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Full Length Research Paper

Paraquat toxicity and its mode of action in some commonly consumed vegetables in Abeokuta, Nigeria

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Paraquat (PQ) is a toxic chemical that is widely used as herbicide in developing countries. This has led to extensive contamination of the environment, foods and food products. Therefore, this study investigated possible occurrence of PQ residues in some commonly consumed vegetables and the mode by which some of them were able to withstand oxidative stress condition associated with PQ toxicity. Levels of PQ residues and constituent antioxidant enzymes activities were determined spectrophotometrically. Paraquat residue concentrations in all vegetables were in the range of 0.04 to 0.27 ppm. A significant ($p<0.05$) increase in the activities of catalase, peroxidase and superoxide dismutase were observed in PQ-treated *Amaranthus caudatus*, *Celocia argentea* and *Chorchorus olitorius* as compared to the control groups during the first week of growth. Vegetables treated with 0.50 mM PQ showed initial signs of wilting without necrotic lesions. A progressive increase in malondialdehyde (MDA) content of PQ-treated vegetables compared to the control was observed. The chlorophyll content of the treated vegetables decreased with increased PQ concentrations. These results did not only revealed that these vegetables showed differential sensitivity to PQ, but also suggested that elevated antioxidant enzyme activities, especially during early stage of growth, is one of the likely mechanistic basis for the observed tolerance withholding capacity of these vegetables to PQ.

Key words: Paraquat, mode of action, leafy vegetables, antioxidant enzymes.

INTRODUCTION

One of the most important tasks of the economy in developing country like Nigeria is to develop the agricultural sector so as to increase and generate employment, promote self sufficiency in food, improve the standard of living, increase gross domestic production and contributes to general development. To attain food sufficiency, government encourages farmers to use improved seeds, fertilizers, irrigation and pesticides. Pesticides are used in most countries around the world to protect agricultural and horticultural crops against pests. They are also used at home and at work to ensure a pest-free environment. As a result of these, many problems such as unsafe use, persistence in environment, toxicity to bees, fish and wild life, contamination of water sources, persistent pesticide accumulating in food chain, negative impact on earthworms and other beneficial organisms has been identified. In developing countries, pesticides are routinely used in unsafe conditions and farmers lack training and resources to increase safety to their own health and environment.

Paraquat ($1,1^1$-dimethyl-$4,4^1$-bipyridinium dichloride) also known as methyl viologen is an important member of bipyridylium family of non-selective herbicide developed by plant protection division of Imperial Chemical Company in 1958 and first marketed for agricultural purposes in the United Kingdom in 1962. It is a quaternary nitrogen herbicide widely used for broadleaf weed control (Chia et al., 1982). Paraquat (PQ) is probably the most effective herbicide that exists and one of the world's worst poisons on the earth. It is a fast acting non-selective compound which destroys tissues of green plants on contact and by translocation within the

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plant (Suntres, 2002). Paraquat has also been demonstrated to be highly toxic for humans and animals and many cases of acute poisoning and death have been reported (Bismuth et al., 1990; Gram, 1997). Paraquat has been banned or restricted by Environmental Protection Agency (EPA) in some countries. For instance, Malaysia Government banned PQ in August 2002, while it was banned in Finland, Sweden and Austria because of its high toxicity and high frequency of poisoning (UNEP/FAO, 1996). The primary use of PQ is to improve crop management by raising the quality of the final harvest products and by decreasing the risk of cross infection from fungi and insects (Dodge, 1989). Worldwide, paraquat's use brings substantial benefits to food production and sustainable agriculture and triggered the growth of minimum and conservation tillage (Bromilow, 2003).

It is translocated to a greater extent especially under condition of low light intensity. When used for pre-planting weed control, new crop emerging picks up traces of the chemical from dead plant debris and soil that it pushes through, thereby leading to residues in the harvested crops. For instance, small amount of PQ (< 0.2 ppm) have been reported to be detectable in the foliage of certain crops such as sugar beet and cereal (Plant Protection Ltd, 1982).

Repeated treatment with PQ led to accumulation in the soil and damage to crops. Multiple spray trials showed PQ residues in soil from 22 to 58 mg/kg. The degradation of PQ in soil by *Lipomyces starkeys* was reported by Burn and Audus (1970) who found the occurrence of this pesticide in cultures containing organic components of the soil. Damanakis et al. (1970) reported that the absorption and mobility of PQ on different soils decreased with the increase of the ratio soil to water. On all soils, residual activity increased rapidly with dose once the minimum phytotoxic dose was reached. Residues of PQ in foods are usually detectable especially when it is used as a preharvest desiccant or pre-plant applications in food crops such as cereals where its levels reached 0.2 mg/kg (Brown et al., 2004).

This study was therefore carried out to investigate possible occurrence of PQ residues in some commonly consumed vegetables in Abeokuta, Nigeria and also probe into mode by which some of these vegetables were able to withstand oxidative stress associated with PQ toxicity.

MATERIALS AND METHODS

Fresh samples of different vegetables: *Amaranthus caudatus, Celocia argentea, Talinium triangulare, Veronia amygdalina, Capsicum frutescens, Lycopersicon esculentum, Raphanus sativus, Basella alba* and *Chorchorus olitorius* were purchased from different markets (Kuto, Omida, Itoku, Elega, Iberekodo and Osiele) in Abeokuta, Nigeria.

Stock paraquat dichloride solution (Gramoxone ® super, manufactured by Syngenta Crop Protection AG Basle, Switzerland) was purchased from C. ZARD, Ijaye, Abeokuta. Nigeria.

Determination of PQ residues in some vegetables

Samples preparation

Five gram of each samples were thoroughly washed and rinsed with deionised water, chopped (that is, the leafy parts removed), blended and homogenized.

Extraction of PQ residues

An extraction procedure adapted from Van Emon et al. (1987) as described by Selisker et al. (1995) was employed. Concentrated tetraoxosulphate (VI) acid (6N H_2SO_4) was added to 2.5 g of sample and then sonicated. Volume was made up to 5 ml with 6 N H_2SO_4 and shaken for about 10 min in an orbital shaker. After centrifugation at 5000 rpm for 10 min, the supernatant was recovered and kept at 4°C until used.

Spectrophotometric determination of PQ residues

The determination of PQ residues was carried out as described by Rai et al. (1997). Twenty microliter of the supernatant was mixed with sodium acetate buffer (0.1 M, pH 5), thereafter 2 ml of 1% aqueous sodium dithionite and 0.1N NaOH were added. The mixture was allowed to stand for about 2 to 5 min and PQ residues were quantified by measuring the absorbance at 600 nm. The amount of PQ residues was then estimated on a standard calibrated graph plotted using commercially available PQ. To examine the efficacy of extraction, three samples of each vegetable were spiked with known concentration of PQ (0.01 to 0.5 ppm) and extraction was performed as previously described and the mean percentage recovery was determined.

Evaluation of PQ-induced oxidative stress in some vegetables

This procedure was carried out by planting vegetables with the highest PQ residues in the previous experiment on pilot plots on which PQ was applied at manufacturer's (0.5 mM) and half-manufacturer's (0.25 mM) concentrations. Viable seeds of *A. caudatus* (A.C), *C. argentea* (C.A), and *C. olitorius* (C.O) were used in this assay.

PQ treatments

Paraquat was used as pre-plant herbicide application by spraying graded concentrations 0.25 and 0.5 mM on 60 cm diameter soil filled pot using knapsack sprayer delivering 280 Lh^{-1} of herbicide solution. This was left for 72 h before 1 g seeds of each vegetable were sown. Batches of vegetables harvested fresh 1, 2, 3 and 4 weeks after germination and stored in cellophane bags kept on ice prior to determination on the same day were used for analysis.

Plant samples extraction

The extraction was carried out according to the procedure of Rani et al. (2004). Briefly, the samples were prepared by grinding one gram (1 g) of each vegetables (control and treated separately) in 50% ethanol in pre-chilled mortal and pestle and the extracts centrifuged at 5000 rpm for 10 min. The supernatant obtained for each sample was used for analysis.

Table 1. Paraquat residues in some vegetables.

Samples	Residues (ppm)
C. olitorius	0.27±0.03
A. caudatus	0.20±0.02
C. argentea	0.15±0.01
C. fruteseens	0.10±0.02
T. triagulare	0.09±0.02
L. esculentum	0.09±0.02
R. sativus	0.09±0.02
V. amygadalina	0.05±0.01
B. alba	0.04±0.01

Each value represented mean ±SEM of six readings

Antioxidants enzymes assays

Superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activities: The assay of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) were carried out according to the procedures described by Das et al. (2000), Aebi (1983) and Rani et al. (2004), respectively.

Protein concentration: The protein concentration of the leaf crude extracts was quantified by Biuret method of Gornall et al. (1949) using bovine serum albumin (BSA) as standard.

Chlorophyll content: The chlorophyll concentration of each vegetable was extracted following the procedure described by Harborne (1993).

Lipid peroxidation: The extent of lipid peroxidation in the vegetable leaves was determined by measuring the amount of malondialdehyde (MDA) formed according to the method of Ohkawa et al. (1997), with slight modifications. Fresh leaves (0.1 g) were homogenized in 1 ml of 5% TCA solution. The homogenates were centrifuged at 5000 rpm for 15 min at room temperature. Equal volume of supernatant and 0.5% thiobarbituric acid (TBA) in freshly prepared 20% TCA solution were added and incubated in a water bath at 95°C for 30 min. The tubes were transferred into an ice bath and then centrifuged at 5000 rprn for 10 min. The absorbance of the supernatant was recorded at 532 nm and malondialdehyde (MDA) content determined using the extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

Statistical analysis

Values were expressed as mean ± standard error of mean. The level of homogeneity among the groups was tested using analysis of variance (ANOVA).

RESULTS

The level of PQ residues detected in some commonly consumed vegetables in Abeokuta is shown in Table 1. From the nine vegetables investigated, *C. olitorius* and *B. alba* have the highest (0.27 ppm) and lowest (0.04 ppm) PQ residues, respectively. Figure 1 shows the results of variation in the enzymatic activities between the control and 0.25 mM PQ treated vegetables. The catalase activities in the PQ-treated vegetables were higher than the control throughout the growth period. The catalase activity in the PQ-treated *A. caudatus* increased progressively throughout the growth period while that of *C. argentea* and *C. olitorius* remained relatively constant during the first three weeks of growth. The peroxidase activity in the PQ-treated *C. olitorius* increased up to the third week after which a decline in activity was observed. The activity of peroxidase in PQ-treated *C. argentea* and *A. caudatus* increased up to second week after which it remained relatively constant. The activities of superoxide dismutase in all the PQ-treated vegetables showed an initial increase in activities during the first and second week of growth. A decrease in the activities of SOD was observed in *C. olitorius* and *A. caudatus* after second week of growth, while an increased activity in *C. argentea* was observed during this period.

The increase in the activities of these enzymes especially during the early growth period could be attributed to enhanced superoxide radical production as a result of PQ action, which SOD acted on to produce hydrogen peroxide. Subsequent actions of catalase and peroxidase on hydrogen peroxide led to its decomposition to water and oxygen. The results of variation in the enzymic activities between the control and 0.50 mM PQ treated vegetables are shown in Figure 2. The CAT activities in PQ-treated vegetables were higher than that of the control throughout the experimental growth period. *A. caudatus* had the highest CAT activity among the control and PQ-treated vegetables. There was an initial increase in CAT activity of the treated vegetables especially during the first and second week of growth. PQ-treated *C. argentea* and *C. olitorius* showed a decline in CAT activities after second and third week of growth, respectively. The POD activities in the control *A. caudatus* and *C. olitorius* declined up to the third week of growth while the pattern was reversed in the PQ-treated ones. *C. argentea* POD activity showed a gradual increase in both the control and PQ-treated vegetable. An enhanced increase in SOD activities was observed in the PQ-treated vegetables compared to the control

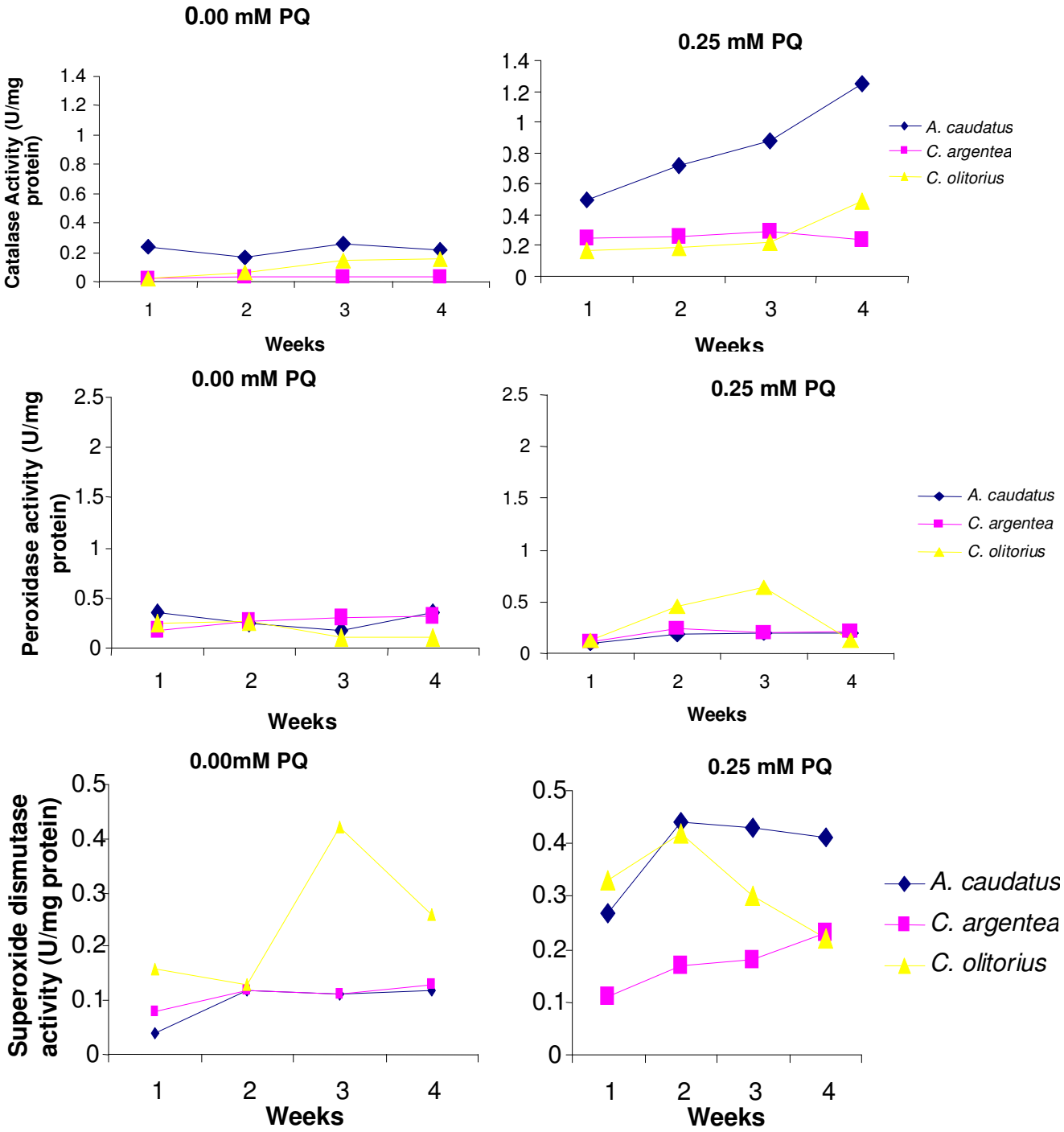

Figure 1. Antioxidant enzymes (CAT, POD and SOD) activities in 0.25 mM PQ treated vegetables.

throughout the growth period except in *C. argentea* which remained relatively constant during the third and the fourth week of growth.

The variation in MDA concentrations and chlorophyll contents in *A. caudatus* under 0.25 and 0.5 mM PQ treatments is shown in Figure 3. An increase in malondialdehyde (MDA) concentration was observed in PQ-treated vegetables compared to the control. The variation in MDA concentrations and chlorophyll contents in *C. argentea* under 0.25 and 0.5 mM PQ treatments during the experimental period is shown in Figure 4. A decrease in chlorophyll levels was observed in the PQ-treated groups compared to the control.

However, an increase in malondialdehyde (MDA)

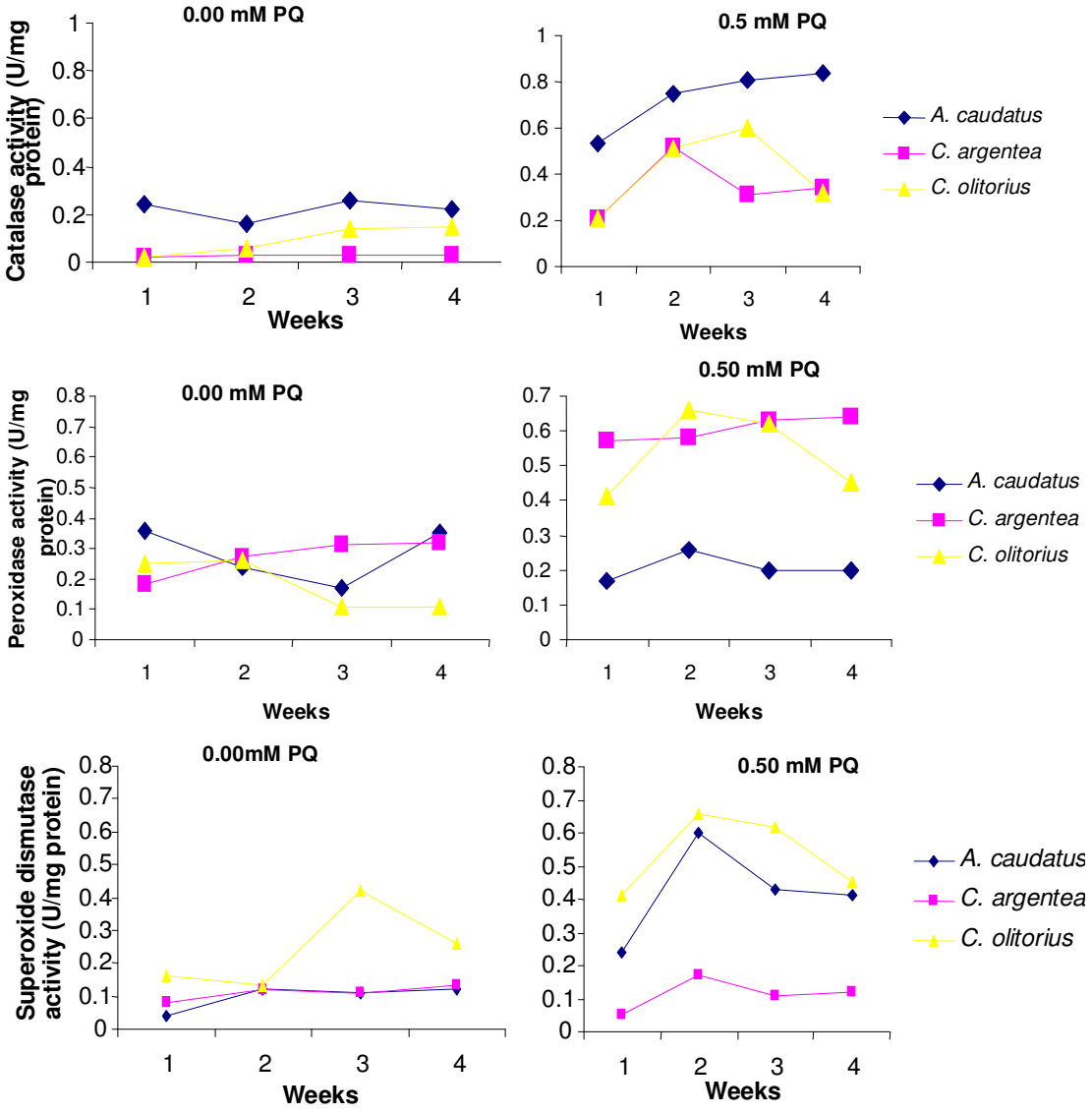

Figure 2. Antioxidant enzymes activities in 0.50 mM PQ treated vegetables.

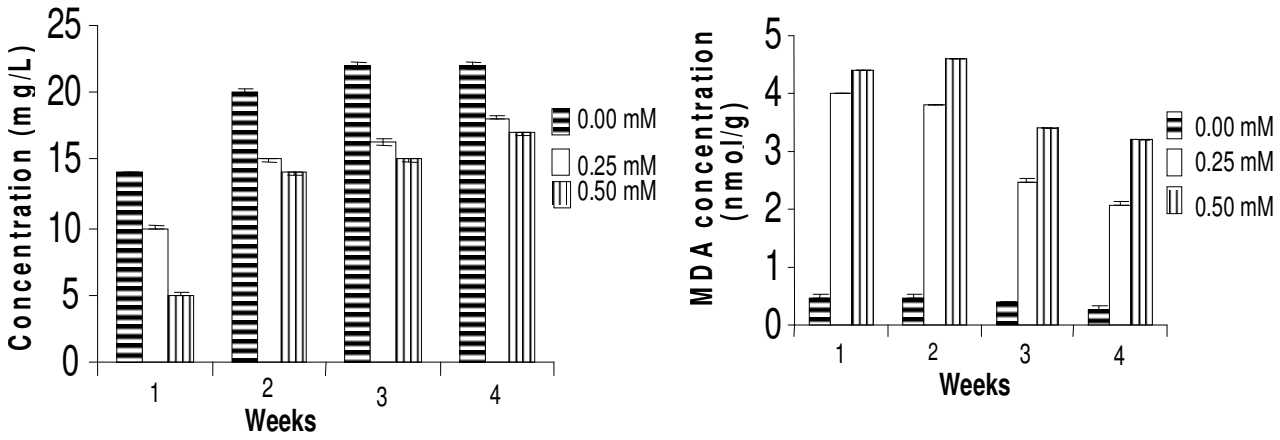

Figure 3. Chlorophyll content and malondialdehyde (MDA) concentrations in *A. caudatus.*

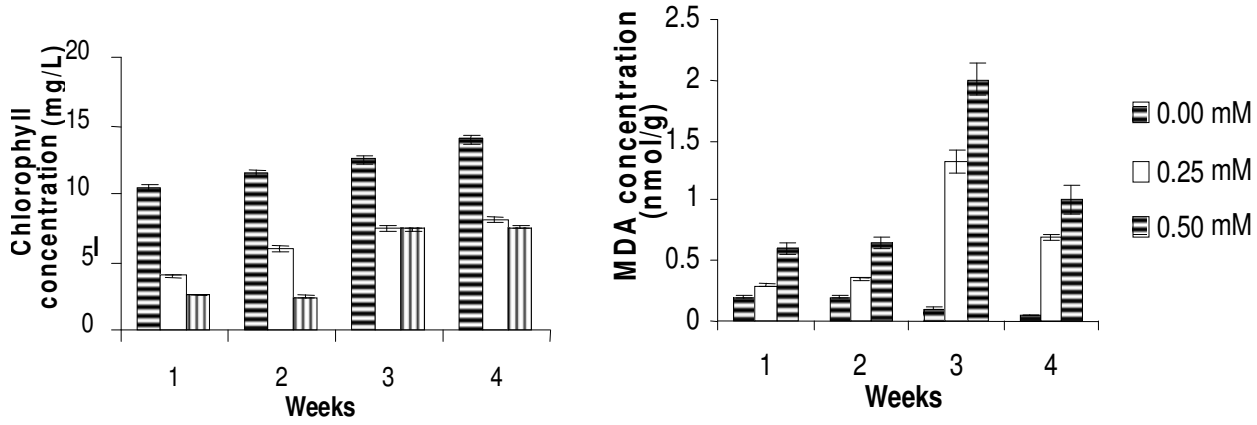

Figure 4. Chlorophyll contents and MDA concentrations in *C. argentea.*

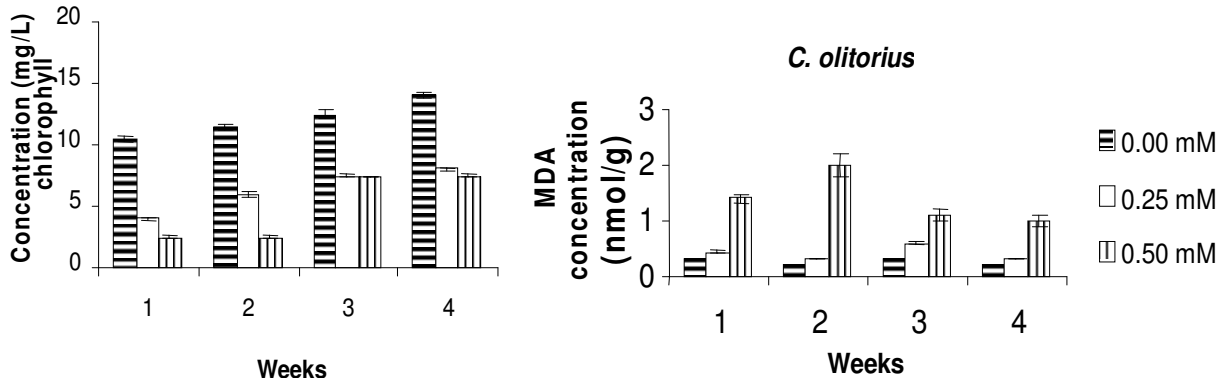

Figure 5. Chlorophyll contents and MDA concentrations in *C. olitorius.*

concentration was observed in PQ-treated vegetables compared to the control. The variation in MDA concentrations and chlorophyll contents in *C. olitorius* under 0.25 and 0.5 mM PQ treatments during the experimental period is shown in Figure 5. Highest chlorophyll level was observed in the control group during the fourth week of growth while the highest MDA concentration was observed during the second week of growth at 0.50 mM PQ treatment.

DISCUSSION

One of the major tasks of the control agencies in most of the developing countries is monitoring for pesticides residues in food. Pesticide residues monitoring is important not only to ensure compliance with the legislated national and international maximum residue limits, but also to assess possible existence of pesticide residues in foods and food products (WHO, 1997; Anderson and Poulsen, 2001; Camoni et al., 2001). Among the vegetables samples analyzed for possible occurrence of PQ residues, *B. alba* and *C. olitorius* had the least and highest PQ residues, respectively. This was conceived to have originated possibly through absorption from the soil and translocation to the leafy parts of the plant. However, these results revealed that PQ residues obtained in these vegetables were below the maximum residue limits (MRLs) of 0.5 ppm set by the UK (Zeneca Agrochemical, 1993). Thus, consumption of these vegetables or their products could be assumed safe.

Paraquat diverts photosynthetic electron transport from reducing NADPH to reduction of the PQ ion. In the presence of oxygen, the univalent reduced PQ radical is re-oxidized to produce superoxide radical, which is normally present in chloroplast and scavenged by superoxide dismutase enzyme. The results of this study clearly confirmed the importance of these antioxidant enzymes in conferring protection against PQ-induced oxidative stress, since the vegetables subjected to PQ treatments were able to survive toxic effect of this herbicide at the applied doses. SOD is considered as the key enzyme involved in the defense mechanism against oxygen toxicity, thus it is acknowledged to play an important role in combating the toxic effects of oxygen free radicals that could be produced by PQ in cellular compartments. Its role has been studied and reported by various authors (Raychaudhuri and Deng, 2000; Ismail et

al., 2001; Alscher et al., 2002). The increase in the total SOD activity in these vegetable especially during the early growth period could be attributed to enhanced superoxide radical production as a result of PQ action.

These results were in agreement with the report of Mckersie et al. (1993), Iannelli et al. (1999), and Iturbe-Ormaetxe (1998) who found that increased total SOD activity improve the tolerance to oxidative stress. The observed higher SOD activities in both PQ-treated vegetables especially during the first week of growth could be attributed to the fact that early developmental stages have been reported to be more sensitive to xenobiotics than later stages, and consequently increased SOD activities. It further buttressed the report of Donahue et al. (1997) who showed that PQ treated young pea leaves had higher SOD activity than older ones. Thus, the induction of SOD expression during the early stage of these vegetables seems to play an important role in removing superoxide radical ($O^{2\cdot}$), thereby minimizing oxidative stress as well as photo-oxidative damage. This observation is in agreement with the reports of Bowler et al. (1994) that plant treated with PQ had increased SOD activity compared to the control plant. It also concurs with the report of Ismail et al. (2001), that a two fold increase in SOD activity was observed in PQ-treated red flower ragleaf *Crassocephalium crepidioide* at the 6 leaves stage. Paraquat applications have also been reported to induce severe oxidative stress in the leaves of wild wheat than cultivated wheat (Ekmekei and Terzioglu, 2005). This could partly support the reason why the cultivated vegetables in this study were able to withstand the oxidative stress induced by PQ. Therefore, the variation in the SOD activity in these vegetables under PQ stress could explain scavenging capacity of $0^{2\cdot}$ as activity of SOD increased. Low levels of SOD activity observed during the fourth week of growth in these vegetables associated with relatively high MDA content could be explained on the premise that in some cases, high concentration of superoxide ion may overload the capacity of SOD to dispose it. Thus, O^2 might have spontaneously disproportionate in acidic thylakoid environment to produce extra hydrogen peroxide which may diffuse through the cell and cause damage.

The elevated levels of CAT and POD may be essential for protection of membrane from oxidative damage under both normal and stressed conditions. The elevated POD activity is important in that it could provide the power to detoxify the peroxides produced by SODs in chloroplasts and other cytosolic compartment stressed plants (Iannelli et al., 1999). The induction of CAT and SOD by PQ in this study is in agreement with the report of Fuerst and Vaughn (1990). It has been reported that an excessive SOD activity with respect to POD activity can potentially lead to enhanced $OH^{\cdot}$ radical formation (Toivonem and Sweeney, 1998). If SOD activity far exceeds the capacity for POD to detoxify the H_2O_2 formed through SOD action on superoxide ions, the H_2O_2 can react with superoxide anions directly to produce singlet oxygen and hydroxyl radicals which are very active in lipid peroxidation. This probably explains the observed early stage increase in the level of lipid peroxidation as measured by malondialdehyde concentration in the PQ-treated vegetables.

The POD activity in *C. argentea* remained relatively constant in the PQ-treated plants throughout the experimental period. Thus, POD therefore appears not to be important in the mechanism of PQ resistance /tolerance in this vegetable. It has been reported that PQ application resulted in increased MDA content (Ekmekci and Terzioglu, 2005). This study also demonstrated initial marked increase in MDA content in PQ-treated group with a gradual decline with age. MDA had been known to react with free amino group of protein, phospholipids and nucleic acids, leading to structural modifications which induce dysfunction of immune systems. As the lipid oxidation of cell membrane increases, the polarity of lipid phase surface change and formation of protein diagnosis increase such that molecular mobility of lipid, number of sulfhydryl groups and resistance to thermal denaturation decreases. This probably explained the observed initial sign of wilting with no observable necrotic lesions in the PQ-treated vegetables.

Biotic and abiotic stresses may cause various types of physiological response and oxidative damage in plants. Some screening techniques are often used to readily quantify the response of different species or different genotypes of the same species towards these stresses and hence to rank them according to their relative stress tolerance. Levels of chlorophyll have been used in photochemical events taking place in leaves under various environmental stresses. In this work, relatively lower levels of total chlorophyll were obtained in the PQ-treated vegetables compared to the control, especially during the first week of growth. This certainly will affect photosynthetic capacity of the vegetable which may deplete the plant's energy resources. However, the level of chlorosis observed was later reversed during the second week of growth in these vegetables. This observation supports the reports of Shaaltiel and Gressel (1998) and Aarti et al. (2006) who showed that chlorophyll content is immediately affected upon treatment of *Conyza* and cucumber with PQ but recovered back to normal as a result of dissipation of PQ from its site of action in photosystem I (PS 1). Varsha and Sujata (1999) reported that when cultures of *Chlamydomonas reinhandii* were treated with PQ, growth and chlorophyll levels decreased.

These results suggest that the response of antioxidants to PQ depends on the severity of stress, species and the age of plant. It also agrees with the findings of Iturbe-Ormaetxe et al. (1998) on defective metabolites against active oxygen species in pea plant exposed to PQ or water deficit. Although, Toivonen and Sweeney (1998)

reported that the rate of chlorophyll loss was associated with lower SOD and POD activities, showing not only their importance against oxygen radical damage but also on peroxide detoxification. Thus, the transient chlorophyll loss observed in these vegetables during the early stage of growth could not be attributed to low SOD and POD activities since these were present at higher levels than during the later stage of growth. Previous studies have established that oxidative stress induced by PQ impedes key steps in chlorophyll biosynthesis by either directly or indirectly inhibiting the activity of these enzymes (Aarti et al., 2006). The inverse relationship observed between malondialdehyde and chlorophyll loss in the PQ treated vegetables is in agreement with the report of Zhuang et al. (1995) on lipid peroxidation which is linked to yellowing as demonstrated in *Bassica olevacea.*

The present study therefore shows that *Corchorus olitorius* is more tolerant to PQ-mediated stress than other vegetables, and this could be attributed not only to the protective role of antioxidant enzymes present in it but also to some endogenous protective mechanism such as high ascorbic acid concentration as reported by Faboya and Ekanem (1997).

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