EFFECT OF SALINITY ON THE SPECIFIC ACTIVITIES OF LIGNINOLYTIC ENZYMES IN *Ganoderma* sp. CULTURES

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EFFECT OF SALINITY ON SPECIFIC ACTIVITIES OF ANTIOXIDATIVE ENZYMES IN *Homalomena sp.* CULTURES

By

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AOS	active oxygen species
APx	ascorbate peroxidase
BSA	Bovine Serum Albumin
CAT	catalase
DNA	deoxyribonucleic acid
GR	glutathione reductase
H_2O_2	hydrogen peroxide
Mg/L	miligram per liter
MS	Murashige and Skoog
NaCl	sodium chloride
$OH^{\bullet}$	hydroxyl radical
$O_2^{\bullet-}$	superoxide radical
$^1O_2$	singlet oxygen
POD	peroxidase
ROS	reactive oxygen species
SOD	superoxide dismutase
g/L	gram per liter
ml	millimeter
mM	milimolar
rpm	revolution per minute
µl	microliter

ABSTRACT

Salinity stress can cause the formation of reactive oxygen species (ROS) which disrupt the metabolic processes in plants. The plant possess enzymatic antioxidant and non-enzymatic antioxidant defence mechanism to prevent the formation of ROS. The objective of this experiment was to investigate the effect of different concentrations of NaCl on the specific activities of antioxidative enzymes in *Homalomena sp.* cultures. *Homalomena* cultures were treated with 0, 25, 50 and 100mM of NaCl for 28 days in Murashige and Skoog (MS) solid medium. Ascorbate peroxidase (APx), catalase (CAT) and peroxidase (POD) specific activities were measured on the 0, 1, 2, 7, 14 and 28 days of treatment periods. NaCl treatment reduced APx specific activity to different extent especially at 24 hours of treatment. Longer treatment periods significantly lowered the APx specific activities. CAT and POD specific activities initially increased in treated leaves up to 7 days and decreased significantly at the later stages of treatment periods. These results suggest that salinity treatment stimulates oxidative stress in *Homalomena sp.* cultures by initially inducing the POD and CAT specific activities associated with the decreased in APx specific activities especially at the later stage of treatment periods.

KESAN SALINITI PADA AKTIVITI SPESIFIK ENZIM ANTIOKSIDATIF PADA KULTUR *Homalomena spesies*

ABSTRAK

Tegasan saliniti menyebabkan penghasilan spesies oksigen reaktif (ROS) yang menganggu proses metabolik dalam tumbuhan. Tumbuhan mempunyai mekanisma pertahanan antioksidan enzimatik dan bukan enzimatik untuk menghalang penghasilan ROS. Tujuan kajian ini dijalankan adalah untuk melihat kesan pelbagai kepekatan NaCl terhadap aktiviti spesifik enzim antioksidatif dalam kultur *Homalomena sp.* Kultur *Homalomena sp.* dirawat dengan 0, 25, 50 dan 100mM NaCl untuk 28 hari dalam media pepejal Murashige and Skoog (MS). Aktiviti spesifik enzim askorbat peroksida (APx), katalase (CAT) dan peroksida (POD) telah diukur pada 0, 1, 2, 7, 14 dan 28 hari rawatan. Rawatan NaCl mengurangkan aktiviti spesifik enzim APx terutamanya 24 jam selepas rawatan. Peningkatn tempoh rawatan mengurangkan aktiviti spesifik enzim APx. Aktiviti spesifik enzim POD dan CAT pada mulanya meningkat dalam kultur rawatan sehingga hari ke tujuh dan menurun secara signifikan pada akhir tempoh rawatan. Keputusan kajian menunjukkan rawatan saliniti merangsang tegasan oxidatif dalam kultur *Homalomena sp.* dengan meningkatkan aktiviti spesifik enzim POD dan CAT semasa permulaan rawatan dan penurunan aktiviti spesifik enzim APx terutamanya pada akhir tempoh rawatan.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Free radicals and other active derivatives of oxygen are inevitable by-products of biological redox reactions. Reactive oxygen species (ROS) inactivate enzymes and damage important cellular components. The increased production of toxic oxygen derivatives is considered to be a universal or common feature of stress conditions. Plant and other organisms have evolved a wide range of mechanisms to contend with this problem (Ajay *et al.*, 2002).

The antioxidant defence system of the plant comprises a variety of antioxidant molecules and enzymes. The capacity and activity of the antioxidative defence system are important in limiting oxidative damage and in destroying ROS that are produced in excess of those normally required for metabolism (Ajay *et al.*, 2002).

A variety of environmental stress (such as chilling, ozone, high light, drought and heat) can severely damage crop plant with consequent high yield losses. A common factor in all these adverse conditions is the occurrences of oxidative stress. ROS can react rapidly with deoxyribonucleic acid (DNA), lipids, and proteins, with cellular damage as a result. Under normal growth conditions, ROS are efficiently scavenged by both enzymatic and nonenzymatic detoxification mechanisms. Nevertheless, during prolonged stress conditions, the defense system becomes saturated and cellular damage is inevitable. The key players in the defense system are superoxide dismutase (SOD), ascorbate peroxidase (APx), catalase (CAT), and glutathione reductase (GR). These antioxidants enzymes directly eliminate ROS (Frank and Inze, 2002).

ROS are generated in plant cells during normal metabolic processes. The photosynthetic electron transport system is the major source of active oxygen in plant tissues, having potential to generate singlet oxygen, $^1O_2$ and superoxide, O_2^- radical. The production of active oxygen is an unavoidable consequence of the operation of

the photosynthetic electron transport chain in an oxygen atmosphere (Ajay *et al.,* 2002).

Oxidative stress is essentially a regulated process, the equilibrium between the oxidative and antioxidative capacities determining the fate of the plant. Under nonstressful conditions the antioxidant defence system provides adequate protection against active oxygen and free radicals. Both natural and man-made stress situations provoke increased production of toxic oxygen derivatives. Some important sites such as the reaction centre protein of PSII (DI) and the apoplastic space appear to have very little protection against oxidative damage (Ajay *et al.,* 2002).

Saline environments are generally correlated with changes in plant lipid metabolism (Kuiper 1985). Salinity reduces substrate water potential, thereby restricting water and nutrient uptake by plants; salinity may also cause ionic imbalance and toxicity. Because substrate salinity fluctuates through the growing season, a plant may be exposed to different salinity levels, at various stages of development, with potentially significant consequences on population dynamics (Gilles *et al.*, 2000). High salinity levels in the external medium are known to affect many physiological and metabolic processes, leading to cell growth reduction (Ashraf *et al.*, 2004).

In this research, *Homalomena sp.* cultures (family araceae) were hizomatous or tuberous herbs. It comprises of 110 genera and 1800 species (pick5.pick.uga.edu/mp/). *Homalomena sp.* is also an ornamental plant, as a living decoration in aquarium and can also balancing water condition in aquarium. *Homalomena* is a perennial type of garden plant found worldwide, especially in the tropical and humid climates of Central America and Asia. It can reach a height of approximately nine inches, with dark, heart-shaped green leaves (http://toptropicals.com/catalog/uid/homalomena_sp.).

1.2 Objective

Antioxidant enzymes play an important role in adaptation to stress conditions. Consequently, the aim of this experiment was to examine the effect of different concentrations of NaCl on the specific activities of antioxidative enzymes, such as catalase (CAT), ascorbic peroxidase (APx) and peroxidase (POD) in *Homalomena sp.* cultures.

CHAPTER 2

LITERATURE REVIEW

2.1 Family of *Araceae*

The *Araceae* are rhizomatous or tuberous herbs comprising about 110 genera and 1,800 species. The hallmark of the *Araceae* is the spathe and spadix inflorescence, a floral structure consisting of a petal-like leaf (the spathe) and a flower-bearing protuberance (the spadix). An individual flower may be bisexual or unisexual, depending on the genus. The spathe might look and behave like a rather odd petal, but it is in fact a modified leaf. Many species betray this origin by having spathes that are green and leaf-like or that turn green and photosynthesis after flowering (Bown, 2000).

In spite of the diversity of sizes, shapes, colours, textures and odour in aroid apathe and spadix, all but the most eccentric are quite easily recognizable as such, leaving little doubt that the plant in question is an aroid. . Most species with compound leaves do not produce their most ornate foliage until plants are mature, so a plant may have leaves of different ages with three, five, seven, or more main lobes or leaflets. Leaf shape also may vary according to conditions, both in the wild and cultivation (Bown, 2000).

Naturally variegated species abound and a high proportion of tropical rain forest species have fascinating glossy or velvety textures. Not only the leaf blades, but even the stalk and undersides of veins are highly ornamented; striped, mottled, bristled, frilled, prickled and all shades imaginable (Bown, 2000).

A high proportion of aroids are aquatic and semi-aquatic. Aroids are found from tropical swamp forests, clear acid blackwater rivers, and tidal areas to temperate ponds and wet woodland. Many of the most popular ornamental plants for aquatic are aroids (Bown, 2000).

2.1.1 *Homalomena species*

The genus *Homalomena* is undergoing revision (Hay, 1999). During the 1990s the number of species was estimated at about 80 (Hay, 1990), nearer 110 (Mayo *et al.*, 1997), and more probably in the region of 150. Most occur in southern Asia and the southwestern Pacific region, but 11 species are known from South America. Many species are rather insignificant and difficult to tell apart, yet some are very striking and can vary greatly in appearance. Several of the South American species are downy (Hay, 1990).

In some Southeast Asian rain forests *Homalomena* are the commonest aroid, and many species can be found within a short distance in clumps or small colonies on the forest floor or on rocks. One such area is Harau in West Sumatra, a valley formed by a fault and walled with sheer cliffs more than 130 ft. (40 m) high, down which waterfalls plunge as spray into pools way below (Hay, 1990).

Cultivation of *Homalomenas* is very few. The best known is *Homalomena wallisii,* as shown in figure 1 (c) from Colombia; it looks like a squat dieffenbachia, with oval leaves heavily splashed with yellow and neat clumps of foliage at ground level. *H. lindenii* also is seen occasionally in collections. It is found wild in New Guinea and has long-stalked olive green heart-shaped leaves and yellow midrib and veins that are almost luminous when wet. In common with many *Homalomenas*, the plant smells strongly of anise. Several New Guinea *Homalomenas* are variegated and some species from that part of the world are also known to be hallucinogenic (Bown, 2000).

The only other genus in the tribe Homalomeneae is *Furtadoa*, which was first described in 1981 (Hotta, 1981). Native to the Malay Peninsula and Sumatra, it has only two species. Both are small creeping plants with elliptic evergreen leaves and boat-shaped, green persistent spathes. The floral anatomy is interesting; both species have rather large zones of female flowers, extending half the length of the spadix, and male flowers with a central pistiilode. *Furtadoa mixta* dwells on the rainforest floor, while the smaller *F. sumatrensis* is a rheophyte, growing on rocks in stream in Sumatra (Bown, 2000).

Figure 1: (a) *Homalomena emerald* Gem, (b) *Homalomena rubescens* and (c) *Homalomena wallisii*

2.2 Oxidative stress tolerance in plant

To limit cellular damage cause by excessive ROS levels, plants have evolved a broad variety of nonenzymatic and enzymatic protection mechanism that efficiently scavenges ROS (Van Montagu, 1995). The best known nonezymatic antioxidants are ascorbate, glutathione, α-tocopherol and carotenoids. They are present in relatively high concentrations within plant cells (Alscher, 1993). While enzymatic antioxidant are ascorbate peroxidase (APx), glutathione reductase (GR), peroxidase (POD), superoxide peroxidase (SOD), and catalase (CAT).

2.2.1 Generation of toxic reactive oxygen species and associated regulatory mechanisms

Molecular oxygen is produced as a result of the oxidation of water by the photosynthetic electron transport chain. The latter, however, can also use oxygen as an electron acceptor. In addition, molecular oxygen is assimilated during photorespiration producing phosphoglycollate. Both of these reactions have positive and negative effects. Superoxide, produced by the transport of electrons to oxygen, is not compatible with metabolism and must be eliminated by the antioxidative defence system while recycling of phosphoglycollate to phosphoglycerate (in order to reenter the Benson Calvin cycle) results in a considerable loss of assimilated carbon. In addition, large amounts of H_2O_2 are produced during the oxidation of the glycollate in the peroxisomes (Rich and Bonner, 1978).

Although much of this H_2O_2 is destroyed by catalase, some chemical decarboxylation of keto acids by H_2O_2 still occurs (Zelitch, 1990). Nevertheless, photosynthesis benefits since photorespiration protects the photosynthetic membrane against light-induced damage at times when carbon assimilation is limited (Egneus *et al.*, 1975; Heber *et al.*, 1978). This may indeed be regarded as the principal function of photorespiration, which is far more effective than electron transport to oxygen (termed pseudocyclic electron flow or the Mehler reaction) in protecting against photoinhibition (Wu *et al.*, 1991).

2.2.1.a Formation of singlet oxygen

The chlorophyll pigments associated with the electron transport system are the primary source of singlet oxygen, $^1O_2$. Singlet oxygen may also arise as a by-product of lipoxygenase activity. Like the hydroxyl radical, $OH^{\bullet}$, $^1O_2$ is highly destructive, reacting with lost biological molecules at near diffusion-controlled rates (Knox and Dodge, 1985; Cadenas, 1989). The lifetime of excited chlorophyll singlet state is short within these aggregates, but varies according to physiological conditions. The excited singlet state of chlorophyll is used for the transfer of energy or electrons. However, there are two other possible routes of electron excitation, radiative decay (fluorescence) and conversion to the triplet chlorophyll state. The latter interacts with oxygen to produce $^1O_2$ (Rich and Bonner, 1978).

There are two strategies for defence against $^1O_2$ in the thylakoid membranes. The first is the regulation of the light-harvesting apparatus to minimize triplet chlorophyll production, and the second is the rapid quenching of both the triplet chlorophyll state and $^1O_2$ by membrane- bound quenchers. Two major processes decrease the lifetime of excited singlet-state chlorophyll; the first is photochemistry and electron transport in the reaction centres and the second process involves thermal dissipation of excess excitation energy that quenches singlet- excited chlorophyll to the ground state. Thermal energy dissipation plays a pivotal role in photoprotection since it limits the rate of reduction of the first stable electron acceptor of PSII (QA) (Rich and Bonner, 1978).

2.2.1.b Superoxide production

Photo-reduction of dioxygen in chloroplasts was first shown by the production of acetaldehyde in the presence of ethanol and catalase and the photo-reduced product was assumed to be hydrogen peroxide (Mehlar, 1951). Under most circumstances, the control of electron flow between PSII and PSI regulates the reduction state of the acceptor side of PSI. This means that the redox state of PSI acceptors does not significantly limit electron transport (Harbinson and Hedley, 1993). The regulated activation of Benson–Calvin cycle and control of the rate of electron flow are

important factors determining the redox state of the ferredoxin pool (Foyer *et al.*, 1990; Harbinson and Hedley, 1993).

There are two sites of $O_2^{\bullet-}$ production on the reducing side of PSI (Mehlar, 1951; Badger, 1985). The majority of O_2 reduction *in vivo* is thought to proceed via reduced ferredoxin (Fdred), which reduces molecular oxygen to the superoxide radical;

$$2O_2 + 2Fd_{red} \longrightarrow 2O^{\bullet}_2{}^{-} + 2Fd_{ox}$$

Hydrogen peroxide is then formed through dismutation of $O_2^{\bullet-}$;

$$2O^{\bullet}_2{}^{-} + 2H^{-} \longrightarrow H_2O_2 + O_2$$

The latter occurs spontaneously, but the velocity of the reaction is greatly increased by SOD;

$$2O^{\bullet}_2{}^{-} + 2H^{-} \xrightarrow{SOD} H_2O_2 + O_2$$

2.2.1.c Production and scavenging of hydrogen peroxide in chloroplasts

Hydrogen peroxide is produced by the dismutation of superoxide radicals in a reaction mostly catalysed by superoxide dismutase (Asada *et al.*, 1974). In leaf cells, catalase is exclusively localized in peroxisomes and has not been found in chloroplasts. The hydrogen peroxide in chloroplasts is scavenged by a peroxidase reaction using the photo-reductant produced in the thylakoid as the electron donor (Nakano and Asada, 1981; Asada and Badger, 1984). Thus, diffusion of hydrogen peroxide from chloroplasts to peroxisomes and its scavenging by catalase are very unlikely to occur. The electron donor for the peroxidase reaction has been identified as ascorbate (Foyer and Halliwell, 1976).

$$2H_2O_2 \xrightarrow[\text{Peroxisomes}]{\text{Catalase}} 2H_2O + O_2$$

$$H_2O_2 + \text{Ascorbic acid} \xrightarrow[\text{Chloroplast}]{\text{Ascorbate peroxidase}} H_2O + \text{Mono-dehydro-ascorbic acid}$$

2.2.1.d Hydroxyl radical: The most reactive oxidant in cells

Hydrogen peroxide and superoxide radical ($O^{\bullet}_2^-$) by themselves are relatively less damaging, but they can form species damaging the essential cellular components such as hydroxyl radicals ($OH^{\bullet}$) that can initiate lipid peroxidation and also attack DNA, proteins and many small molecules. As according to Haber and Weiss (1934), the hydroxyl radical ($OH^{\bullet}$) are identified as the oxidizing species in these reactions;

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{\cdot} + OH^-$$

The availability of reduced ferrous ion in biological systems may limit the reaction, but ferric ion can be recycled to reduced ferrous state by reducing agents such as $O^{\bullet}_2^-$;

$$Fe^{3+} + O_2^- \longrightarrow O_2 + Fe^{2+}$$

When these mechanisms are summarized, therefore can form as;

$$H_2O_2 + O_2^- \xrightarrow[\text{Haber- Weiss reaction}]{Fe^{2+},\ Fe^{3+}} OH^{\cdot} + OH^- + O_2$$

Superoxide and hydrogen peroxide will form the destructive hydroxyl radical in the presence of trace amounts of iron ion, and initiate the oxidation of organic substrates.

2.2.1.e Oxidation of organic substrates by hydroxyl radical

Two possible reactions oxidation of organic substrates may proceed by; addition of $OH^{\bullet}$ to an organic molecule, or abstraction of a hydrogen atom from it. In the addition reaction the $OH^{\bullet}$ add to organic substrate forming a hydroxylated product, which is further oxidized by Fe^{3+} ion, O_2 or other agents to a stable oxidized product. The hydroxylated product can also dismutate to form cross-linked products (Rich and Bonner, 1978).

2.3 Antioxidant system in plants

Oxidative stress is a general term used to describe the steady state level of oxidative damage in a cell, tissue, or organ, caused by the reactive oxygen species (ROS). This damage can affect a specific molecule or the entire organism. ROS, such as free radicals and peroxides, represent a class of molecules that are derived from the metabolism of oxygen and exist inherently in all aerobic organisms. There are many different sources by which the ROS are generated (www.genox.com).

The aspect of salinity stress in plants is the stress induced production of reactive oxygen species (ROS) including superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$). ROS are a product of altered chloroplast and mitochondrial metabolism during stress. These species cause oxidative damage to different cellular components including membrane lipids, proteins and nucleic acids. The alleviation of this oxidative damage could provide enhanced plant resistance to salt stress (Haliwell and Gutteridge, 1986).

The oxidative stress defense mechanisms in plants are includes superoxide dismutases (SOD), ascorbate peroxidase (APx), catalase (CAT), glutathione reductase (GR) and also peroxidases POD. Because of hydroxyl radicals are too reactive to be directly controlled, aerobic organisms prefer to eliminate the less reactive precursor forms, such as superoxide and H_2O_2, and hence prevent the formation of hydroxyl radicals. SOD scavenges superoxide radicals, whereas catalase and peroxidase remove H_2O_2. Other enzymes that are inolved in the removal of AOS are monodehydroascorbate reductase, dehydroascorbate reductase and glutathione peroxidase (Noctor and Foyer, 1998).

2.3.1 Ascorbate Peroxidases (APx)

Ascorbate is present in chloroplasts, cytosol, vacuole and apoplastic space of leaf cells in high concentrations (Foyer *et al.*, 1991; Polle *et al.*, 1990). It is perhaps the most important antioxidant in plants, with a fundamental role in the removal of hydrogen peroxide (Foyer, 1993). Oxidation of ascorbate occurs in two sequential steps, first

producing mono-dehydroascorbate, and if not rapidly re-reduced to ascorbate, the mono-dehydro-ascorbate disproportionates to ascorbate and dehydro-ascorbate (Figure 3).

Ascorbate peroxidase activity has mainly been reported from chloroplast and cytosol (Chen and Asada, 1989). However some recent studies have also reported its occurrence in mitochondria as well (Gomez, 1999; Anderson, 1995). In the chloroplasts, SOD and ascorbate peroxidase enzymes exist in both soluble and thylakoid-bound forms. Superoxide generated at the membrane surface can thus be trapped and converted immediately to H_2O_2 to be scavenged by the membrane bound ascorbate peroxidase (Nakano and Asada, 1980; Anderson, 1983).

Isolated intact chloroplasts rapidly metabolize exogenously added H_2O_2 (Nakano and Asada, 1980; Anderson, 1983), indicating that *in situ* the chloroplasts may eliminate H_2O_2 generated both internally and externally. Two enzymes are involved in the regeneration of reduced ascorbate, namely mono-dehydro-ascorbate reductase (E.C. 1.6.5.4) which uses NAD(P)H directly to recycle ascorbate and dehydro-ascorbate reductase. However, the situation is further complicated because mono-dehydro-ascorbate itself is an efficient electron acceptor (Foyer, 1993). Mono-dehydro-ascorbate is reduced directly to ascorbate using electrons derived from the photosynthetic electron transport chain as follows:

$$4\ \text{Mono-dehydro-ascorbate} + 2H_2O \xrightarrow[\text{Electrontransport}]{\text{Light}} 4\ \text{Ascorbate} + O_2$$

The regeneration of ascorbate within the chloroplast provides a putative mechanism for the regulation of electron transport (Neubauer and Schreiber, 1989). This is a potent mechanism for preventing photo-oxidation. The Mahler peroxidase reaction sequence helps to generate the low lumen pH values required for the formation of zeaxanthin (Hager, 1969; Pfundel and Dilley, 1993). This xanthophyll pigment has been consistently shown to be involved in the mechanisms of thermal energy dissipation (Demmig-Adams and Adams, 1992).

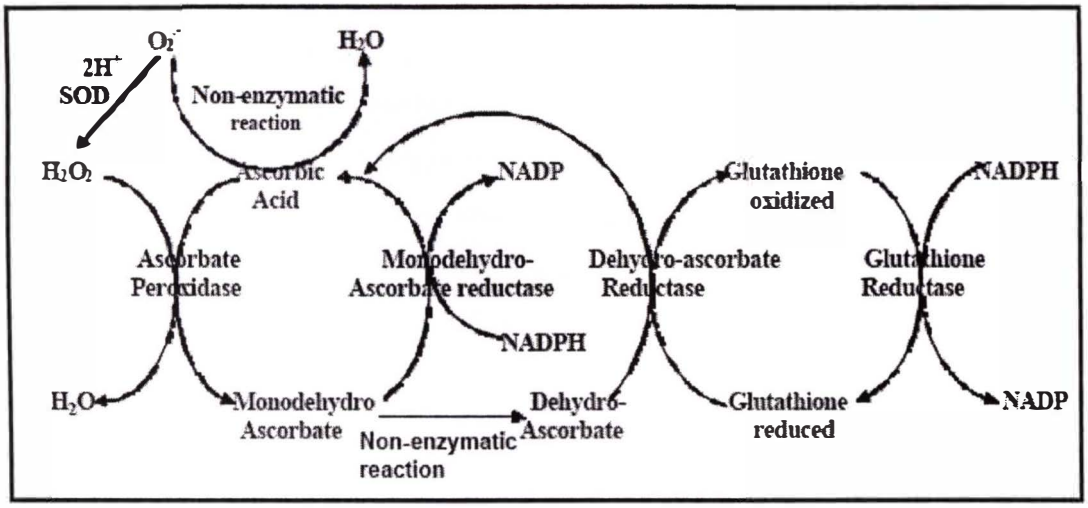

Figure 3: Asada–Halliwell pathway of hydrogen peroxide scavenging and ascorbic acid regeneration involving various antioxidant enzymes. (Source: Foyer, 1993).

2.3.2 Catalases (CAT)

Plants, unlike animals, have multiple forms of catalases (H_2O_2: H_2O_2 oxidoreductase) that are mainly found in peroxisomes and glyoxisomes. Catalases activity was also found in the mitochondria of maize. Catalases directly consume H_2O_2 or oxidize substrates such as methanol, ethanol, formaldehyde, and formic acid. There are some striking similarities in the organization of the catalase gene family in different species (Willekens *et al.*, 1994: Guan *et al.*, 1996).

Catalases can be divided into three classes according to their expression. Class I is most prominent in photosynthetic tissues, where they are involved in the removal of photorespiratory H_2O_2. Class II catalases are highly expressed in vascular tissues, where they might play a role in lignifications, but their exact biological role remains unknown. Class III is abundant only in seeds and young seedlings and its activity is linked to the removal of excessive H_2O_2 that is produced during fatty acid degradation in the glyoxylate cycle in the glyoxisomes (Ashraf *et al.*, 2004).

2.3.3 Peroxidase (POD)

Peroxidases are ubiquitous enzymes found in plants. Besides the peroxidases, whose oxidation products play mainly physiological roles (lifnification, cross-linking of cell wall matrices), a second class is also part of the AOS defense system (Maranon *et al.*, 1994).

Peroxidases are haem-containing glycoproteins found in animal and plant tissues, as well as in microorganisms. There is a family of class III plant peroxidases (POX, EC 1.11.1.7) encoded by a large multigene family that comprises a number of peroxidase isoenzymes. Generally, peroxidases catalyse the oxidoreduction between hydrogen peroxide and reductants (Hiraga *et al.*, 2001). Catalytic mechanism involves the formation of two intermediates called compounds I and II, which can react with organic co-substrates, finally producing the radical product (Maranon and Huystee, 1994). Since a variety of organic and inorganic molecules can be oxidised by peroxidases it has been suggested that peroxidases play an important role in a wide

range of biochemical processes. As reported by Gaspar *et al.* (1991), peroxidases are involved in auxin and ethylene metabolism, redox reactions in plasma membranes, cell wall modifications (lignification and suberinization) as well as in developmental and defence processes.

2.4 Salinity effects on aquatic plant

Salinity imposes two stresses on plant tissues: a water-deficit that results from the relatively high solute concentrations in the soil, and ion-specific stresses resulting from altered K^+/ Na^+ ratios and Na^+ and Cl^- concentrations that are inimical to plants. As salinity stress is a continuing and increasingly deleterious obstacle to the growth and yield of crop plants, owing to irrigation practices and increasing demands on fresh water supply, the engineering of salt tolerant crop plants has been a long-held and intensively sought objective (Apse and Blumwald, 2002).

The generation of toxic oxygen species is increased under stress conditions (Elstner *et al.,* 1988). Plants which are exposed to severe stress have been shown to increase susceptibility to photo-inhibition with subsequent development of chlorosis. Photo-oxidative damage is exacerbated by salinity stress (Wise and Naylor, 1987).

Salinity presents a stress condition for growth of the plants. Under natural conditions of growth and development, plants are inevitably exposed to different types of stress, which may cause increased production of active oxygen species (AOS) (Smirnoff, 1993).

Plants have evolved various protective mechanisms to eliminate or reduce AOS. In plant cells, one such protective mechanism is an antioxidant system, composed of both non-enzymatic and enzymatic antioxidants (Foyer *et al.,* 1994b). The capacity of the antioxidant defense system is often increased under stress conditions (Gressel and Galun, 1994), but in most situations the response is moderate (Foyer *et al.*, 1994a).

Salinity is an ecological factor of considerable importance, influencing the types of organisms that live in a body of water. As well, salinity influences the kinds of plants

that will grow either in a water body, or on land fed by water (or by a groundwater). Salt is difficult to remove from water, and salt content is an important factor in water use (such as potability) (http://en.wikipedia.org./wiki/Salinity).

When a plant experiences environmental stress such as salinity, the critical balance between the formation of active oxygen species (AOS) and quenching activity of the antioxidants is disturbed. AOS generated due to salt stress unbalances the cellular redox system in favor of oxidized forms resulting in oxidized damage to lipids, proteins and nucleic acid. Plant possesses a number of antioxidant enzymes and osmoprotectants to protect themselves from these potential cytotoxic effects (Halliwell and Gutteridge, 1989).

2.4.1 Effects of salinity on the antioxidative enzymes and antioxidants

Activities of antioxidative enzymes such as ascorbate peroxidase, glutathione reductase, monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and Mn-SOD increase under salt stress in wheat, while Cu/ Zn-SOD remains constant and total ascorbate and glutathione content decrease (Hernandez *et al.*, 2000).

Lechno *et al.*, (1997) reported that NaCl treatment increases the activities of the antioxidative enzymes such as catalase and glutathione reductase and the content of the antioxidants ascorbic acid and reduced glutathione but does not affect the activity of SOD in cucumber plants.

In radish, NaCl stress decreases praline oxidase activity and increases protease, gamma glutamyl kinase, and ATPase activities (Muthukumarasamy *et al.,* 2000). According to Liang, (1999) SOD activity in plant leaves of barley and H^+ ATPase activity in plant roots increase by salinity, whereas malondialdehyde (MDA) concentration in plant leaves decreases.

Four other key enzymes involved in oxdative stress as tissue degradation (catalase, polyphenol oxidase, SOD, and lypoxygenase) are also significantly increased as a result of NaCl treatment (Kennedy and Fillippis, 1999).

CHAPTER 3

MATERIALS AND METHODS

3.1 Plant materials

Cultures of *Homalomena sp.* were obtained from Biotechnology Laboratory (Institute of Oceanography (INOS), UMT) (Plate 1 c and b). Rhizomes of *Homalomena sp.* were transferred onto a fresh Murashige and Skoog (MS) medium (plate 1 a). Subsequent subcultures were carried out every 2 months and cultures were incubated in 12h/ 12h (light/ dark) photoperiod under cool, white fluorescent lamps at 27 $\pm$ 2°C.

3.2 Preparation of culture media

Culture media used was Murashige and Skoog (1962) basal medium (appendix 1) supplemented with B5 vitamin, 2-IP hormone and 30 g/L sucrose. The culture medium was solidified with 2.5 g/L gelrite agar and was adjusted to pH 5.7 – 5.8, prior to autoclaving (HVE-50 Hiclave TM) at 121° C for 15 minutes.

3.3 NaCl treatment

Treatment medium contained Murashige and Skoog (1962) basal medium added with the appropriate amount of NaCl to prepare a final concentration of 0, 25, 50, and 100 mM NaCl (Plate 1 c and d). 15 ml of treatment medium of each concentration was poured into each flat bottom culture tube and the medium was autoclaved at 121° C for 15 minutes.

Ascorbate peroxidase, catalase and guaiacol peroxidase specific activities were measured every 0, 1, 2, 7, 14, and 28 days of treatment periods.

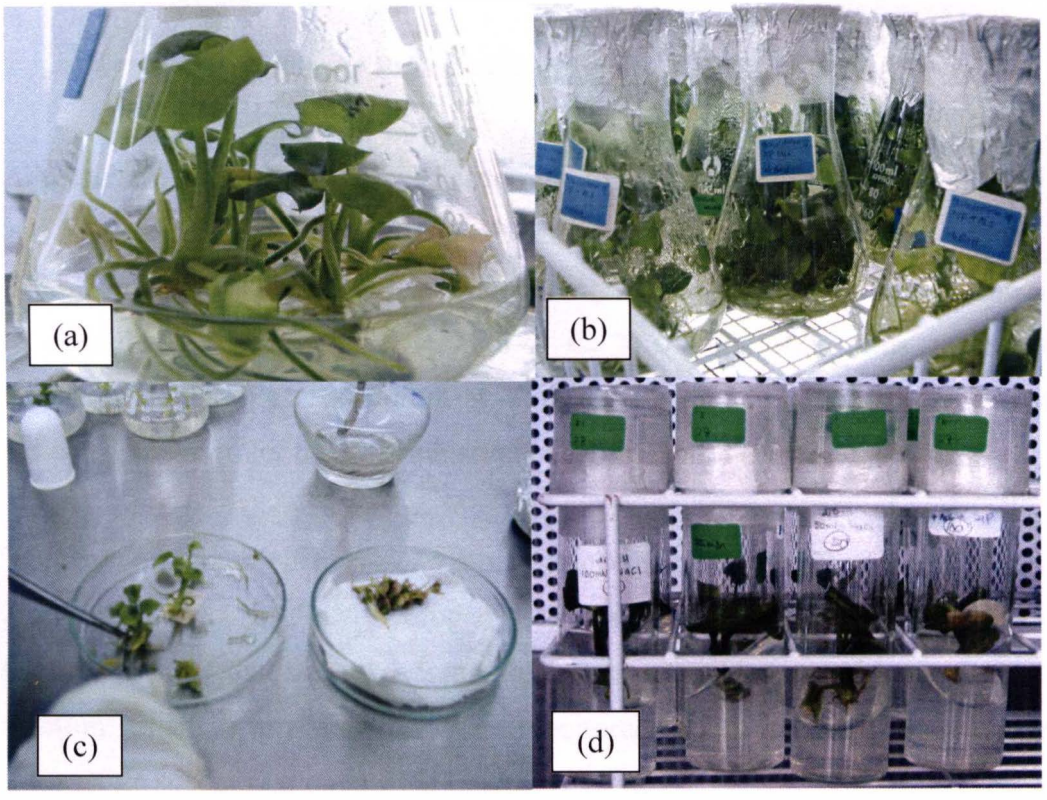

Plate 1 : Show the *in vitro* plant of *Homalomena sp.* cultures (a), after two months of proliferation, the plants were ready to use for salinity treatments (b), under sterile conditions, the plants materials were transferred onto the fresh treatment medium with different concentration NaCl 0, 25, 50 and 100mM NaCl for 28 days of treatment periods (c) and about 0.10 gram of leaves were taken in the determination on the specific activities of APx, CAT and POD on day 0, 1, 2, 7, 14 and 28 days of treatment periods (d).

3.4 Determination of specific activites of antioxidative enzymes

3.4.1 Ascorbate peroxidase (APx) specific activity

APx specific activity was assayed according to the method of Sairam *et al.,* (1998) and Nakawa *et al.*, (1981). 0.10g of leaf tissues was ground up with 1.0 ml of 100mM phosphate buffer (pH 7.0) containing 1mM ascorbic acid and clean sand in prechilled mortar and pestle at 0-4° C. The homogenate was centrifuged (centrifuge 5804R) at 10 000rpm at 4° C for 10 minutes. The supernatant was collected and used for the determination of APx specific activity.

The reaction mixture consists of 1.5 ml 100mM phosphate buffer (pH 7.0), 0.1 ml 3mM EDTA, 0.5 ml 3mM hydrogen peroxide, 400µl of enzyme extract, 0.3 ml deoinized water and 0.2 ml 1.5 mM hydrogen peroxide was added to start the reaction. Changes in absorbance were monitored at 290 nm for 3 minutes using UV-visible spectrophotometer (Shimadzu UV-1601).

APx specific activity was expressed as µmol ascorbate oxidized per hour per mg protein,

$$\text{Specific activity} = \frac{(\Delta\ A/\ \text{min}) \times (\text{ml reaction mix}) \times (10^6 \mu\text{mol}/\ \text{mol}) \times (60\ \text{min}/\ \text{hour})}{(E) \times (\text{mg protein}) \times (1000\ \text{ml}/\ \text{l})}$$

$$= (\text{units}/\ \text{mg protein})$$

Where E is the molar extinction coefficient of ascorbate ($2.8 \times 10^3\ M^{-1}\ cm^{-1}$).

3.4.2 Catalase (CAT) specific activity

CAT specific activity was assayed according to the method of Clairbone (1985). 0.10g of leaves tissue was ground with 1.0 ml of 50mM phosphate buffer (pH7.4) and clean sand in prechilled mortar and pestle at 0-4° C. The mixture was centrifuged (centrifuge 5804R) at 10 000rpm at 4° C for 10 minutes. The supernatant was collected and used for the determination of CAT specific activity.

The reaction mixture consists of 3 ml of reaction buffer (19mM hydrogen peroxide in 50mM phosphate buffer, pH 7.0) and 100 µl of enzymes extract was added to start the reaction. Changes of absorbanc were monitored at 240 nm for 3 minutes using UV-visible spectrophotometer (Shimadzu UV-1601). Catalase specific activity was expressed as µmoles of hydrogen peroxide consumed per minute per mg protein.

$$\text{Specific activity} = \frac{(\Delta\ A/\ \text{min}) \times 1000}{(E) \times (\text{mg protein/ ml reaction mixture})}$$

$$= (\text{units/ mg protein})$$

Where E is the molar extinction coefficient of peroxide (43.6 M^{-1} cm^{-1}).

3.4.3 Peroxidase (POD) specific activity

POD specific activity was assayed following the method of Agrawal and Parwardhan (1993). A 0.10g leaves tissue was ground up with 1 ml 100mM phosphate buffer (pH7.0) and clean sand in prechilled mortar and pestle at 0-4° C. The mixture was centrifuged (centrifuge 5804R) at 10 000rpm at 4° C for 10 minutes. The supernatant was collected and used for the determination of POD specific activity.

A 200 µl of enzymes extract was added into reaction mixture containing 1 ml 50mM phosphate buffer (pH 7.5), 1 ml 20mM guaiacol and 1 ml 30 mM hydrogen peroxide. Changes in absorbance were monitored at 470nm for 3 minutes using UV-visible spectrophotometer (Shimadzu UV-1601).

POD specific activity was expressed as µmoles guaiacol oxidized per hour per mg protein.

$$\text{Specific activity} = \frac{(\Delta\ A/\ \text{min}) \times 1000}{(E) \times (\text{mg protein/ ml reaction mixture})}$$

$$= (\text{units/ mg protein})$$

Where E is the molar extinction coefficient of guaiaciol (26.6 M^{-1} cm^{-1}).

3.4.4 Soluble Protein content

Protein concentration was determined according to the method by Bradford (1976). Bradford reagent was prepared by dissolving 100 mg Coomassie Brilliant Blue G-250 in 50 ml 95 % ethanol added with 50 ml of 85% orto-phosphoric acid and followed by deionized water to 1 liter solution.

The reagent were filtered through a filter paper (Whatman no.1) and stored at room temperature in light- proof bottles. For protein determination, 100 µl of enzymes extract was added to 3 ml of Bradford's reagent and mixed gently. The absorbance was measured at 595 nm after 10 minutes. The protein concentration was calculated according to a standard curve prepared with various concentration of BSA (Bovine Serum Albumin) (Appendix 2).

3.4.5 Statistical analysis

Data obtained was analysis using analysis of variance (One-way ANOVA). Multiple comparisons were performed using Turkey-test and at 0.05 as significant level using SPSS 10.0 for windows.

CHAPTER 4

RESULTS

4.1 Ascorbate peroxidase specific activity

Figure 4 and Table 1 (appendix 7) shows the changes in ascorbate peroxidase specific activities of *Homalomena sp.* cultures treated with different concentrations of NaCl. APx specific activities were generally higher in control compared to the treated cultures. NaCl treatment significantly increased the APx specific activities. Longer treatment period sharply decreased the APx specific activities in treated and control cultures. No significant differences ($p>0.05$) were observed in leaves treated under 50 and 100 mM NaCl at day 14 to 28 days of treatment. APx specific activities decreased significantly ($p<0.05$) at the later stages of treatment periods.

4.2 Catalase specific activity

Figure 5 and Table 2 (appendix 7) shows the changes in catalase specific activities of *Homalomena sp.* cultures treated with different concentrations of NaCl CAT specific activities fluctuated over 28 days of treatment periods. The maximum CAT specific activities were observed under treatment with 25mM NaCl at 7 days of treatment periods and decreased significantly ($p<0.05$) at the later stages of treatment periods. After 14 days treatment, catalase specific activities increased significantly in cultures treated with 100mM NaCl compared to other treatments.

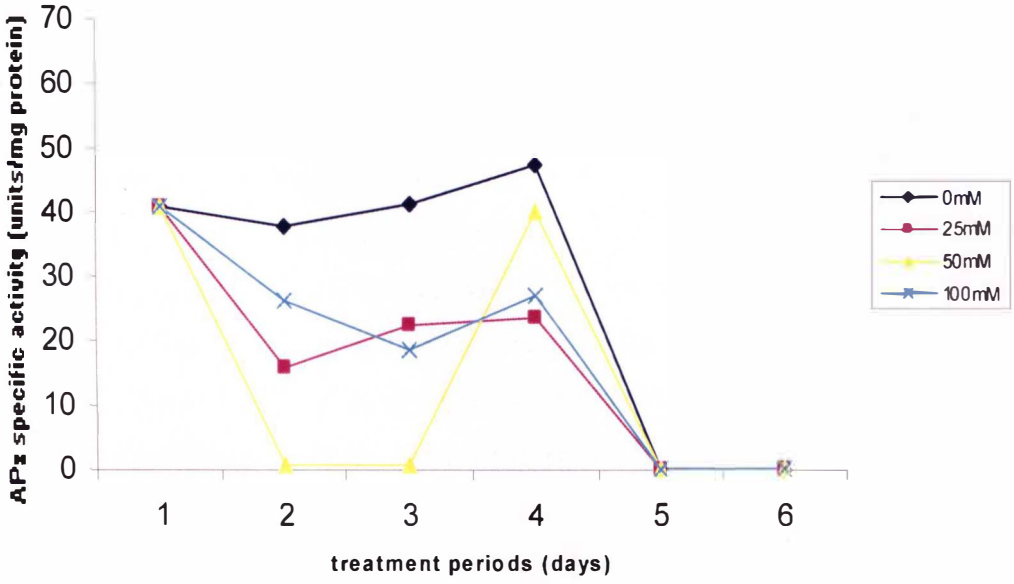

Figure 4: The changes in ascorbate peroxidase specific activities of *Homalomena sp.* cultures treated with different concentrations of NaCl. Data are means ± SE (n = 3).

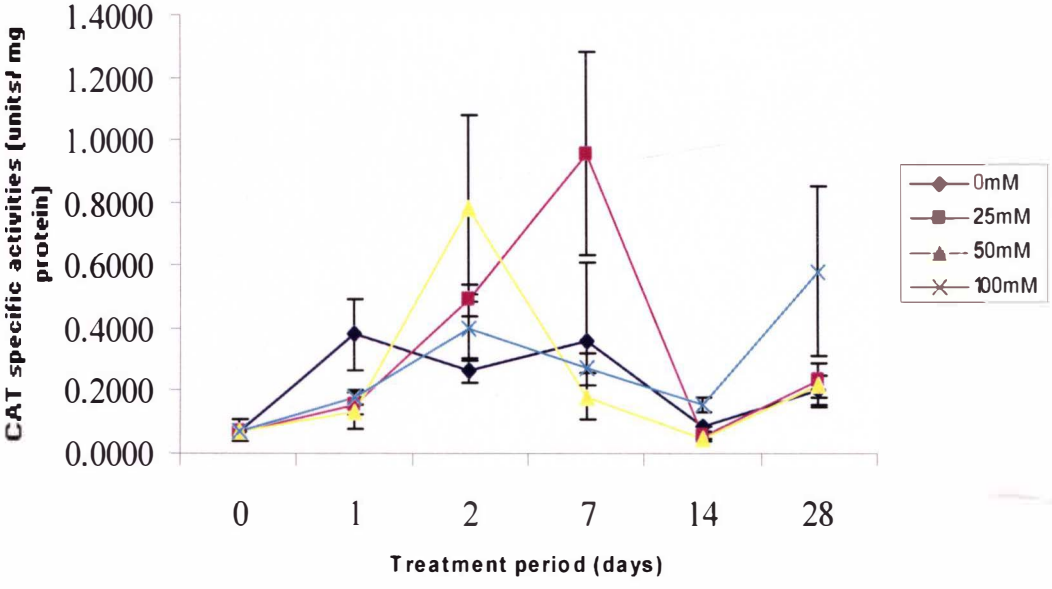

Figure 5: The changes in catalase specific activities of *Homalomena sp.* cultures treated with different concentrations of NaCl. Data are means ± SE (n = 3).

4.3 Peroxidase specific activity

Figure 6 and Table 3 (appendix 7) shows the changes in peroxidase specific activities of *Homalomena sp.* cultures treated with different concentrations of NaCl. POD specific activities markedly increased ($p<0.05$) after 24 hours to 7 days of treatment with 25 and 100mM NaCl as well as control except for cultures treated with 50mM NaCl. No significant differences ($p>0.05$) were observed between control and leaves treated with 25mM NaCl compared to other treatment. At 7 to 14 days, the specific activities in treated and untreated cultures showed significantly decreased ($p<0.05$) in POD specific activity. From 14 to 28 days, the POD specific activity showed the increasing in treated and untreated cultures in all concentrations except for 100mM of NaCl treatment.

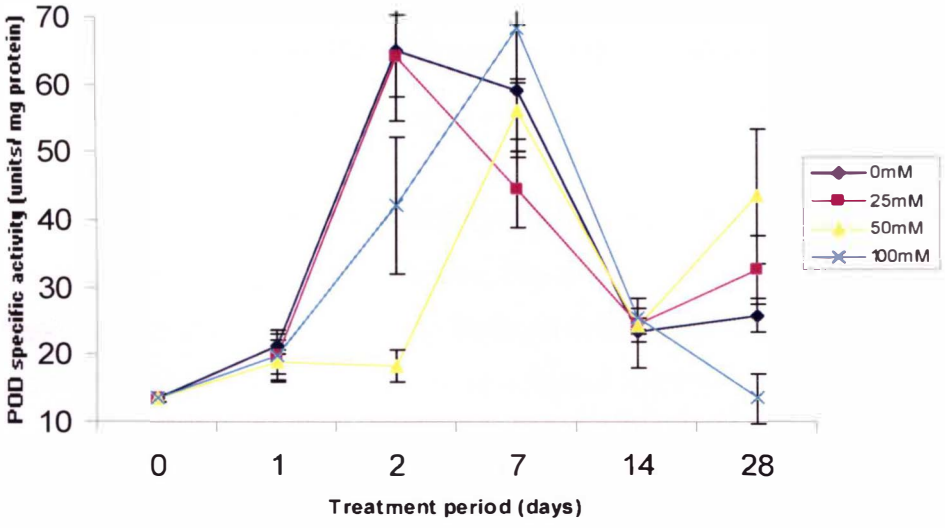

Figure 6: The changes in peroxidase specific activities of *Homalomena sp.* cultures treated with different concentrations of NaCl. Data are means ± SE (n = 3).

CHAPTER 5

DISCUSSIONS

Antioxidant plays an important role in protecting plants under stress conditions. In this study, salinity stress reduced the APx specific activities while activities of CAT and POD were induced.

From the previous study, the oxidative damage such as high light intensity, extremes temperature, drought, high salinity, herbicide treatment or mineral deficiencies has been reported to have greater resistance to these oxidative in plants with having high levels of antioxidants, either constitutive or induced (Harper and Harvey, 1978; Dhindsa and Matowe, 1981; Wise and Naylor, 1987; Spychalla and Desborough, 1990).

From the result, the NaCl treatment decreased the APx specific activities in the later stages of treatment periods (Figure 4). The control and treated of *Homalomena sp.* give the different results which they showed no significantly differences between them. Parida *et al.* (2004) reported that salinity causes increases in APx activity. H_2O_2 has been shown to induce cytosolic APx, therefore, the H_2O_2 accumulation under high salinity conditions may be a signal for adaptive response to the stress. H_2O_2 in connection with other signal molecules may contribute to the control of plant growth and development at specific checkpoints of the cell cycle.

Enhancement of APx activity is an important sign of higher salinity tolerance; Bor *et al.* (2003) also found that induced APx activity in salt tolerant wild beet and Mittova *et al.* (2002) also found same relation between APx and salt tolerance in tomato. From this view it may be estimated that although APx activity in cultivar *Orhangazi* increased under salt treatment this was not enough to overcome the inhibitory effects of NaCl on growth. Over expression of APx gene in plants has been reported to increase protection against oxidative stress (Wang *et al.*, 1999).

According to the figure 5, the NaCl treatment fluctuated the CAT specific activities and decreased at later stages of treatment periods. The increasing in CAT specific increased scavenging production of H_2O_2 which convert H_2O_2 to water. Van and Vranova (2001) reported that, CAT activity has been seen to be crucial for the cellular defence against salt-induced photorespiration in peroxisomes of leaves, in transgenic *Nicotiana tabacum* with low CAT activity, H_2O_2, arising from photorespiration, has been demonstrated to be an important mediator of cellular toxicity during environmental stress. Azevedo Neto *et al.* (2006) found higher CAT activity in two maize cultivars differing in salt tolerance.

The peroxisomal CAT protein is very sensitive to salt and high temperatures stress (Foyer and Noctor, 2000) probably because, an imbalance that occur between its synthesis and degradation. Some reports by Polidoros and Scandalios (1997), demonstrated that leaf CAT is also sensitive to high radiation levels.

According to figure 6, POD specific activities initially increased and longer treatment (14 to 28 days) periods reduced the POD specific activities. The longer treatment periods gives the increasing of POD specific activities to scavenge the production of H_2O_2. PODs protect cells against harmful concentration of hydroperoxides (Sudhakar *et al.,* 2001). Lin and Kao (1999) state that POD activity in the stressed roots is in accordance with that found in roots of rice seedlings Roots are the first organs, which come in contact with salt and are thought to play a critical role in plant salt tolerance.

Results indicated that CAT and POD specific activity were higher compared to APx in the leaves *Homalomena sp.* cultures. The higher POD activity indicated that it had a higher capacity for the decomposition of H_2O_2 generated by SOD. Excessive tissue levels of H_2O_2 in principle are minimized through the activity of metabolizing enzymes such as ascorbate peroxidase and catalase. Thus, CAT and POD were favorable compared to APx.

The activities of the antioxidative enzymes such as catalase (CAT), ascorbate peroxidase (APX), guaicol peroxidase (POD), glutathione reductase (GR), and superoxide dismutase increase under salt stress in plants and a correlation of these enzyme levels and salt tolerance exists in cotton (Gossett *et al.*, 1994), pea plants

(Hernandez *et al.*, 1995, 2000), oak seedling (Sehmer *et al.*, 1995), *Grevillea ilicifolia* and *Grevillea arenaria* (Kennedy and Fillippis, 1999), fox-tailed millet (Sreenivasulu *et al.*, 2000), *Solanum tuberosum* (Benavides *et al.*, 2000), rice (Lee *et al.*, 2001) and wild tomato species, *Lycopersicon pennellii* (Mittova *et al.*, 2002).

Specific activity was not same and differ depends on their function in the antioxidative enzyme that involved. In previous research, they stated that the mechanisms of antioxidative enzymes under salt stress are not clear. Expression of stress proteins is an important adaptive strategy of environmental stress tolerance (Schoffl *et al.*, 1999; Sanmiya *et al.*, 2004; Wahid and Close, 2006). There are different hypothesis about the salt tolerance mechanisms of plants, as Greenway and Munns (1980) proposed that adaptation to saline environments depends mostly on the ability of salt exclusion by plants. According to Yahya (1998), maintenance of low sodium concentration in the leaves and shoots is related to the limitation of its transport from roots.

Many changes have been found in the activities of antioxidant enzymes in plants under salinity. It has been reported that salt-tolerance plants, besides being able to regulate the ion and water movements, also exhibit a strong antioxidative enzymes system for effective removal of ROS (Rout and Shaw, 2001). The increased production of ROS in chloroplasts of plants under salt stress has also been previously reported in responses of shoot and roots of wheat (Meneguzzo *et al.,* 1999).

According to Azevedo (2006), in plants a number of enzymes regulate H_2O_2 intracellular levels, but CAT, APx and GPX are considered the most important. Salt stress resistance may depend, at least in part, on the enhancement of the antioxidative defense system, which includes antioxidant compounds and several antioxidative enzymes. Salt stress is known to result in extensive lipid peroxidation, which has often been used as indicator of salt-induced oxidative damage in membranes (Hernandez and Almansa, 2002). From the previous study, the efficiency of the latter process is dependent upon the plant antioxidant defense mechanisms.

Changes in the level of antioxidant molecules are signals of plant tolerance or adaptation to stress conditions. Therefore, changes in the activity of these enzymes are

correlated into oxidative stress tolerance of plants (Lee *et al.*, 2001; Sudhakar *et al.*, 2001). Variations in the antioxidant levels can serve as a signal for the modulation of ROS scavenging mechanisms and ROS signal transduction (Foyer *et al.*, 1997; Mittler, 2002).

CHAPTER 6

CONCLUSIONS

Salinity stress caused by NaCl treatments would trigger the antioxidative enzymes specific activities in *Homalomena sp.* cultures. Above results indicated that salinity treatment effected the specific activities of antioxidative enzymes; APx, CAT and POD in *Homalomena sp.* cultures. Longer treatment periodssignificantly reduce the APx specific activities while in CAT and POD specific activities were significantly induced.

REFERENCES

Agrawal, R. and Parwardhan, M.V. 1993. Production of peroxidase enzymes by callus culture of *Citurs aurantifolia S.* Journal Science Food Agriculture. 61: 377-378.

Ajay, A., Sairam, R.K., and Srivastava, G.C. 2002. Oxidative stress and antioxidative systems in plants. Division of Plant Physiology. 82:1227-1238.

Alscher, R.G.and Hess, J.L. 1993. Antioxidants in higher plants. Boca Raton, FL: CRC Press.

Anderson, M. D., Prasad, T. K. and Stewart, C. R. 1995. Changes in isozyme profiles of catalase. Plant Physiology. 109: 1247–1257.

Anderson, J. W., Foyer, C. H., and Walker, D. A. 1983. Light dependent. Reduction of hydrogen peroxide by intact spinach chloroplasts. Biochemistry Biophysiology Acta. 724: 69–74.

Asada, K., Kiso, K. and Yoshikawa, K. 1974. Univalent reduction of molocular oxygen by spinach chloroplasts on illumination. Journal Biology Chemistry. 249: 2175–2181.

Asada, K. and Badger, M. R. 1984. Photoreduction of $18O_2$ and H_2O_2 with a concomitant evolution of 18O2 in intact spinach. Plant Cell Physiology. 25: 1169–1179.

Asada, K. 1992. Ascorbate peroxidase – a hydrogen peroxidase scavenging enzyme in plants. Physiology Plant. 85: 235-241.

Ashraf, M. and Harris, P.J.C. 2004. Potential biochemical indicators of salinity tolerance in plants. Plant Science. 166: 3-16.

Azevedo, N. 2006. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. Environmental and Experimental Botany. 56: 87–94.

Benavides, M.P., Marconi, P.L., Gallego, S.M., Comba, M.E., and Tomaro, M.L. 2000. Relationship between antioxidant defence systems and salt tolerance in Solanum tuberosum. Aust. Journal Plant Physiology. 27: 273–278.

Bor, M., Ozdemir, F., and Turkan, I. 2003. The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet Beta vulgaris L. and wild beet *Beta maritima L. Plant Science.* 164: 77–84.

Bown, D. 2000. Aroids. Plants of the Arum Family. Portland, Oregon, Timber Press, pp. 114-116.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgrm quantities of protein utilizing the principle of protein of protein-dye binding. Anal Biochemistry. 72: 248-254.

Cadenas, E. 1989. Biochemistry of oxygen toxicity. Annual Revision Biochemistry. 58: 79–110.

Chen, G. X., and Asada, K. 1989. Peroxidase in tea leaves: occurrence of two isozymes. Plant Cell Physioliogy. 30: 987– 998.

Clairbone, A. 1985. Catalase activity. In: *Handbook of method for oxygen radical research.* (E. A. Greenwald, ed). CRS Press, Boca Raton. pp: 283-284.

Demmig-Adams, B., and Adams, III W. W. 1992. Photoprotection and other responses of plants to high light stress. Annual Revision Plant Physiology Plant Molecular Biology. 43: 599–626.

Dhindsa, R.S., and Matowe, W., 1981. Drought tolerance in two mosses: correlated with enzymatic defense against lipid peroxidation. Journal Experiment Botanical. 32: 79–91.

Egneus, H., Heber, U., Matthiesen,U. and Kirk, M. 1975. Reduction of oxygen by the electron transport chain of chloroplasts. Biochemistry Biophysiology Acta. 408: 252–268.

Elstner, E. F., Wagner, G. A. and Schutz, W. 1988. Activated oxygen in green plants in relation to stress. Current Topics Plant Biochemistry Physiology. 7: 159–187.

Foyer, C. H. and Halliwell, B. 1976. The presence of glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta. 133: 21–25.

Foyer, C. H., Furbank, R. T., Harbinson, J. and Horton, P. 1990. The mechanisms contributing to photosynthetic control of electron transport by carbon assimilation in leaves. Photosynthesis Resistance. 25: 83–100.

Foyer, C. H., Lelandais, M., Edwards, E. A., and Mullineaux, P. M. 1991. In Active Oxygen, Oxidative Stress and Plant Metabolism: Current Topics in Plant Physiology (eds Pell, E. and Steffen, K.), Am. Soc. Plant Physiologists, Rockville, MD. pp. 131–144.

Foyer, C. H. and Lelandais, M. 1993. In: *Photosynthetic Responses to the Environment* (ed. Yamamoto, H. Y.), Am. Soc. Plant Physiologists, Rockville, MD. 8: 88–101.

Foyer, C.H., Descourvieres, P., Kunert, K.J. 1994a. Protection against oxygen radicals: an important defense mechanism study in transgenic plants. Plant Cell Environment. 17:507–23.

Foyer, C.H., Lelandais, M., and Kunert, K.J. 1994b. Photooxidative stress in plants. Physiology Plant. 92:696–717.

Foyer, C.H., Lopez-Delgado, H., Dat, J.F., and Scott, I.M. 1997. Hydrogen peroxide and glutathione-associated mechanisms of acclamatory stress tolerance and signaling. Physiology Plant. 100: 241– 254.

Foyer, C.H, and Noctor, G. 2000. Oxygen processing in photosynthesis: regulation and signalling. New Phytology. 146: 359–88.

Frank Van and Inze, D. 2002. Transgenic plants expressing tolerance toward oxidative stress. Ghent University, Belgium.

Gaspar, T., Penel, C., Hagege, D., and Greppin, H. 1991: Peroxidase in plant growth, differentiation and developmental processes. *In:* Biochemical, molecular and physiological aspects of plant peroxidases, J. Lobarzewski, H. Greppin, C. Pennel, T. Gaspar (Eds.), Lublin, Poland and Geneva, Switzerland. pp. 249–280.

Gilles, H., Loic, M., Christina, E., Reynolds, and Julie, S. 2000. The effect of salinity on different developmental stages of an endemic annual plant, *Aster laurentianus* (Asteraceae). American Journal Botany. 88: 62-67.

Gomez, J. M., Hernandez, J. A., Jiménez, A., del Rio, L. A. and Sevilla, F. 1999. Differential response of antioxidative enzymes of chloroplasts and mitochondria to long-term NaCl stress of pea plants. Free Radical Resistance Supplement. 31: 11–18.

Gossett, D.R., Millhollon, E.P., Lucas, M.C. 1994. Antioxidant response to NaCl stress in salt tolerant and salt sensitive cultivars of cotton. Crop Sciencea. 34: 706–714.

Greenway, H., and Munns, R., 1980. Mechanisms of salt tolerance in non halophytes. Annual Revision Plant Physiology. 31: 149–190.

Gressel, J., and Galun, E. 1994. Genetic controls of photooxidant tolerance. *In*: Foyer C.H., Mullineaux P.M, editors. Causes of photooxidative stress and amelioration of defense systems in plant. Boca Raton: CRC Press; pp. 237–74.

Guan, L.Q. and Scandalios, J.G. 1996. Molecular evolution of maize catalases and their relationship to other eukaryotic and prokaryotic catalases. Journal Molecular Evolution. 42: 570- 579.

Haber, F. and Weiss.1934. The catalytic decomposition of hydrogen peroxide by iron salts. Journal Proc. Royal Social. 147: 332.

Haliwell, B., and Gutteridge, J.M.C. 1986: Oxygen frees radicals and iron in relation to biology and medicine: some problems and concepts. Arch Biochemistry Biophysiology. 246:501-514.

Harbinson, J. and Hedley, C. L. 1993. Changes in P-700 oxidation during the early stages of the induction of photosynthesis. Plant Physiology. 103: 649–660.

Hager, A. 1969. Light dependent decreased of pH value in a chloroplast compartment causing enzymatic interconversion of violaxanthin to zeaxanthin-relations to photophosphorylation. Planta. 89: 224–243.

Harper, D.B., and Harvey, B.M.R., 1978. Mechanisms of paraquat tolerance in perennial ryegrass II. Role of superoxide dismutase, catalase, and peroxidase. Plant Cell Environment. 1: 211–215.

Hay, A. 1990. Aroids of Papua New Guinea. Madang, Papua New Guinea: Christensen Research Institute.

Hay, A. 1999. Revision of *Homalomena* (*Araceae-Homalomena*) in New Guinea, the Bismark Archipelago and Solomon Islands. Blumea. 44: 41-71.

Heber U., Egneus H., Hanch, U., Jensen, M. and Koster, S. 1978.Photooxidative stress in plants. Planta. 143: 41–53.

Hernandez, J.A., Olmos, E., Corpas, F.J., Sevilla, F., and del Rio, L.A. 1995. Salt-induced oxidative stress in chloroplasts of pea plants. Plant Sciences. 105: 151–167.

Hernandez, J.A., Campillo, A., Jiménez, A., Alacon, J.J., and Sevilla, F. 1999. Response of antioxidant systems and leaf water relations to NaCl stress in pea plants. New Phytology. 141: 241–251.

Hernandez, J., Jimenez, A., Mullineaux, P., and Sevilla, F. 2000. Tolerance of pea plants (*Pisum sativum*) to long-term salt stress is associated with induction of antioxidant defences. Plant Cell Environment. 23: 853–862.

Hernandez, J.A., and Almansa, M.S. 2002. Short-term effects of salt stress on antioxidant systems and leaf water relations of pea leaves. Physiology Plant. 115: 251–257.

Hertwig, B., Streb, P., and Feierabend, J. 1992. Light dependence of catalase synthesis and degradation in leaves and the influence of interfering stress conditions. Plant Physiology. 100:1547–53.

Hiraga, S., Sasaki, K., Ito, H., Ohashi, Y., and Matsui, H. 2001. Guaiacol peroxidases in carrot (*Daucus carota* L.) Root. Plant Cell Physiology. 42:462–468.

Hotta, M. 1981. New species of the genus *Homalomena* (Araceae) from Sumatra with a short note on the genus *Furtadoa.* Gardens' Bulletin Singapore. 38 (1): 43-54.

Kennedy, B.F., and De Fillippis, L.F., 1999. Physiological and oxidative response to NaCl of the salt tolerant *Grevillea ilicifolia* and the salt sensitive *Grevillea arenaria*. Journal Plant Physiology. 155: 746–754.

Kim, S.Y., Lim, J.H., Park, M.R., Kim, Y.J., Park, T.I.I., and Seo, Y.W., *et al.* 2005. Enhanced antioxidant enzymes are associated with reduced hydrogen peroxide in barley roots under salt stress. Journal Biochemistry Molecular Biology. 38: 218–24.

Knox, J.P. and Dodge, A.D. 1985. Singlet oxygen and plants. Phytochemistry. 24: 889– 896.

Lechno, S., Zamski, E., and TelOr, E., 1997. Salt stress-induced responses in cucumber plants. Journal Plant Physiology. 150: 206–211.

Lee, D.H., Kim, Y.S., and Lee, C.B., 2001. The inductive responses of the antioxidant enzymes by salt stress in the rice (*Oryza sativa* L.). Journal Plant Physiology. 158: 737–745.

Liang, Y.C., 1999. Effects of silicon on enzyme activity and sodium, potassium and calcium concentration in barley under salt stress. Plant Soil. 209: 217–224.

Lin, C.C., and Kao, C.H. 1999. NaCl induced changes in ionically bound peroxidases activity in roots of rice seedlings. Plant Soil. 216:147– 153.

Maranon, M.J.R., and Van Huystee, R.B. 1994. Guaiacol peroxidases in carrot (*Daucus carota* L.) Root. Phytochemistry. 37: 1217–1225.

Mayo, S., Bogner, J., and Boyce, P.C. 1997. Gearum rediscovered. The Genera of Araceae. Royal Botanical Gardens, Kew.

Mehler, A. H. 1951. Studies on reactions of illuminated chloroplasts. Arch. Biochemistry Biophysiology. 33: 65–77.

Meneguzzo, S., and Navarilzzo, I. 1999. Antioxidative responses of shoots and roots of wheat to increasing NaCl concentrations. Journal Plant Physiology. 155: 274– 280.

Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Science. 22: 561–569.

Mittova, V., Tal, M., Volokita, M., and Guy, M. 2002. Salt stress induces up-regulation of an efficient chloroplast antioxidant system in the salt-tolerant wild tomato species *Lycopersicon pennellii* but not in the cultivated species. Physiology Plant. 115: 393–400.

Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiology Plant. 15: 473-497.

Muthukumarasamy, M., Gupta, S.D., and Pannerselvam, R., 2000. Enhancement of peroxidase, polyphenol oxidase and superoxide dismutage activities by triadimefon in NaCl stressed *Raphanus sativus* L. Biology Plant. 43: 317–320.

Nakano, Y. and Asada, K. 1981. Hydrogen peroxide in scavenged by ascorbate – specific peroxidase in spinach chloroplast. Plant Cell Physiology. 22: 867–880.

Noctor, G. and Foyer, C.H. 1998. Ascorbate and glutathione: Keeping active oxygen under control. Annual Revision Plant Physiology. Plant Molecular Biology. 49: 249- 279.

Parida, A., Das, A.B., and Da, P. 2002. NaCl stress causes changes in photosynthetic pigments, proteins and other metabolic components in the leaves of a true mangrove, *Bruguiera parviflora*, in hydroponic cultures. Journal Plant Biology. 45: 28–36.

Pfundel, E.E., and Dilley, R.A. 1993. The pH dependence of violaxanthin deepoxidation in isolated pea chloroplasts. Plant Physiology. 101: 65– 71.

Polidoros, A.N., and Scandalios, J.G. 1997. Response of the maize catalases to light. Free Radical Biology Medicine. 23: 497–504.

Polle, A., Chakrabarti, K., Schurmann, W., and Rennenberg, H. 1990. Composition and properties of hydrogen peroxide decomposing systems in extracellular and total extracts from needles of Norway spruce (*Picea abies* L., Karst.). Plant Physiology. 94: 312–319.

Rich, P.R., and Bonner, W.D. 1978. sites of superoxide anion gener ation in higher plant mitochondria . Arch. Biochemistry Biophysiology. 188: 206–213.

Rout, N.P., and Shaw, B.P., 2001. Salt tolerance in aquatic macrophytes: possible involvement of the antioxidative enzymes. Plant Science. 160: 415–423.

Sairam, R.K., Shukla, D.S., and Sayena, D.C. 1998. Stress induced injury and antioxidant enzymes in relation to drought tolerance in wheat genotypes. Biology Plant. 40: 357-36.

Sanmiya, K., Suzuki, K., Egawa, Y., and Shono, M. 2004. Mitochondrial small heat-shock protein enhances thermotolerance in tobacco plants. FEBS Lett. 557:265–8.

Schoffl, F., Prandl, R., and Reindl, A. 1999. Molecular responses to heat stress. In: Shinozaki K, K Yamaguchi-Shinozaki K, editors. Molecular responses to cold, drought, heat and salt stress in higher plants. Texas: KRG Landes Co. pp: 81–98.

Sehmer, L., Alaoui-Sosse, B., and Dizengremel, P., 1995. Effect of salt stress on growth and on the detoxyfying pathway of pedunculate oak seedlings (*Quercus robur* L.). Journal Plant Physiology. 147: 144–151.

Smirnoff, N. 1993. The role of active oxygen in the response of plants to water deficit and desiccation. New Phytology. 125:27–58.

Smirnoff, N., and Wheeler, G.L. 2000. Ascorbic acid in plants: biosynthesis and function. Critical Revision Plant Science. 19: 267–290.

Spychalla, J.P., and Desborough, S.L. 1990. Superoxide dismutase, catalase, and alpha- tocopherol content of stored potato tubers. Plant Physiology. 94: 1214–1218.

Sreenivasulu, N., Grimm, B., Wobus, U., and Weschke, W. 2000. Differential response of antioxidant compounds to salinity stress in salt-tolerant and salt-sensitive seedlings of fox-tail millet (*Setaria italica*). Physiology Plant. 109: 435–442.

Sudhakar, C., Lakshmi, A., and Giridarakumar, S. 2001. Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. Plant Science. 161: 613–619.

Van, B.F., Vranova, E., Dat, J.F., and Inze, D. 2001.The role of active oxygen species in plant signal transduction. Plant Science. 161:405–414.

Van Montagu, D. 1995. Oxidative stress in plants. Current Opinoin Biotechnology. 6: 153-158.

Wahid, A., and Close, T.J. 2006. Expression of dehydrins (DHNs) under heat stress and their relationship with water relations of sugarcane leaves. Biology Plant in press.

Wang, J., Zhang, H., Allen, R.D. 1999. Overexpression of an *Arabidopsis* peroxisomal ascorbate gene increase protection against oxidative stress. Plant Cell Physiology. 40: 725–732.

Willekens, H., Villarroel, R., Van Montagu, M., Inzé, D., and Van, Camp M. 1994. Molecular identification of catalases from *Nicotiana plumnaginifolia* (L). FEBS Lett. 352: 79-83.

Wise, R. and Naylor, A.W. 1987. Chilling enhanced photooxidation. Plant Physiology. 83: 278–282.

Wu, J., Nelnanis, S. and Heber, U. 1991. Photorespiration is more effective than the Mehler reaction in protecting the photosynthesis apparatus against photoinhibition. Botanical Acta. 104: 283– 291.

Yahya, A. 1998. Responses to soil salinity of Sesame (*Sesamum indicum* L.) and Sugar beet (*Beta vulgaris* L.). Doctoral Thesis-Uppsala.

Zelitch, I. 1990. In Perspectives in biochemical and genetic regulation of photosynthesis (ed. Zelitch, I.), Liss, Inc., New York. pp. 239–252.

APPENDICES

Appendix I

(MS Media : 1 liter)

Macronutrients (100 ml for each solution)

Chemicals	**g/ L**
NH_4NO_3	16.5
KNO_3	19.0
CaCl	4.4
$MgSO_4.7H_2O$	3.7
KH_2PO_4	1.7

Micronutrients (10 ml for each solution)

Chemicals	**g/ ½ L**
H_3BO_4	3.100
$MnSO_4.H_2O$	11.150
$ZnSO_4.7H_2O$	4.300
KI	0.415
$Na_2MoO_4.2H_2O$	0.125
$CuSO_4.5H_2O$	0.125
$CoCl_3.6H_2O$	0.125

Vitamins B5 (10 ml for each solution)

Chemicals	g/ 100ml
Glysin	0.20
A. nicotinic	0.08
Piridoksin HCl	0.05
Tianin HCl	0.01
Myoinisitol	0.10

Fe EDTA	**10 ml/L**
2-IP Hormone	**10 ml/L**
Sucrose	**30 g/L**
Phytogel	**2.5 g/L**

NaCl for treatment

Molarity	g/L
O mM	0.000
25 mM	1.461
50 mM	2.922
100 mM	5.844

Appendix 2

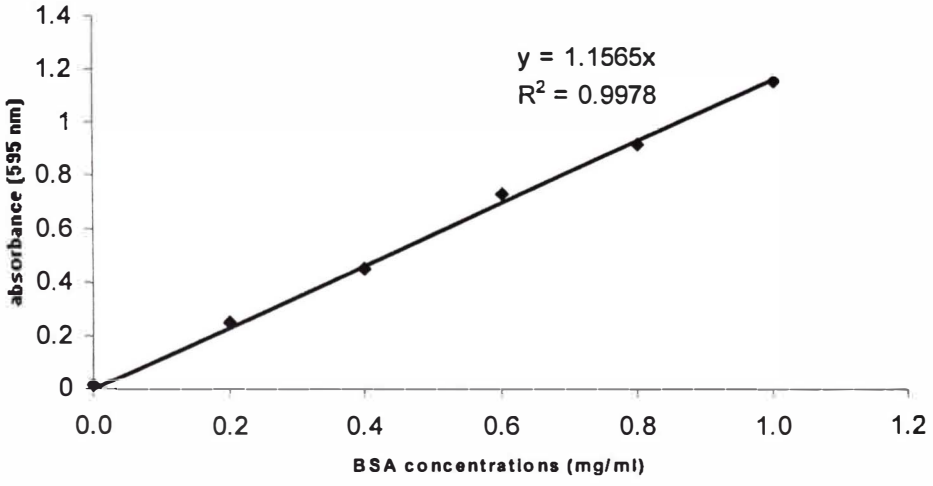

Figure 4: Protein standard curve for ascobate peroxidase assay

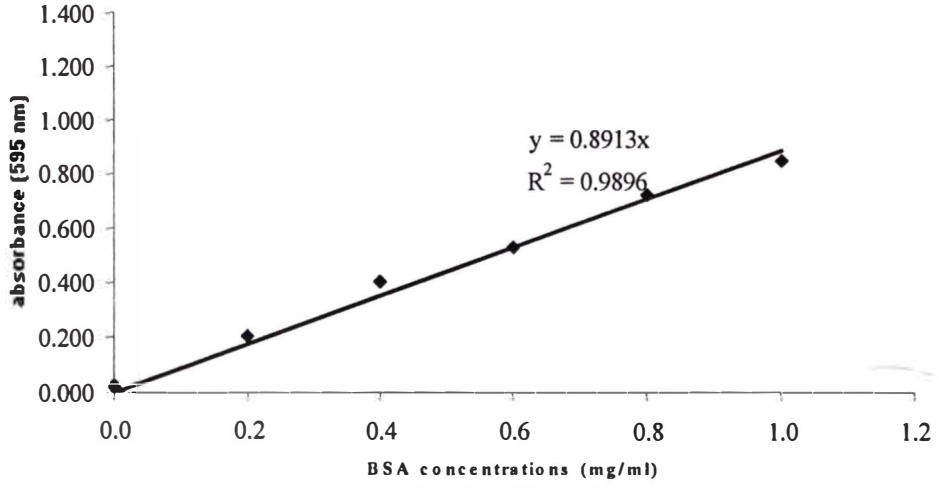

Figure 5: Protein standard curve for catalase assay

Appendix 3

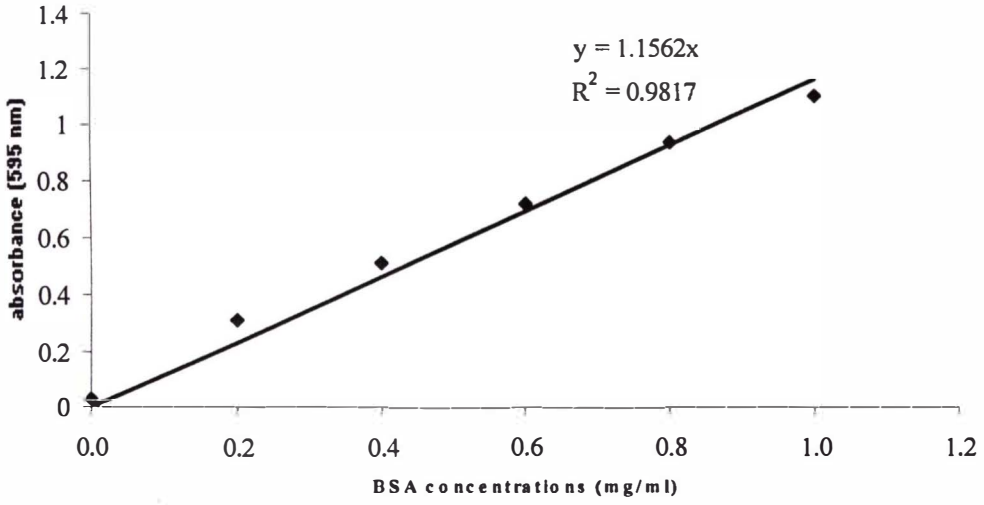

Figure 6: Protein standard curve for peroxidase assay

Appendix 4

ANALYSIS FOR ASCORBATE PEROXIDASE SPECIFIC ACTIVITY USING ONE-WAY ANOVA

1. Day 1

ANOVA

ACTAPX1

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1743.517	3	581.172	16.789	.001
Within Groups	276.931	8	34.616		
Total	2020.449	11			

Multiple Comparisons

Dependent Variable: ACTAPX1

Tukey HSD

(I) CONCENTR	(J) CONCENTR	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	25	16.87217*	4.80392	.032	1.48811	32.25622
	50	31.88900*	4.80392	.001	16.50494	47.27306
	100	6.44593	4.80392	.565	-8.93812	21.82999
25	0	-16.87217*	4.80392	.032	-32.25622	-1.48811
	50	15.01683	4.80392	.056	-.36722	30.40089
	100	-10.42623	4.80392	.211	-25.81029	4.95782
50	0	-31.88900*	4.80392	.001	-47.27306	-16.50494
	25	-15.01683	4.80392	.056	-30.40089	.36722
	100	-25.44307*	4.80392	.003	-40.82712	-10.05901
100	0	-6.44593	4.80392	.565	-21.82999	8.93812
	25	10.42623	4.80392	.211	-4.95782	25.81029
	50	25.44307*	4.80392	.003	10.05901	40.82712

*. The mean difference is significant at the .05 level.

2. Day 2

ANOVA

ACTAPX2

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2460.995	3	820.332	1.533	.279
Within Groups	4280.143	8	535.018		
Total	6741.138	11			

Multiple Comparisons

Dependent Variable: ACTAPX2
Tukey HSD

(I) CONCENTR	(J) CONCENTR	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	25	18.63920	18.88594	.761	-41.84111	79.11951
	50	40.32707	18.88594	.221	-20.15324	100.80738
	100	22.39923	18.88594	.651	-38.08108	82.87954
25	0	-18.63920	18.88594	.761	-79.11951	41.84111
	50	21.68787	18.88594	.673	-38.79244	82.16818
	100	3.76003	18.88594	.997	-56.72028	64.24034
50	0	-40.32707	18.88594	.221	-100.80738	20.15324
	25	-21.68787	18.88594	.673	-82.16818	38.79244
	100	-17.92783	18.88594	.780	-78.40814	42.55248
100	0	-22.39923	18.88594	.651	-82.87954	38.08108
	25	-3.76003	18.88594	.997	-64.24034	56.72028
	50	17.92783	18.88594	.780	-42.55248	78.40814

3. Day 7

ANOVA

ACTAPX7

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1201.133	3	400.378	1.758	.233
Within Groups	1821.771	8	227.721		
Total	3022.905	11			

Multiple Comparisons

Dependent Variable: ACTAPX7
Tukey HSD

(I) CONCNETR	(J) CONCNETR	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	25	24.88053	12.32129	.258	-14.57717	64.33824
	50	7.13743	12.32129	.936	-32.32027	46.59514
	100	20.47813	12.32129	.400	-18.97957	59.93584
25	0	-24.88053	12.32129	.258	-64.33824	14.57717
	50	-17.74310	12.32129	.511	-57.20080	21.71460
	100	-4.40240	12.32129	.983	-43.86010	35.05530
50	0	-7.13743	12.32129	.936	-46.59514	32.32027
	25	17.74310	12.32129	.511	-21.71460	57.20080
	100	13.34070	12.32129	.709	-26.11700	52.79840
100	0	-20.47813	12.32129	.400	-59.93584	18.97957
	25	4.40240	12.32129	.983	-35.05530	43.86010
	50	-13.34070	12.32129	.709	-52.79840	26.11700

4. Day 14

ANOVA

ACTAPX14

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.802E-03	3	6.006E-04	.532	.673
Within Groups	9.026E-03	8	1.128E-03		
Total	1.083E-02	11			

Multiple Comparisons

Dependent Variable: ACTAPX14

Tukey HSD

(I) CONCENTR	(J) CONCENTR	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	25	-3.260E-02	2.74E-02	.650	-.12043	5.5230E-02
	50	-1.367E-02	2.74E-02	.957	-.10150	7.4163E-02
	100	-2.470E-02	2.74E-02	.805	-.11253	6.3130E-02
25	0	3.2600E-02	2.74E-02	.650	-5.52300E-02	.12043
	50	1.8933E-02	2.74E-02	.898	-6.88966E-02	.10676
	100	7.9000E-03	2.74E-02	.991	-7.99300E-02	9.5730E-02
50	0	1.3667E-02	2.74E-02	.957	-7.41633E-02	.10150
	25	-1.893E-02	2.74E-02	.898	-.10676	6.8897E-02
	100	-1.103E-02	2.74E-02	.977	-9.88633E-02	7.6797E-02
100	0	2.4700E-02	2.74E-02	.805	-6.31300E-02	.11253
	25	-7.900E-03	2.74E-02	.991	-9.57300E-02	7.9930E-02
	50	1.1033E-02	2.74E-02	.977	-7.67966E-02	9.8863E-02

5. Day 28

ANOVA

ACTAPX28

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.873E-03	3	1.624E-03	.734	.560
Within Groups	1.769E-02	8	2.212E-03		
Total	2.257E-02	11			

Multiple Comparisons

Dependent Variable: ACTAPX28

Tukey HSD

(I) CONCENTR	(J) CONCENTR	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	25	-2.800E-03	3.84E-02	1.000	-.12577	.12017
	50	-5.067E-02	3.84E-02	.577	-.17364	7.2305E-02
	100	-1.957E-02	3.84E-02	.955	-.14254	.10340
25	0	2.8000E-03	3.84E-02	1.000	-.12017	.12577
	50	-4.787E-02	3.84E-02	.618	-.17084	7.5105E-02
	100	-1.677E-02	3.84E-02	.970	-.13974	.10620
50	0	5.0667E-02	3.84E-02	.577	-7.23046E-02	.17364
	25	4.7867E-02	3.84E-02	.618	-7.51046E-02	.17084
	100	3.1100E-02	3.84E-02	.848	-9.18713E-02	.15407
100	0	1.9567E-02	3.84E-02	.955	-.10340	.14254
	25	1.6767E-02	3.84E-02	.970	-.10620	.13974
	50	-3.110E-02	3.84E-02	.848	-.15407	9.1871E-02

Appendix 5

ANALYSIS FOR CATALASE SPECIFIC ACTIVITY USING ONE-WAY ANOVA

1.Day 1

ANOVA

ACTCAT1

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.116	3	3.878E-02	2.290	.155
Within Groups	.135	8	1.693E-02		
Total	.252	11			

Multiple Comparisons

Dependent Variable: ACTCAT1

Tukey HSD

(I) CONCENTR	(J) CONCENTR	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	25	.22830	.10624	.217	-.11192	.56852
	50	.24487	.10624	.176	-9.53557E-02	.58509
	100	.19977	.10624	.308	-.14046	.53999
25	0	-.22830	.10624	.217	-.56852	.11192
	50	1.6567E-02	.10624	.999	-.32366	.35679
	100	-2.853E-02	.10624	.993	-.36876	.31169
50	0	-.24487	.10624	.176	-.58509	9.5356E-02
	25	-1.657E-02	.10624	.999	-.35679	.32366
	100	-4.510E-02	.10624	.973	-.38532	.29512
100	0	-.19977	.10624	.308	-.53999	.14046
	25	2.8533E-02	.10624	.993	-.31169	.36876
	50	4.5100E-02	.10624	.973	-.29512	.38532

2. Day 2

ANOVA

ACTCAT2

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.433	3	.144	1.413	.308
Within Groups	.818	8	.102		
Total	1.251	11			

Multiple Comparisons

Dependent Variable: ACTCAT2
Tukey HSD

(I) CONCNETR	(J) CONCNETR	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	25	-.22623	.26101	.822	-1.06208	.60962
	50	-.51897	.26101	.268	-1.35482	.31688
	100	-.13877	.26101	.949	-.97462	.69708
25	0	.22623	.26101	.822	-.60962	1.06208
	50	-.29273	.26101	.687	-1.12858	.54312
	100	8.7467E-02	.26101	.986	-.74838	.92332
50	0	.51897	.26101	.268	-.31688	1.35482
	25	.29273	.26101	.687	-.54312	1.12858
	100	.38020	.26101	.503	-.45565	1.21605
100	0	.13877	.26101	.949	-.69708	.97462
	25	-8.747E-02	.26101	.986	-.92332	.74838
	50	-.38020	.26101	.503	-1.21605	.45565

3. Day 7

ANOVA

ACTCAT7

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.013	3	.338	1.932	.203
Within Groups	1.398	8	.175		
Total	2.411	11			

Multiple Comparisons

Dependent Variable: ACTCAT7
Tukey HSD

(I) CONCENTR	(J) CONCENTR	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	25	-.56880	.34134	.398	-1.66190	.52430
	50	.17453	.34134	.954	-.91856	1.26763
	100	8.5967E-02	.34134	.994	-1.00713	1.17906
25	0	.56880	.34134	.398	-.52430	1.66190
	50	.74333	.34134	.209	-.34976	1.83643
	100	.65477	.34134	.293	-.43833	1.74786
50	0	-.17453	.34134	.954	-1.26763	.91856
	25	-.74333	.34134	.209	-1.83643	.34976
	100	-8.857E-02	.34134	.993	-1.18166	1.00453
100	0	-8.597E-02	.34134	.994	-1.17906	1.00713
	25	-.65477	.34134	.293	-1.74786	.43833
	50	8.8567E-02	.34134	.993	-1.00453	1.18166

4. Day 14

ANOVA

ACTCAT14

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.229E-02	3	7.430E-03	7.794	.009
Within Groups	7.627E-03	8	9.533E-04		
Total	2.992E-02	11			

Multiple Comparisons

Dependent Variable: ACTCAT14

Tukey HSD

(I) CONCNETR	(J) CONCNETR	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	25	2.7967E-02	2.52E-02	.694	-5.27668E-02	.10870
	50	3.6567E-02	2.52E-02	.506	-4.41668E-02	.11730
	100	-7.300E-02	2.52E-02	.077	-.15373	7.7334E-03
25	0	-2.797E-02	2.52E-02	.694	-.10870	5.2767E-02
	50	8.6000E-03	2.52E-02	.985	-7.21334E-02	8.9333E-02
	100	-.10097*	2.52E-02	.017	-.18170	-2.02332E-02
50	0	-3.657E-02	2.52E-02	.506	-.11730	4.4167E-02
	25	-8.600E-03	2.52E-02	.985	-8.93334E-02	7.2133E-02
	100	-.10957*	2.52E-02	.011	-.19030	-2.88332E-02
100	0	7.3000E-02	2.52E-02	.077	-7.73343E-03	.15373
	25	.10097*	2.52E-02	.017	2.0233E-02	.18170
	50	.10957*	2.52E-02	.011	2.8833E-02	.19030

*. The mean difference is significant at the .05 level.

5. Day 28

ANOVA

ACTCAT28

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.297	3	9.906E-02	1.201	.370
Within Groups	.660	8	8.249E-02		
Total	.957	11			

Multiple Comparisons

Dependent Variable: ACTCAT28

Tukey HSD

(I) CANCENTR	(J) CANCENTR	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	25	-3.173E-02	.23451	.999	-.78272	.71925
	50	-1.627E-02	.23451	1.000	-.76725	.73472
	100	-.37850	.23451	.423	-1.12949	.37249
25	0	3.1733E-02	.23451	.999	-.71925	.78272
	50	1.5467E-02	.23451	1.000	-.73552	.76645
	100	-.34677	.23451	.491	-1.09775	.40422
50	0	1.6267E-02	.23451	1.000	-.73472	.76725
	25	-1.547E-02	.23451	1.000	-.76645	.73552
	100	-.36223	.23451	.457	-1.11322	.38875
100	0	.37850	.23451	.423	-.37249	1.12949
	25	.34677	.23451	.491	-.40422	1.09775
	50	.36223	.23451	.457	-.38875	1.11322

Appendix 6

ANALYSIS FOR PEROXIDASE SPECIFIC ACTIVITY USING ONE-WAY ANOVA

1. Day 1

ANOVA

ACTPOD1

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.099	3	2.700	.086	.966
Within Groups	251.339	8	31.417		
Total	259.438	11			

Multiple Comparisons

Dependent Variable: ACTPOD1

Tukey HSD

(I) CONCENTR	(J) CONCENTR	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	25	1.50903	4.57656	.987	-13.14694	16.16501
	50	2.26477	4.57656	.958	-12.39121	16.92074
	100	1.49067	4.57656	.987	-13.16531	16.14664
25	0	-1.50903	4.57656	.987	-16.16501	13.14694
	50	.75573	4.57656	.998	-13.90024	15.41171
	100	-1.837E-02	4.57656	1.000	-14.67434	14.63761
50	0	-2.26477	4.57656	.958	-16.92074	12.39121
	25	-.75573	4.57656	.998	-15.41171	13.90024
	100	-.77410	4.57656	.998	-15.43008	13.88188
100	0	-1.49067	4.57656	.987	-16.14664	13.16531
	25	1.8367E-02	4.57656	1.000	-14.63761	14.67434
	50	.77410	4.57656	.998	-13.88188	15.43008

2. Day 2

ANOVA

ACTPOD2

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4387.873	3	1462.624	5.836	.021
Within Groups	2005.004	8	250.626		
Total	6392.877	11			

Multiple Comparisons

Dependent Variable: ACTPOD2
Tukey HSD

(I) CONCERT	(J) CONCERT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	25	.66870	12.92609	1.000	-40.72579	42.06319
	50	46.57007*	12.92609	.029	5.17558	87.96455
	100	22.79273	12.92609	.355	-18.60175	64.18722
25	0	-.66870	12.92609	1.000	-42.06319	40.72579
	50	45.90137*	12.92609	.031	4.50688	87.29585
	100	22.12403	12.92609	.378	-19.27045	63.51852
50	0	-46.57007*	12.92609	.029	-87.96455	-5.17558
	25	-45.90137*	12.92609	.031	-87.29585	-4.50688
	100	-23.77733	12.92609	.324	-65.17182	17.61715
100	0	-22.79273	12.92609	.355	-64.18722	18.60175
	25	-22.12403	12.92609	.378	-63.51852	19.27045
	50	23.77733	12.92609	.324	-17.61715	65.17182

*. The mean difference is significant at the .05 level.

3. Day 7

ANOVA

ACTPOD7

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	869.491	3	289.830	1.441	.301
Within Groups	1608.977	8	201.122		
Total	2478.468	11			

Multiple Comparisons

Dependent Variable: ACTPOD7
Tukey HSD

(I) CONCERT	(J) CONCERT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	25	14.76700	11.57935	.601	-22.31471	51.84871
	50	2.99550	11.57935	.993	-34.08621	40.07721
	100	-9.04410	11.57935	.861	-46.12581	28.03761
25	0	-14.76700	11.57935	.601	-51.84871	22.31471
	50	-11.77150	11.57935	.745	-48.85321	25.31021
	100	-23.81110	11.57935	.245	-60.89281	13.27061
50	0	-2.99550	11.57935	.993	-40.07721	34.08621
	25	11.77150	11.57935	.745	-25.31021	48.85321
	100	-12.03960	11.57935	.732	-49.12131	25.04211
100	0	9.04410	11.57935	.861	-28.03761	46.12581
	25	23.81110	11.57935	.245	-13.27061	60.89281
	50	12.03960	11.57935	.732	-25.04211	49.12131

4. Day 14

ANOVA

ACTPOD14

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.042	3	2.347	.061	.979
Within Groups	306.756	8	38.345		
Total	313.798	11			

Multiple Comparisons

Dependent Variable: ACTPOD14

Tukey HSD

(I) CONCENTR	(J) CONCENTR	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	25	-1.31387	5.05599	.993	-17.50516	14.87743
	50	-1.10353	5.05599	.996	-17.29483	15.08776
	100	-2.14803	5.05599	.973	-18.33933	14.04326
25	0	1.31387	5.05599	.993	-14.87743	17.50516
	50	.21033	5.05599	1.000	-15.98096	16.40163
	100	-.83417	5.05599	.998	-17.02546	15.35713
50	0	1.10353	5.05599	.996	-15.08776	17.29483
	25	-.21033	5.05599	1.000	-16.40163	15.98096
	100	-1.04450	5.05599	.997	-17.23579	15.14679
100	0	2.14803	5.05599	.973	-14.04326	18.33933
	25	.83417	5.05599	.998	-15.35713	17.02546
	50	1.04450	5.05599	.997	-15.14679	17.23579

5. Day 28

ANOVA

ACTPOD28

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1416.346	3	472.115	3.296	.079
Within Groups	1145.821	8	143.228		
Total	2562.167	11			

Multiple Comparisons

Dependent Variable: ACTPOD28

Tukey HSD

(I) CONCENTR	(J) CONCENTR	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	25	-6.89453	9.77165	.892	-38.18725	24.39818
	50	-17.65370	9.77165	.337	-48.94641	13.63901
	100	12.27207	9.77165	.612	-19.02065	43.56478
25	0	6.89453	9.77165	.892	-24.39818	38.18725
	50	-10.75917	9.77165	.699	-42.05188	20.53355
	100	19.16660	9.77165	.277	-12.12611	50.45931
50	0	17.65370	9.77165	.337	-13.63901	48.94641
	25	10.75917	9.77165	.699	-20.53355	42.05188
	100	29.92577	9.77165	.061	-1.36695	61.21848
100	0	-12.27207	9.77165	.612	-43.56478	19.02065
	25	-19.16660	9.77165	.277	-50.45931	12.12611
	50	-29.92577	9.77165	.061	-61.21848	1.36695

Appendix 7

Table 1: Changes in ascorbate peroxidase (APx) specific activities. The data are the means of at least three independent replicates. Data are means ± SE (n = 3).

Treatment Periods (days)	Concentrations of NaCl (mM)			
	0	25	50	100
0	40.609±0.566a	40.609±0.566a	40.609±0.566a	40.609±0.566a
1	32.758±3.877c	15.866±1.1988ab	0.869±0.1320a	26.312±4.258bc
2	41.016±13.278a	22.377±10.794a	0.689±0.048a	18.616±15.563a
7	47.2740±10.1556a	22.393±3.621a	40.137±7.877a	26.796±7.031a
14	0.023±0.008a	0.056±0.011a	0.037±0.018a	0.048±0.025a
28	0.039±0.012a	0.042±0.013a	0.090±0.036a	0.059±0.024a

Same letter at same raw indicates there were no significant differences ($p<0.05$) between the days of treatment under different concentrations.

Table 2: Changes in catalase (CAT) specific activities of different concentration of NaCl. The data are the means of at least three independent replicates. Data are means ± SE (n = 3).

Treatment Periods (days)	Concentrations of NaCl (mM)			
	0	25	50	100
0	0.071±0.035a	0.071±0.035a	0.071±0.035a	0.071±0.035a
1	0.3810±0.112a	0.153±0.025a	0.136±0.058a	0.181±0.025a
2	0.264±0.040a	0.490±0.051a	0.783±0.295a	0.403±0.106a
7	0.358±0.249a	0.630±0.322a	0.183±0.076a	0.272±0.051a
14	0.085±0.001b	0.058±0.016a	0.049±0.001a	0.159±0.024ab
28	0.203±0.044a	0.235±0.052a	0.220±0.0711a	0.582±0.2700a

Same letter at same raw indicates there were no significant differences ($p<0.05$) between the days of treatment under different concentrations.

Table 3: Changes in peroxidase (POD) specific activities of different concentration of NaCl. The data are the means of at least three independent replicates. Data are means ± SE (n = 3).

Treatment Periods (days)	Concentrations of NaCl (mM)			
	0	25	50	100
0	13.610±0.649a	13.610±0.649a	13.610±0.649a	13.610±0.649a
1	21.205±0.990a	19.696±3.477a	18.940±1.807a	19.715±3.884a
2	64.861±10.331b	64.193±6.173ab	18.291±2.326a	42.069±10.019b
7	59.146±9.807a	44.379±5.600a	56.150±4.150a	68.190±7.508a
14	23.219±5.290a	24.583±2.519a	24.323±1.163a	25.367±1.632a
28	25.798±2.548a	32.693±5.059a	43.452±9.870a	13.526±3.706a

Same letter at same raw indicates there were no significant differences ($p<0.05$) between the days of treatment under different concentrations.

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EFFECT OF SALINITY ON THE SPECIFIC ACTIVITIES OF ANTIOXCIDATIVE ENZYMES IN HOMALOMENA SP. CULTURES - ADIBAH ISMAIL