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Full Length Research Paper

Antimicrobial and antioxidant activities of drought-endemic plants and enzymes production in *Schefflera arboricola* plants under pesticides stress

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Antioxidant enzymes comprise the immune system in plants against abiotic and biotic stresses. In one part of the current study, the level of activity of four antioxidants has been examined in four unexplored Egyptian xerophytes. Their antimicrobial activity against seven virulent bacteria was also determined. In a second part of the study, the level of antioxidant enzymes was compared to their levels in *Schefflera arboricola* plant upon the applications of three pesticides. It was believed that the biosynthesis of these enzymes under the effect of pesticide treatments was pesticide-specific. From total number of pesticides, two only led to a significant oxidative damage. The herbicide 'roundup' caused an abrupt increase in antioxidants in Schefflera leaf and hence to a protective mechanism against oxidative damage. Subsequently, the level of lipid peroxidation has decreased. In addition, our data have viewed that glutathione (GSH) was not efficient in scavenging the oxidative damage in Schefflera.

Key words: Antioxidant enzymes, drought, malonyldialdehyde, pesticides, *Schefflera.*

INTRODUCTION

Recently, drought deleterious effects on cultivated plants metabolic pathways including antioxidant enzymes pathways was intensively reported (Manivannan et al., 2008). However, under this investigation, we focused on studying the antioxidants that are abundant group naturally present in drought-endemic, desert-plants in Suez desert of Egypt. The antioxidant level in these xerophytic plants was compared to the same set of antioxidants that were synthesized upon pesticides applications to a mesophytic plant from the ornamentals called *Schefflera arboricola*. Reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals are known to synthesize from normal ($OH^{\bullet}$) pathways in all cellular organelles (Scandalios, 1993). At low concentrations, ROS are involved for optimizing H_2O_2 concentration to be suitable for cell wall biosynthesis-which is an important physiological function (Elstner, 1987). At higher concentrations, ROS often act as energetically reactive radicals (Neill et al., 2002) and capable for modulating normal cellular metabolism through oxidative damage to lipids, proteins and nucleic acids (Imlay, 2003). Plants responded to unfavorable environmental conditions by inducing ROS and malonyldialdehyde (MDA) (Hernandez et al., 1993; Radic et al*.,* 2006). Therefore, MDA is the biomarker for lipid peroxidation and an indicator for oxidative damage (Meloni et al., 2003).

Pastori and Foyer (2002) had identified the so-called 'cross-tolerance' which is a phenomenon that describes the involvement of common pathways in plants to detoxify the endangered effects of ROS. Some of these are the antioxidant enzymes that are functioning to detoxify the effects of ROS in plants. However, anti-

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Abbreviations: CAT, Catalase; **GSH,** glutathione; **MDA,** malonyldialdehyde; **POD,** peroxidase; **SOD,** superoxide dismutase; **ROS,** reactive oxygen species; **GSSG,** oxidized glutathione; **TBA,** thiobarbituric acid; **TCA,** trichloracetic acid.

oxidants are not exclusively enzymes but could also be primary component, such as phenolic compounds, glutathione (GSH), carotenoids, ascorbate (vitamin C) and tocopherols (vitamin D) (Alguacil et al*.,* 2006; Chanwitheesuk et al., 2005).

The enzyme superoxide dismutase (SOD) was determined in plants tissue scavenging the superoxide anion-which is formed after the release of H_2O_2. The later was detected upon crop plants exposure to abiotic stresses (Wang et al., 2010). The enzyme peroxidase (POD) decomposes H_2O_2 through consuming its oxidation capacity in the oxidation of phenolics and antioxidants (Boldt and Scandalios, 1997). The activity of POD was found to increase over time in tissues (Van Assche and Clijsters, 1990; Miteva et al*.,* 2005; Stroinski, 1995; Mocquot et al., 1996; Miteva and Peycheva, 1999; Weeckx and Clijsters, 1996) especially after plant's prolonged exposure to toxic substances such as excess H_2O_2 (Meloni et al., 2003) or to salt stress. Catalase (CAT) enzyme is known to dismutates H_2O_2 into water and oxygen (Boldt and Scandalios, 1997).

Extracts from desert plants were examined for their antimicrobial activities against the pathogenesity of seven virulent bacteria. For example; Enterobacteriaceae including *Escherichia coli* and *Enterobacter*-Gram-negative bacteria that cause diarrhea and kidney failure; *Staphylococcus aureus* which is a Gram-positive bacterium (Santoro et al., 2006) causes pneumoniaee; *Klebsiella pneumoniaee*, which causes acute prostatitis and various lung diseases (Anderson and Janoff, 1998); *Bacillus megaterium* that causes eye diseases (Ramos-Esteban et al., 2006) etc.

The crop toxicity which resulted from various applications of pesticides during agriculture was examined in this study through examining pesticides effects on a mesophytic plant. *Schefflera*, is inedible plant, used to underline a relationship between the type/level of antioxidant enzyme induced in response to specific pesticides. We endeavored that pesticides doses were in similar amounts used by farmers, we then focused on the physiological changes which had occurred in *Schefflera* after the treatments.

In this study, a set of antioxidants enzymes from drought-adapted plants was determined and compared to the same set of enzymes produced in an ornamental plant upon treat-ments with three pesticides. The anti-microbial activity of the methanolic extracts of some desert plants was detected.

MATERIALS AND METHODS

Experimental procedures

Reagents

The pesticides stocks were diluted with water and the concentrations were similar to local concentrations used by farmers. The pesticides were named as follows: The 'insecticide' malathion (75%), a cholinesterase-inhibiting organophosphate; the 'herbicide' roundup (48%), including the surfactant polyethoxylated tallow amine (POEA), and the 'fungicide' fenfuram (72%), whch contains Furan-carboxamides as a chemical group. These chemicals were a gift from 'the Pesticide Center' affiliation to the Ministry of Agriculture-Cairo-Egypt. The other chemicals were purchased from sigma fine chemicals.

Equipment

The absorption values were collected from a Unicam UV–visible double beam spectrophotometer from Helios Company. It employed a tungsten filament light source and a deuterium lamp, which has a continuous spectrum in the ultraviolet region. The spectrophotometer is equipped with a temperature-controller cell holder. The centrifugation was Beckman high speed, USA.

Microbial cultures and plant materials

he bacterial cultures (*B. megaterium*, *B. subtilis*, *E. coli*, *S. aureneus*, *Endobacter*, *K. pneumoniae* and *Pseudomonas aerogenosa*) were isolated from sterile stocks in the 'National Research Center' from cultures that regenerated on regular basis. Desert plants (*Anabasis articulate, Hammada elegans, Rumex dentatus, Zygophyllum album*) were collected arbitrary from Suez desert some 60 km far from Cairo and from the depth of the desert to avoid the runway complications and polluted effects on these plants. Sampling was acheived during April/2009. Two-months-old *S. arboricola* (octopus tree or local name *cheflera pequena*) was purchased from the 'Agricultural Research Center' in Cairo. *Schefflera* sp., leaves were detached and dipped in either water or in solutions of malathione, roundup or fenfuram for ten days. Fresh desert plants were used immediately for testing their antioxidant enzymes activities in adition to MDA. Another fresh mass patch of desert plants was air dried at ambient conditions, grinded and extracted for antimicrobial test.

Extraction of desert plants for antimicrobial activity

A xerophyte was used for antioxidants analysis and antimicrobial efficiency. 20 g from air-dried mass was grinded to powder, then the extraction process proceeded slowly using over night reflux with 1 L of 70% (*v/v*) methanol, ethanol, water and ethyl acetate at ambient temperature. The four suspensions were filtered and the filtrate was fixed for evaporation to dryness using Büchi Rotavapor R-200 with "V" assembly (vertical water condenser) and the crude extracts were weighed accurately, dissolved in dimethyl sulfoxide (DMSO) and raised to lease equal volums. The extracts were stored in -20°C until used. The following concentrations: 5, 15, 25 and 35 mg/ml were applied to agar plates according to agar diffusion method (Maruhenda and Gemenez, 1986). The volumes were loaded to discs and discs diffusion method was applied to microbial cultures. The inhibition zones were measured in millimeters.

Activity assay of glutathione (GSH)

One gram (1 g) fresh mass from whole plant biomass was immediately homogenized at 4°C in ice-cold 5% (*w/v*) trichloroacetic acid with purified sand in a cold mortar. The homogenate was centrifuged at 12,000 x g for 15 min, the supernatants were used for total (GSH + GSSG) and oxidized glutathione (GSSG) determinations as reported by Sgherri and Navari-Izzo (1995).

GSSG was determined after GSH had been removed by derivatization with 2-vinylpyridine. Changes in absorbance of the reaction mixtures were detected at 412 nm. The amount of GSH was calculated by subtracting the GSSG amount, as GSH equivalents, from the total GSH amount. A standard calibration curve where GSH equivalents (1–10 nmol) was plotted against the rate of change in A 412 was made.

Activity assay of superoxide dusmutase (SOD) and catalase (CAT)

Detection of CAT activity was undertaken using a slight modification of the method of Cakmak and Marschner (1992). The reaction mixture, in a total volume of 3 ml, contained 1.5 ml of 50 mM sodium phosphate buffer (pH 7.0) and 1 ml of 0.2% H_2O_2. The reaction was initiated by adding 0.5 ml of the enzyme extract, and the activity determined by measuring the initial rate of disappearance of H_2O_2 at 240 nm.

SOD activity was estimated spectrophotometrically as the inhibition of photochemical reduction of nitro blue tetrazolium (NBT) at 560 nm, a minor modification of the method of Beauchamp and Fridovich (1971). The reaction mixture consisted of 0.3 ml each of 0.75 mM NBT, 130 mM methionine, 0.1 mM Ethylenediaminetetraacetic acid (EDTA)-Na_2, 0.02 mM Riboflavin, and sterilized water and 1 ml of 50 mM Na-phosphate buffer (pH 7.8). The reaction was initiated by adding 0.5 ml of the enzyme extract and carried out for 20 min at 25°C under a light intensity of about 300 $\mu mol^{-1}\ m^{-2}\ s^{-1}$. One unit of SOD was defined as that which inhibited photoreduction of NBT to 50%. The results of CAT and SOD assays were expressed as U mg^{-1} protein.

Activity assay of peroxidase (POD)

POD activity was measured in crude extracts. Fresh mass from desert plants was homogenized with cold 0.1 tris–HCL buffer (pH 8.0), containing 17% sucrose (1:5 w/v) and Dowex 1 X 8 (200–400 mesh) (1:10 w/v). The homogenate was centrifuged at 11, 000 g for 50 min and the supernatant was used for enzyme assay. POD was determined spectrophotometricaly at 470 nm with guaiacol as hydrogen donor, according to the method described by (Gavrilenko et al., 1975).

Measurements of lipid peroxidation (MDA)

Lipid peroxidation was determined by measuring MDA using the thiobarbituric acid (TBA) method (Placer et al.,1968). Fresh tissues (100 mg) were homogenized in 1.5 ml of 0.25% TBA in 10% trichloracetic acid (TCA). The mixture was heated at 95°C for 30 min and then quickly cooled in an ice bath. After centrifugation at 10,000 g for 10 min, the absorbance of the supernatant was read at 532 nm and correction for unspecific turbidity was done by subtracting the apsorbance at 600 nm. A total of 0.25% TBA in 10% TCA served as blank. The MDA content was calculated according to its extinction coefficient of 155 $mM^{-1}\ cm^{-1}$.

Statistical analysis

All values were means of three or four replicates extracted from independent specimens. The standard deviations (SD), standard errors (SE) and the variance (one-way ANOVA) were analyzed using the software SPSS 12.0 for windows.

RESULTS AND DISCUSSION

The production of ROS in plants from oxidation is a process took place under normal conditions. These ROS often act for energizing multiple processess in plants. However, the overproduction of ROS is deleterious; as they attack fats and membrane lipids causing oxidative damage (Kim et al., 2010). CAT and SOD are involved to protect plants against stresses- although they are not potential during extreme stresses (Borneo et al., 2009). Alternatively, POD and SOD were reported as first defence line; their amounts or activity changes were identified as marks of redox status formed upon some stresses such as a metallic stress (Simonovicová et al., 2004; Ali et al., 2008). It should be noted that *schefflera* leaves became dry after ten days from treaments with pesticides, and probably were burned as their color became brown (data not shown).

The increase of CAT level of activity was pronounced in *schefflera* not the xerophytes (Figure 1). The highest value was 250 U mg^{-1} Fwt under the effect of malathione, followed by roundup treatment which had acheived 216.6 U mg^{-1} Fwt. It seemed that fenfuram fungicide was not CAT-inducing-agent, as the level of CAT activity had reached 60.7 U mg^{-1} Fwt with fenfuram application in *schefflera*. Level of CAT activity in a detached *schefflera* leaf dipped in water for the ten days was 32.8 U mg^{-1} Fwt and has not significantly increased above CAT level in the intact control leaf, the later had the lowest value for CAT (14.5 U mg^{-1} F wt).

An evidence of a differential behavior was observed by comparing these findings with CAT level of induction in desert-plants subjected to permanent environmental stresses, such as drought and temperature. Except for *Zygophyllum*, CAT level of induction in the xerophytes were declined (Figure 1). Moreover, CAT value was as same as CAT value that determined in the detached *Schefflera* leaf dipped in water. CAT values in desert plants were as follows: 37.7, 30.7 and 48.9 U mg^{-1} Fwt in *Anabasis* sp., *Hamada* sp. and *Rumex* sp., respectively. In *Zygophyllum* sp. CAT level was 62.5 U mg^{-1} Fwt, in value similar to CAT value induced in *Schefflera* leaf by fenfuram.

Here, we confirmed that toxic effects of pesticide applied to mesophytic plant were highly exceeded drought effects in desert plants.The activity change of POD was disscused in xerophytic plants and *Schefflera* plant leaf and illustrated in Figure 2. Unexpectedly, POD level of induction in either the xerophytes or *Schefflera* was relatively high and similar, with few exceptions This implies to the general sensitivity of POD biosynthetic pathway either in the xerophytic exposed to permenant environmental stress or the mesophytic plants exposed to toxic stress (Figure 2).

Malathione treatment seemed to be not very toxic to POD biosynthetic pathway in *Schefflera* leaf. The level of activity of POD under malathione treatment was 24.8 U

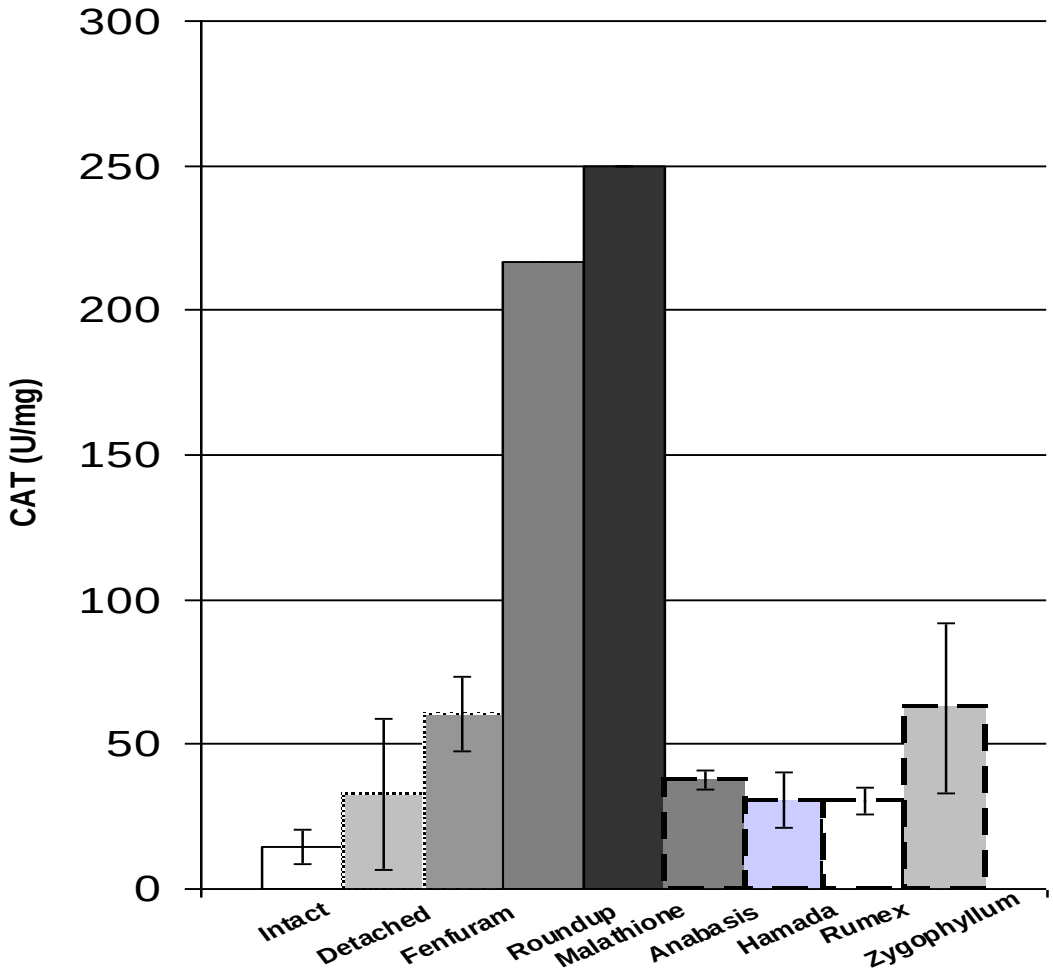

Figure 1. The variations in CAT enzyme content in *Schefflera* leaf and four desert plants. *Schefflera* leaf was incubated for 10-days in concentrations of the fungicide (Fenfuram), the herbicide (Roundup) and the insecticide (Malathione), the detached leaf was incubated in water (detached) and the intact leaf was control (intact). The desert plants are *Anabasis*, *Hamada*, *Rumex* and *Zygophyllum*. Error bars represented ± SD of the mean.

mg^{-1} Fwt. On the other hand, POD biosynthetic pathway was not sensitive to drought in *Rumex* sp. as POD value was significantly very low (17.8 U mg^{-1} F wt, Figure 2). SOD is a class of proteins including metal, it activated superoxide anions dismutation into H_2O_2 and molecular oxygen, preventing hydroxyl radicals (Scandalios, 1993). Compared to malathione which caused the lowest level of SOD induction (27.6 U mg^{-1} Fwt), the metabolic induction of SOD in *Schefflera* plant was sensitive to both fenfuram and round up treatments (43.2 and 89.9 U mg^{-1} F wt, respectively). This level of SOD activity upon malathione treatment had mimicked the same value in the detached *Schefflera* leaf and was 28.3 U mg^{-1} Fwt. Our data were in contrast to Ballester et al. (2005) who discovered that high humidity leads to both SOD and CAT increase in citrus fruit. Here, desscication and high temperature had affected desert plants by decreasing the level of antioxidants particularly CAT and SOD. The activity of SOD production in the four xerophytic plants had ranged between 22.2 and 32.4 U mg^{-1} Fwt. These values were still higher than the value of intact *Schefflera* palnt (14.5 U mg^{-1} Fwt, Figure 3). In agreement with the literature, SOD level of activity was decreased upon treaments with low doses of chromium and increased under higher concentrations (Sinha et al*.,* 2005). These data have provided a new insight into the different physiological strategies of plants to acclimate to their exposure to different stresses.

The activity level of GSH was decresed sponta-neousley, under both the drought stress and the toxic stress of pesticides (Figure 4). The activity level of GSH in intact and detached *Schefflera* leaf was higher than GSH level induced upon fenfuram and roundup treatments. It was reported (Drążkiewicz et al., 2003;

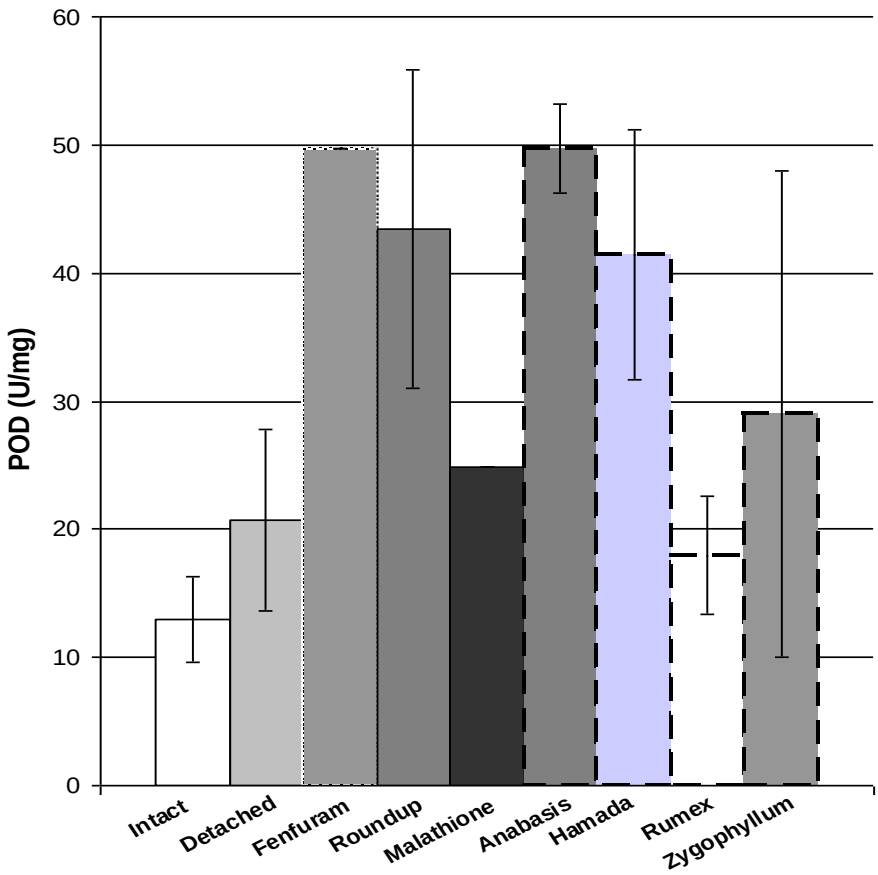

Figure 2. The variations in POD enzyme content in *Schefflera* leaf and four desert plants. *Schefflera* leaf was incubated for 10-days in concentrations of the fungicide (Fenfuram), the herbicide (Roundup) and the insecticide (Malathione); the detached leaf was incubated in water (detached) and the intact leaf was the control (intact). The desert plants are *Anabasis*, *Hamada*, *Rumex* and *Zygophyllum*. Error bars represented ± SD of the mean.

Khatun et al., 2008) that GSH level had declined below the control in response to external stress factors such as copper concentrations. Furthermore, it was suggested that the mechanism of antioxidant defense was facilitated by the depletion of GSH and its conversion to another form. This fact was further reached by Dazy et al. (2008) who observed that some enzymes participated in ascorbat-glutathione-cycle were decrease under stress conditions and thereby, lead to GSH inhibition. Here, the values of GSH per µmol g^{-1} Fwt were as follows: 0.87 in intact *Schefflera* leaf, 0.99 in detached *Sheflera*, 0.67 in fenfuram-treated *Schefflera* leaf, 0.45 in rounup-treated leaf and finally 1.2 in malathione-treated *Schefflera* leaf.

Levels of GSH activities in the xerophytic plants were low and determined per µmol g^{-1} Fwt as follows: 0.6 in *Anabasis*, 0.7 in *Hamada*, 0.7 in *Rumex*, whereas the lowest value was 0.35 detected in *Zygophyllum*.

It appeared from these data (Figure 4) that GSH induction in both intact and detached *Schefflera* leaf had exceeded GSH level of induction in case of fenfuram and roundup treatments. Furthermore, GSH level was high only with malathione treatment in *Schefflera* leaf despite that the level of lipid peroxidation (MDA) was significantly high with malathione treatment (Figure 5). We suggested that GSH was not playing a potent role in protecting against oxidative damage caused by either enviromental factors or toxic stress.

MDA is a decomposition product of polyunsaturated fatty acids hydroperoxides, a biomarker for lipid peroxidation in the leaf (Khatun et al., 2008) and an indicator of cell injury resulted from ROS. In addition, MDA is a quantity that reflected the degree of lipid peroxidation occured to the membrane (Wang et al., 1999). We illustrated data of MDA in *Schefflera* plant only and not in the xerophytes because xerophytes are not susceptible to lipid peroxidation within their native environment. The highest value of lipid peroxidation was observed in *Schefflera* leaf treated with fenfuram (1.22 nmol g^{-1} Fwt) followed by malathione (1.18 nmol g^{-1} Fwt). Traces of MDA (0.4 nmol g^{-1} Fwt) were detected in intact

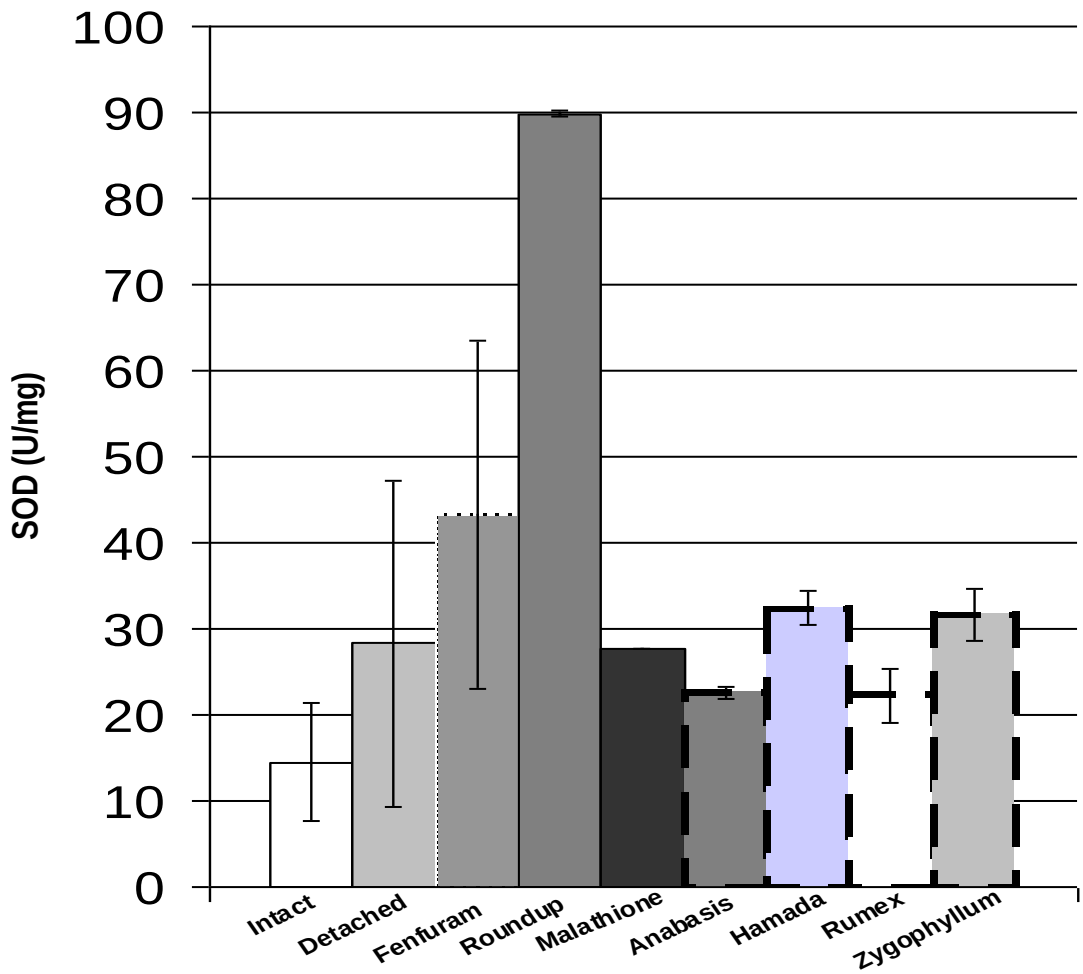

Figure 3. The variations in SOD enzyme content in *Schefflera* leaf and four desert plants. *Schefflera* leaf was incubated for 10-day in concentrations of the fungicide (Fenfuram), the herbicide (Roundup) and the insecticide (Malathione), the detached leaf was incubated in water (detached) and the intact leaf was the control (intact). The desert plants are *Anabasis*, *Hamada*, *Rumex* and *Zygophyllum*. Error bars represented ± SD of the mean.

Schefflera leaf, whereas the MDA value in a detached *Schefflera* leaf and in roundup-treated leaf was 0.66 nmol g^{-1} Fwt and 0.54 nmol g^{-1} Fwt, respectively (Figure 5).

Roundup treatment had triggered a lipid peroxidation decrease instead of an increase in *Schefflera* leaf. However, this could still be acceptable particularly that a progressive increase of most antioxidants enzymes was observed upon roundup treatments in *Schefflera* (Figures 1 to 3). These antioxidants must have scavenged ROS from exerting severe oxidative damages. It was previosly dicovered that preventing H_2O_2 from leaking out from the vacuole to the cytosol is a mechanism sometimes used for protection by antioxidant enzymes (Tang et al., 2010).

Using two-ways ANOVA test as statistical analyses to compare the level of activities of antioxidant enzymes either in intact, detached or treated leaf with pesticides, we see that the results were highly significant, as had recorded (0.0) at $p < 0.05$. On the other hand, the same data were non-significant (0.031) at $p < 0.01$. The SE values were ± 0.17 for lipid peroxidation, ± 13 for SOD, ± 0.12 for GHS, ± 6.96 for POD, and for CAT, SE ± 49.1. In terms of plants, the antioxidants had possessed SE ± 2.8 in SOD, SE ± 0.00 in GSH, SE± 6.97 in POD, and in CAT, SE ± 7.5. The ANOVA test had displayed 0.23 (non-significant) at $p < 0.05$ and was significant (0.001) at $p < 0.01$.

To understand the relationship between antioxidant enzymes activities in xerophytes and their antimicrobial role, a series of different concentrations from the crude extracts of these xerophytes was applied separately to cultures of seven common important pathogenic strains of bacteria grown in Petri dishes under sterile conditions. The inhibition zone per mm was measured and listed in Table 1. The area of the inhibition zones throughout the treatments was generally moderate. *Hamada* extract was lacking any inhibitory impacts on bacteria growth, even

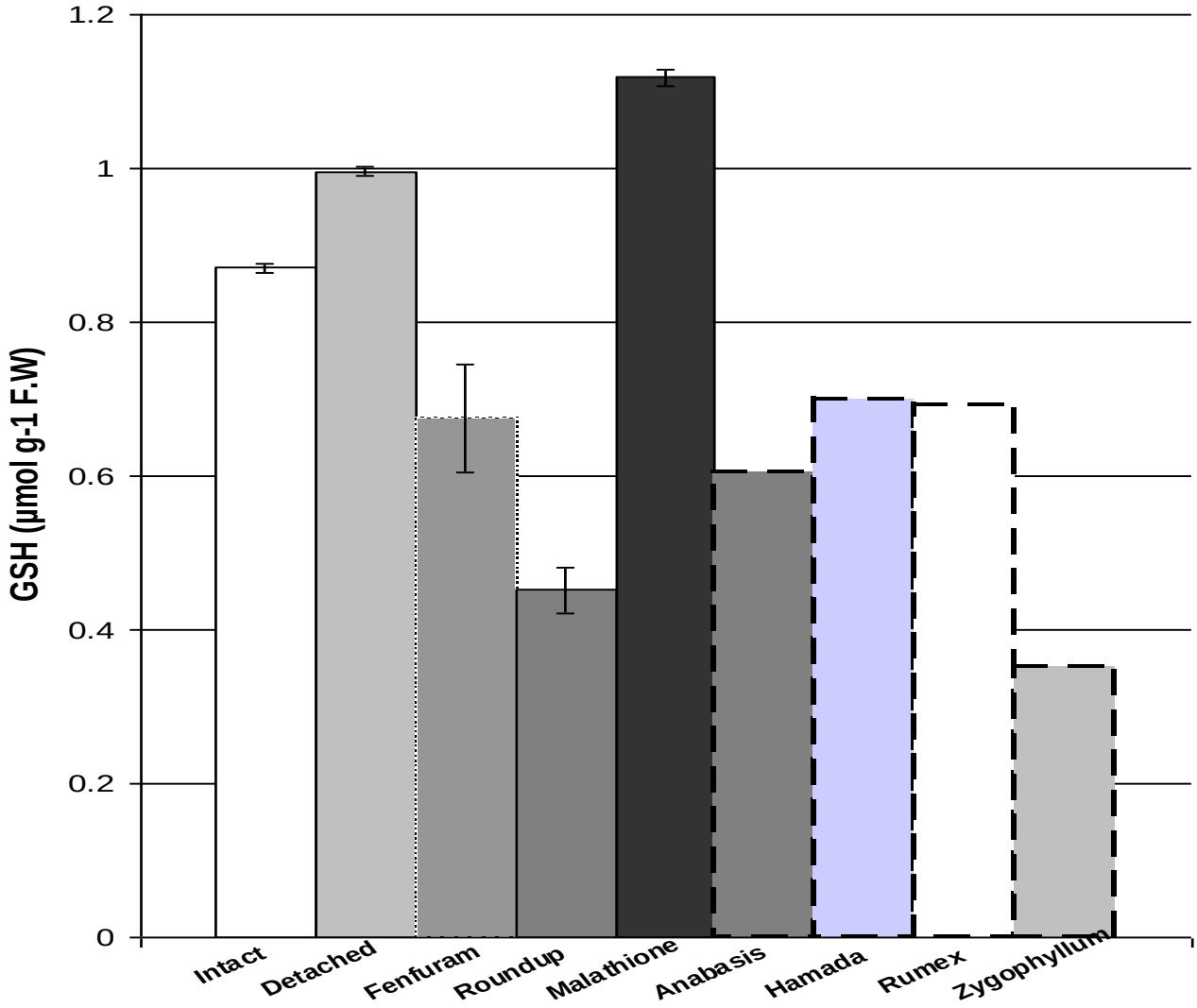

Figure 4. The variations in GSH enzyme content in *Schefflera* leaf and four desert plants. *Schefflera* leaf was incubated for 10-days in concentrations of the fungicide (Fenfuram), the herbicide (Roundup) and the insecticide (Malathione), the detached leaf was incubated in water (detached) and the intact leaf was the control (intact). The desert plants are *Anabasis*, *Hamada*, *Rumex* and *Zygophyllum*. Error bars represented ± SD of the mean.

with high concentrations.

On the other hand, *Zygophyllum* extract caused growth-inhibition of two virulent bacteria strains (*S. aureneus* and *P. aerogenosa*). In most of the cases, the inhibition zone was concentration-dependent manner (Table 1). *Anabasis* and *Rumex* showed inhibitory disc diffusion against three bacterial strains. Both plants had shared inhibitory effects against *B. subtilis* and *Endo-bacter*. The inhibition zone was even larger in *Anabasis* than in *Rumex*. The growth of *E. coli* was sololy inhibited by *Anabasis* extract (Table 1).

Desert plants have shown significant differences in their potentiality as acting as antimicrobial agents. The values were zero both at $p < 0.05$ and $p < 0.01$. In *Anabasis* sp. SE±1.17, in *Hamada* sp. SE±0.00, in *Rumex* sp., SE±0.86 and in *Zygophyllum* sp. SE±0.45.

Conclusion

In the present study, the activity levels of antioxidant enzymes were low in drought-endemic plants (desert plants). The highest activity level of CAT was detected in response to malathione applications in *Schefflera* plant leaf, followed by roundup application whereas fenfuram was not CAT-inducing pesticide. POD level of induction was high in *Anabasis* sp. and *Hamada* sp. with values similar to those determined under the effects of fenfuram and roundup in *Schefflera*. SOD was induced under only roundup treatment in *Schefflera* leaf. GSH was not an efficient bio-indicator for pesticide stress as its level was not significantly changed. Owing to the antioxidant enzymes protection conferred to *Schefflera* plants treated with roundup, the lipid peroxidation (MDA) value was

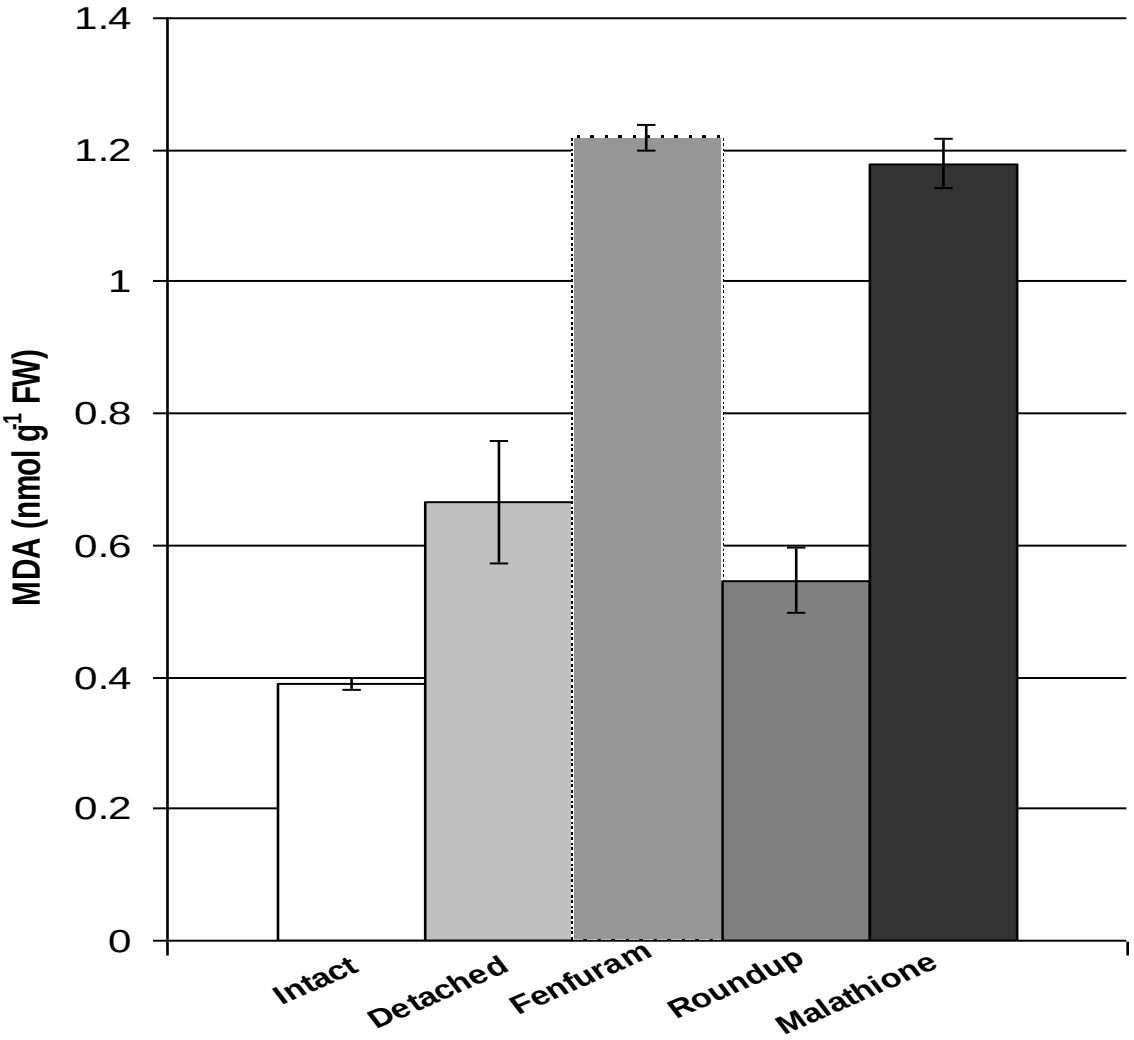

Figure 5. Effect of treatments with different pesticides on MDA concentration of *Schefflera* leaf. The leaf petiole was dipped for 10-days in concentrations of the fungicide (Fenfuram), the herbicide (Roundup) and the insecticide (Malathione). The detached leaf was incubated in water (detached) and the intact leaf was the control (intact). Error bars represented ± SD of the mean.

Table 1. The antimicrobial activities measured using the disc diffusion assay of some desert plants against seven virulent bacteria (*B. megaterium, B. subtilis, E. coli*, *S. aureus*, *Enterobacter*, *K. pneumoniae and P. aerogenosa*).

	Dry wt (mg/ml)			
Bacteria	***Rumex* sp.**	***Anabasis* sp.**	***Zygophyllum* sp**	***Hamada* sp.**
Strain	**5, 15, 25, 35**	**5, 15, 52, 35**	**5, 15, 52, 35**	**5, 15, 25, 35**
B. megaterium	-, -, 9, 9	Null	Null	Null
B. subtilis	9, 11, 11, 11	12, 13, 13, 13	Null	Null
E. coli	Null	10, 13, 15, 15	Null	Null
S. aureneus	Null	Null	9, 10, 10, 10	Null
Endobacter	7, 7, 7, 7	10, 10, 10, 10	Null	Null
K. pneumoniae	Null	Null	Null	Null
P. aerogenosa	7, 9, 9, 9	Null	8, 8, 9, 9	Null

* Each value is a mean of three replicates. Series of concentrations; 5, 15, 25 and 35 mg/ml from crude extracts of desert plants (*A. articulate, H. elegans, R. dentatus, Z. album*) was applied to bacterial cultures and the inhibition zones were measured in mm.

relatively very low in the leaf.

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