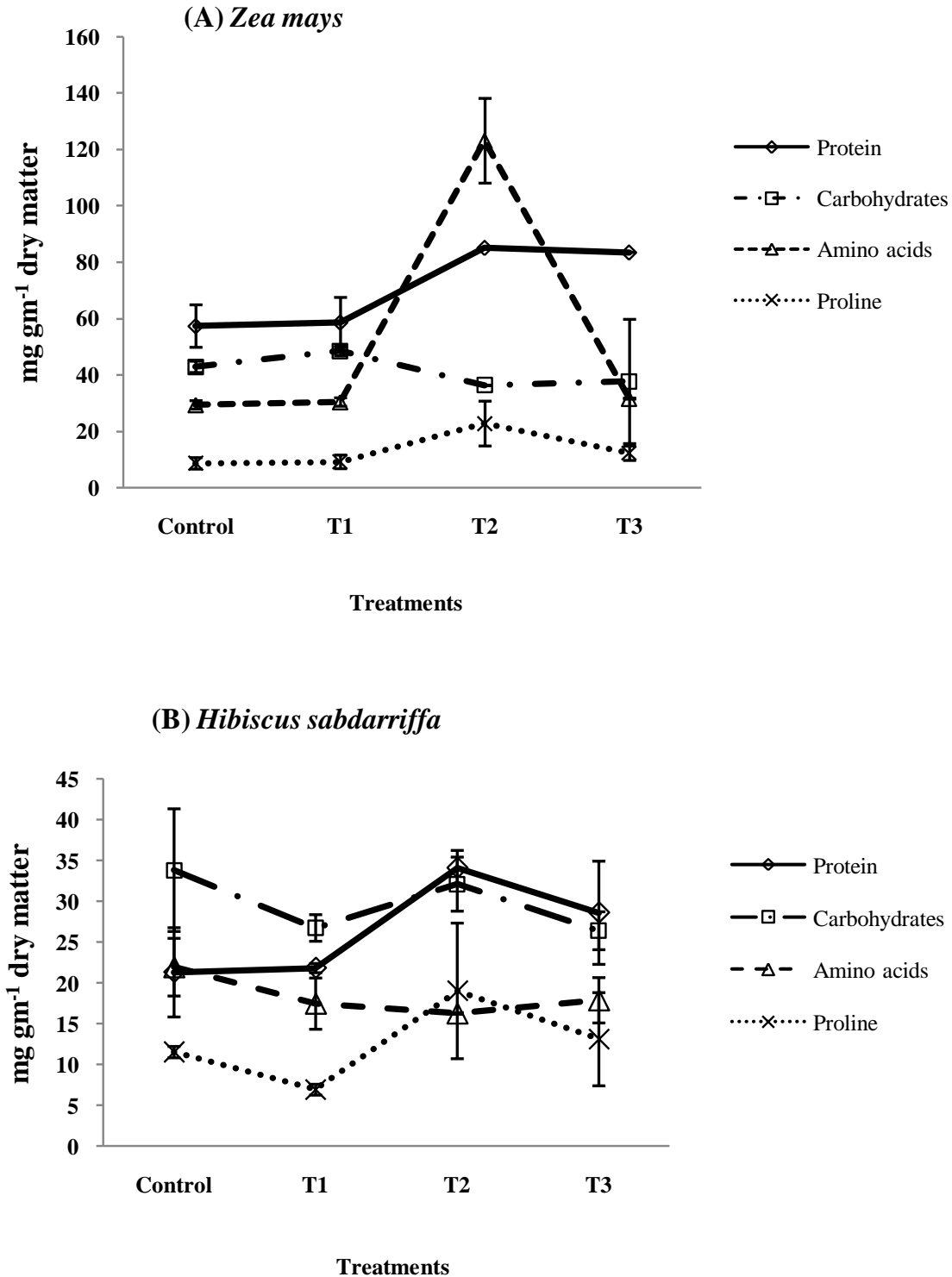


**Figure 3.** Fresh and dry weights of *Z. mays* and *H. sabdarriffa* cultivated in soil amended with 10% compost. T1 = Plant + compost, T2 = Plant + fungus, T3 = plant + fungus + compost.

antimicrobial compounds among which oxido-reductive enzymes such as PODs, CAT and APX that have been implicated in cellular protection and disease resistance (Lvanov et al., 2005). The present study showed that CAT activities decreased in both infected plants cultivated in soil amended with compost compared to those without compost (Figure 5A, B). The inhibition of CAT activity results in  $H_2O_2$  accumulation that may induce lipid peroxidation in the cell (Chai et al., 2005) APX activity increased in *Z. mays* when amended with

compost (T3) as compared to infected plant without compost (Figure 5A). This increase in APX activity might decrease  $H_2O_2$  concentration in this plant (Baek et al., 2005). But when *H. sabdarriffa* amended with compost (T3), a significant decrease was observed (150%) as compared to infected plant without compost (T2) (Figure 5B). The increase in (APX) activity was more obvious in *Z. mays* plants than *H. sabdarriffa* because *Z. mays* might be more resistant than *H. sabdarriffa* to pathogenicity.

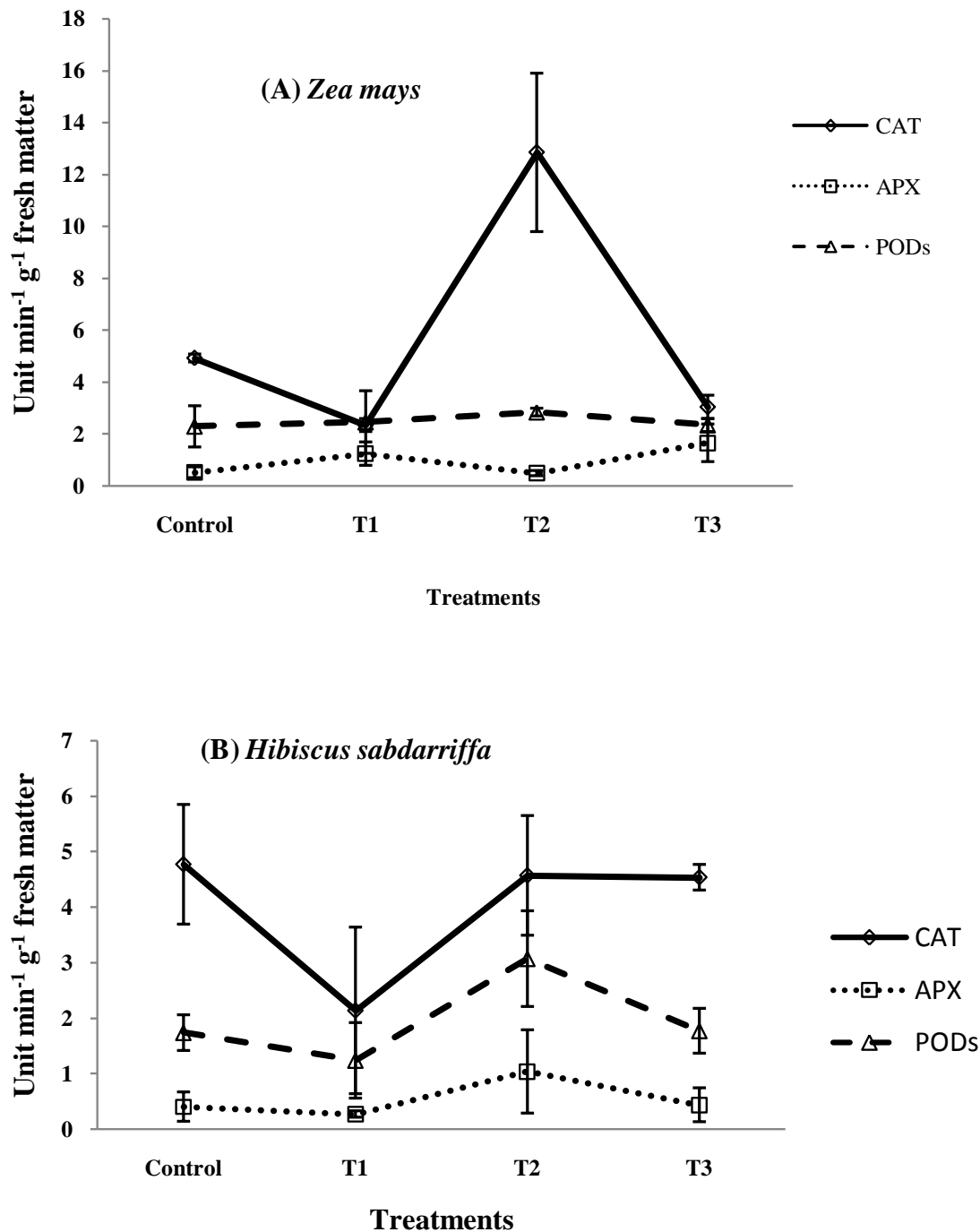




**Figure 4.** Changes in proteins, carbohydrates, amino acids, and proline contents in *Z. mays* and *H. sabdarriffa* cultivated in soil amended with 10% compost. Control= plant without compost. T1 = Plant + compost. T2 = Plant + fungus. T3 = plant + fungus + compost.

The increase in (PODs) activity might be associated with chlorophyll degradation and was likely induced by increased levels of superoxide radicals resulting from

decline in (SOD) and (CAT) activities (Liua and Huanga, 2000). Accordingly, the PODs activities in infected *H. sabdarriffa* decreased with compost compared to the



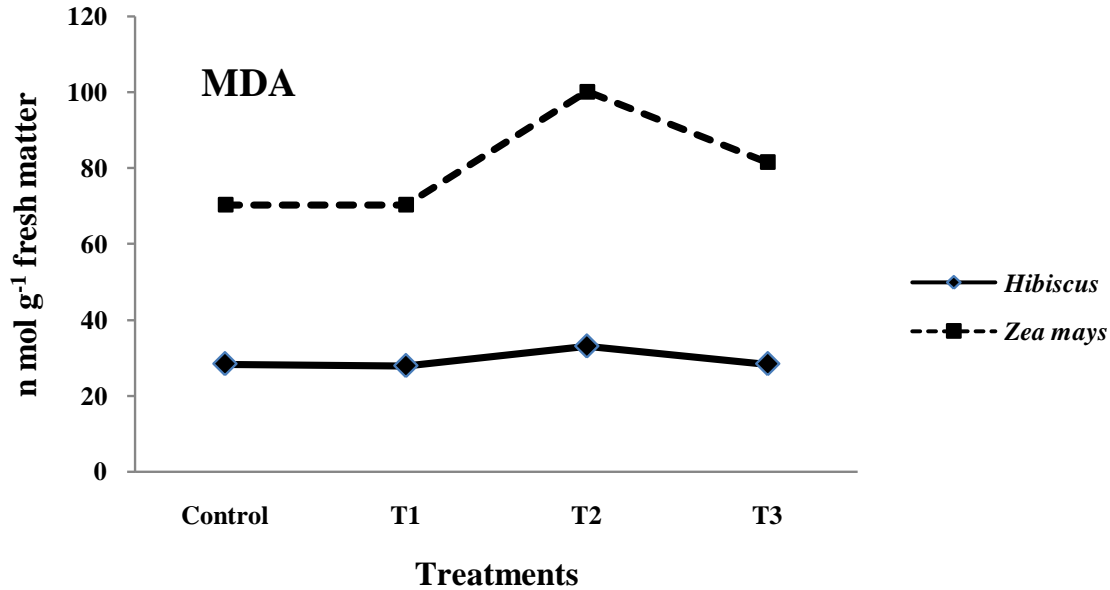
**Figure 5.** Changes in antioxidant enzymes activities in *Z. mays* and *H. sabdarriffa* cultivated in soil amended with 10% compost. Control= plant without compost. T1 = Plant + compost. T2 = Plant + fungus. T3 = plant + fungus + compost.

values recorded for the same plant without compost. Similar results were observed in *Hibiscus* cultivars when treated with some phyto-hormones which reduced PODs activities (Abou, 2007).

The lower MDA content, in case of compost addition (Figure 6) indicates that both infected plants are more

tolerant to fungal infection under these conditions compared to plants without compost.

We conclude that the observed disease suppression in compost-amended soil was associated with the reduction in soil pathogen population and increase in the activity and population of other microorganisms. Further studies,



**Figure 6.** Changes in malondialdehyde (MDA) content in *Z. mays* and *H. sabdariffa* cultivated in soil amended with 10% compost. Control = plant without compost. T1 = Plant + compost. T2 = Plant + fungus. T3 = plant + fungus + compost.

however, are needed to determine the influence of these composts on the microbial community and on pathogen activity in different soils. The feasibility of these pathogen suppressive actions of compost on a field scale needs to be verified.

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