Antioxidative Potential of Garlic on Lead-Induced Oxidative Stress and Effect on Enzyme Activity in Rice Plants

Tugbobo Oladimeji S1*, Idowu Kayode S1, Ajao Oluwaseyi I2

Affiliation
1Department of Science Technology, Federal Polytechnic, Ado-Ekiti, Nigeria
2Department of Mathematics and Statistics, Federal Polytechnic, Ado-Ekiti, Nigeria

Corresponding author: Tugbobo Oladimeji S, Associate Professor of Biochemistry, Toxicology and Plant Biochemistry Unit, Department of Science Technology, Federal Polytechnic, Ado-Ekiti, Nigeria, Tel: +2348035303701; Email: tugbobooladimeji1@gmail.com

Citation: Tugbobo OS, Idowu KS and Oluwaseyi AI. Antioxidative potential of garlic on lead-induced oxidative stress and effect on enzyme activity in rice plants (2018) Edelweiss Appl Sci Tech 2: 79-83

Received: Dec 04, 2017
Accepted: Jan 25, 2018
Published: Jan 31, 2018

Copyright: © 2018 Tugbobo OS, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract
Seedling of Ofada rice (Oryza sativum L) were raised in sand (swampy) and clay (upland) cultures under 500mg/kg lead acetate and 500mg/kg 2

Oxidative stress and effect on enzyme activity in rice plants.

Experimental Design

Collection of Soil Sample
Soil samples were collected from five states in South-West region of Nigeria. Two locations (Toga and Owode) in Ogun state were considered for the research due to their appreciable lead levels after a comprehensive soil test.

Preparation of Garlic Extract
500g of the powdered sample (garlic cloves) was extracted via maceration for 48hrs using the method of Aguawa and Mittal [9].

Introduction
Lead occurs naturally in soil and water. Plants absorb soil lead through their roots and thus, all plants contain small amount of this metal [1]. The relationship between plants and soil lead varies with factors ranging from chemical forms of the element in soil, soil properties, climate, plant species, etc. [2]. Lead, a toxic heavy metal and pollutant of the environment that originates from various sources like mining, pottery, casting and soldering, metallurgy, aerosols and dust from smelters, ammunition and automobile-exhaust, gasoline, etc. [3]. However, increased lead levels in the soil environment inhibits seed germination, stunts seedling growth and threatens plant metabolic reactions for proper growth and development resulting in low yields (4). High levels of heavy metals in the soil normally result in oxidative damage to plants either directly or indirectly by triggering an increased level of reactive oxygen species generation that generally cause damage to the biological molecules such as proteins, membrane lipids, chloroplast pigments, enzymes, nucleic acids, etc.[5]. These free radicals include superoxide radical (O2⋅−) hydroxyl radical (OH⋅−) and hydrogen peroxide (H2O2) that are produced as by products during membrane linked electron transport reaction and by associated metabolic pathways [6]. Oxidative stress occurs where there is imbalance between the production of reactive oxygen species and the biological system’s ability to readily detoxify the reactive intermediates produced or failure to easily repair the damage [7]. Also several studies have reported that organosulphur and polyphenolic compounds in plants protect against oxidative stress [8]. To salvage the rice plants from lead-induced oxidative stress, this study was designed to assess the uptake and distribution of lead in root and shoot of rice plants, determine the inhibitory potential of garlic as well as possible alternation in the activity of some defensive enzymes of rice plants.

Materials and Methods

Preparation of Garlic Extract
500g of the powdered sample (garlic cloves) was extracted via maceration for 48hrs using the method of Aguawa and Mittal [9].

Citation: Tugbobo OS, Idowu KS and Oluwaseyi AI. Antioxidative potential of garlic on lead-induced oxidative stress and effect on enzyme activity in rice plants (2018) Edelweiss Appl Sci Tech 2: 79-83
In both locations (Toga and Owode areas), rice seeds were surface sterilized with 0.1% sodium hypochlorite solution for 10mins and then rinsed with distilled water. After 24hrs imbibitions of seeds in water, the seedlings were raised in clay (Owode area) and sand (Toga area) cultures respectively in lead free plastic pots. The pots received respective treatment solutions and were carefully maintained under control for seedling growth in a Biological Oxygen Demand (B.O.D) and optimum relative humidity with 12hrs photoperiod. In each location, the pots were grouped into four groups A, B, C, and D. ‘A’ contained seedlings treated with distilled water only and served as positive control. ‘B’ seedlings were treated with 500mg/kg lead acetate only, and seedling in ‘C’ received 500mg/kg lead acetate with 500mg/kg garlic extract simultaneously while seedling in ‘D’ were treated with 500mg/kg garlic extract only.

### Determination of Lead in Rice Seedlings

Fresh root and shoot samples were surface sterilized with 1M HCl and then with 1mM Na2EDTA for the surface bound lead and then dried in oven at 70°C for 5-days. Dried samples were ground to a fine powder in mortar and pestle and digested with concentrated H2SO4. Digested samples were dissolved in de-ionized distilled water and lead content was estimated using atomic absorption spectrophotometer (AAS).

### Oxidative Stress Assay

The level of lipid peroxidation products was determined using the method of Heath and Packer [10]. Fresh root and shoot samples were ground in 0.25% thiobarbituric acid (TBA) in 10% TCA using mortar and pestle. The mixture was heated at 95°C for 30min then cooled in an ice bath and centrifuged at 10 000 x g for 10min. The absorbance of the supernatant was read at 532nm while total of 0.25% TBA in 10% TCA served as blank. The concentration of lipid peroxides together with the oxidative-modified proteins of plants were quantified and expressed as total TBARS as nmol g\(^{-1}\) fresh weight using an extinction coefficient of 155mM\(^{-1}\) cm\(^{-1}\).

### Evaluation of Garlic Inhibitory Potential

The production and inhibition of lipid peroxides from rice roots and shoots was determined using method described by [11]. The roots and shoots were ground in cold saline (1/10 w/v) with 10 up-and-down strokes in mortar and pestle. The homogenate was centrifuged at 10 000xg for 10mins to obtain the supernatant and also incubated with lead acetate and garlic extract at varied concentrations together with de-ionized water at total volume 300µl at 37°C for 1hr. The color reaction was monitored by adding 200, 250 and 500µl each of 8.1% Sodium Dodecyl sulphate (SDS), acetic acid at pH 3.4 and 0.6% TBA respectively. The solution was incubated at 97°C for 1hr and absorbance was read at 532nm.

### Superoxide dismutase Assay

The activity of Superoxide Dismutase (SOD) was determined according to method described by [12]. About 200 mg fresh tissue (root/shoot) were homogenized in 5ml of 100 mM K-phosphate buffer at pH 7.8 containing 0.1 mM EDTA. 0.1% (v/v) Triton X-100 and 2% (w/v) polyvinyl pyrrolidone (PVP). The extract was filtered and centrifuged at 22 000 x g for 10mins at 4°C and after which the supernatant was used for the enzyme assay. The assay mixture in total volume of 1.5ml contained 1000µl of 100µl enzyme at 240 nm.

### Catalase Assay

The activity of catalase was examined according to [13]. 200 mg Fresh tissue (roots/shoot) were homogenized in 5ml of 50 mM EDTA. 2% (w/v) PVP and 0.5% (v/v) Triton X -100. The homogenate was centrifuged at 22 000 x g for 10mins at 4°C and after which the supernatant was used for the enzyme assay. The assay mixture in total volume of 1.5ml contained 1000µl of 100µl enzyme at 240 nm.

### Glutathione reductase Assay

Glutathione reductase was assayed according to [14]. 200mg Fresh tissue (root/shoot) were homogenized using chilled mortar and pestle in 5ml of 50 mM Tris-HCl buffer (pH 7.6). The homogenate was centrifuged at 22 000 x g for 30mins at 4°C and the supernatant was used for the enzyme. The reaction mixture in a total volume of 1ml contained 50 mM Tris-HCl buffer (pH 7.6), 0.15 mM NADPH, 1 mM GSSG, 3 mM MgCl\(_2\) and 200µl enzyme extract. The activity of the enzyme was monitored with absorbance at 340nm.

### Results

<table>
<thead>
<tr>
<th>Rice Pots</th>
<th>lead levels in root (mg/kg)</th>
<th>lead levels in shoot (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>15.2</td>
<td>7.25</td>
</tr>
<tr>
<td>B</td>
<td>20.5</td>
<td>12.5</td>
</tr>
<tr>
<td>C</td>
<td>16.7</td>
<td>10.8</td>
</tr>
<tr>
<td>D</td>
<td>12.4</td>
<td>8.5</td>
</tr>
</tbody>
</table>

Table 1: Uptake of lead by growing rice seedlings (Sand) in Toga area for 40-days.

### Table 2: Uptake of lead by growing rice seedlings (clay) in Owode area for 40-days.

![Figure 1](image-url)
Citation: Tugbobo OS, Idowu KS and Oluwaseyi AI. Antioxidative potential of garlic on lead-induced oxidative stress and effect on enzyme activity in rice plants (2018) Edelweiss Appli Sci Tech 2: 79-83
Oxidation in plant levels.

The antioxidant activity has been reported to be concomitant with the development of reducing power [16] and this is due to garlic extract hydrogen donating ability [17]. In this study, garlic antioxidant activity was demonstrated against the reactive oxygen species generated and thus, inhibits lipid peroxidation due to its scavenging potential. Besides, plants generally possess inherent antioxidant defense system used naturally to combat the oxidative damage. In view of this, Table 7 shows effect of lead uptake on Superoxide-Dimutase (SOD) activity in rice seedling where a significant (P<0.05) increase in SOD activity (26.51 and 17.99) was observed in roots of rice seedlings of pots C and B treated with lead acetate.

Meanwhile similar effect was observed in shoots of rice seedlings of pots C and D treated with lead acetate where significant (P<0.05) increase (39.91 and 20.57) in SOD activity respectively. However, SOD activity was observed higher in shoot than root because root absorbs more lead concentrations than shoot. Hence, the excessive lead has the propensity of reducing the defensive potential of SOD in plant root. SOD activity has been reported to be increased under water stress [18], heavy metal toxicity [19]. This increase in response to stress could be due to de novo synthesis of the enzyme [20]. The catalase (CAT) activity observed for lead treated rice seedlings in B was significantly (P<0.05) lower than control for both root (0.15) and shoot (0.22) respectively. However, in pots C and D treated with the sample extract, CAT activity was appreciably higher in the plant tissues when compared to control.

The decline in CAT activity in pot B could be attributed to lead toxicity which could possibly delay the removal of hydrogen peroxide and peroxides mediated by catalase which in turn enhances free radical mediated lipid peroxidation in plant tissues [21]. Besides, a decline in catalase activity has been attributed to the inactivation of enzyme protein due to deleterious activity of reactive oxygen species which either decrease the enzyme synthesis or cause alteration in assembly of enzyme subunits [22]. On the contrary, Glutathione Reductase (GR) activity was higher in both root (1.56) and shoot (1.21) of rice seedlings treated with lead acetate in pot B compared with control. Similarly, GR activity was significantly (P<0.05) higher in pot D treated with extract sample than tissues of rice seedlings in pot C. This could be attributed to antioxidant potential of the extract (garlic) which also compliments the GR antioxidant activity [23]. In addition, the increased GR activity suggests possible involvement of GR in regenerating GSH from GSSG under lead toxicity in order to increase GSH/GSSG ratio and thus, increasing total glutathione pool [24]. The study above clearly suggests that lead toxicity induces oxidative stress in rice plants which could be modulated by garlic antioxidant effect, while antioxidant enzymes play a pivotal role in combating oxidative stress in the plants.

**Citation:** Tugbobo OS, Idoowu KS and Oluwaseyi AI. Antioxidative potential of garlic on lead-induced oxidative stress and effect on enzyme activity in rice plants (2018) Edelweiss Appli Sci Tech 2: 79-83
Acknowledgement

The authors appreciated the financial aid and facilities provided by tertiary education trust fund (TETFUND) via Federal Government of Nigeria.

References


Citation: Tugbobo OS, Idowu KS and Oluwaseyi AI. Antioxidative potential of garlic on lead-induced oxidative stress and effect on enzyme activity in rice plants (2018) Edelweiss Appli Sci Tech 2: 79-83