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Allelopathic effects of *Nypa fructican* Leaf and Roots extracts on the antioxidant enzyme activities of *Rhizophora mangle*, *Tridax procumbens* and *Cyperus rotundus*

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ABSTRACT

The allelopathic properties of *Nypa fructican* leaf and root extract on antioxidant enzymes: superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activity of *Rhizophora mangle*, *Tridax procumbens* and *Cyperus rotundus* was investigated. Pot experiment was performed with different concentrations (0%, 25%, 50%, 75% and 100%) of the leaf and root extracts of *Nypa fructican*. Distilled water was used as control (0%). The antioxidant enzymes (SOD), (POD) and (CAT) activities were measured using standard methods. The results showed a concentration dependent increase in the antioxidant enzyme; superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities. Allelochemicals disrupt the balance of reactive oxygen species (ROS) and antioxidants, altering the physiological condition of plants.

Keywords: Alleloparthy, *Nypa fructican*, *Enzyme Activity*, Chlorophyll Content, Bioherbicides, Antioxidant, *R. mangle*.

Introduction

Allelopathy is a phenomenon whereby plants directly or indirectly release secondary metabolites known as allelochemicals which interfere with the growth and development of other plants. Studies in alleloparthy have shown that it can be used to control weeds and to reduce the use of synthetic herbicides in crop production (Farooq et al., 2020). Antioxidant enzymes are a family of powerful free radical scavenging proteins, naturally synthesized by all aerobic organisms. These oxidative enzymatic machineries sequentially sequester singlet oxygen (O^{2-}) and Hydrogen Peroxide, which are the well-known reactive oxygen species (ROS). Increase in ROS production and an induction of oxidative stress are general plants' responses to various allelochemicals (Steszek et al., 2021). To cope with stress conditions, plants possess several scavenging enzymes, such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), which are activated to strengthen the plants' resilience against both abiotic and biotic stressors.

Allelopathic compounds, especially phenolics, are known to enhance the activity of antioxidant enzymes like SOD, CAT, and POD as plants respond to reactive oxygen species (ROS) (Nethravathy, 2021).

Nypa fructicans is an invasive plant that belongs to the family Arecaceae and grows along tidal streams in brackish swamps. Its invasive nature has been linked to the presence of phytochemicals like phenolic compounds. Yusoff *et al*, 2015, mentioned that the extracts of *N. fructicans* contain phenolic and flavonoids compounds. These compounds may confer strong alleloparthic effects influencing the physiological processes of neighboring plants (Numbere, 2018).

Rhizophora mangle, commonly known as red mangrove, is a widely distributed intertidal species with significant ecological and economic importance in tropical coastal regions. It thrives in the sheltered intertidal zones of tropical and subtropical shorelines, often dominating these environments.

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Cyperus rotundus L., an invasive sedge from the Cyperaceae family found worldwide, It is commonly called purple nutsedge or nutgrass. In Nigeria it is locally called Giragiri (Hausa) and Danda (Yoruba). Reports have indicated that *Cyperus rotundus* can cause a 35-89% reduction in the yields of cabbage, tomato, cucumber, green bean, carrot, okra, bell pepper, onion, garlic and many other crops, its aggressive growth competes with crops, creating challenges for agricultural sustainability.

Tridax procumbens, commonly known as coat buttons or tridax daisy, belongs to the Asteraceae family. *T. procumbens* is regarded as an economically significant weed. It is widely recognized as a weed in agricultural and disturbed areas, significantly impacting soybean production in Brazil and reducing crop yields in Sri Lanka (Latif *et al.,* 2017).

The rampant spread of weeds along with concerns about the environmental impact of synthetic herbicides highlights the need for eco-friendly alternatives like allelopathic plant extracts, which can inhibit weed growth by synthesis and triggering oxidative stress responses (Zhang *et al.,* 2020).

Materials and Methods

Equipments /Apparatus

Water bath (Grant, England), hot air oven (Gallenpkam, England), Bench centrifuge (Clay adams, USA), beakers, digital weighing balance (Mettler PT 320-Wagen, Switzerland), funnel, measuring cylinder, pH meter (Hanna, HI 98106), magnetic stirrer (Lensor, USA), No 1 Whatman Filter paper, Rotary evaporator (Buchi Rotavapour Switzerland).

Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grades

Plant material, collection and Identification

Fresh and healthy leaves and roots of *Nypa fructican* and seedlings of Rhizophora mangle were collected from Ekede in Andoni Local Government Area of Rivers State.

Seeds of *Cyperus rotundus* and *Tridax procumbens* were collected from a farm in the Agricultural Development Area in Rumuodomaya, in Obio Akpor Local Government Area of Rivers State.

The samples collected were identified and certified by Dr Wisdom Barade a taxonomist in the Department of Department of Science Laboratory Technology, School of Applied Sciences, Kenule Beeson Saro-Wiwa Polytechnic, Bori, Rivers State, Nigeria and voucher numbers were given**,** *Nypa fructican* KSPT/2023/330**,** *Rhizophora mangle* KSPT/2023/325, *Tridex procumbens* KSPT/2023/321, *Cyperus rotundus* KSPT/2023/321**.**

Preparation of Plant (Leaf and Root) Extracts

The leaves and roots were washed in running tap water to remove the surface contaminants and dust and dried at room temperature for a period of one month, ground into powder and packed in a paper bag prior to experiment. Extracts were prepared following the method of Ngondya *et al.* (2016) with some modifications: 100 g of *Nypa fruticans* leaf and root powder were soaked separately in 1 L of distilled water for 72 hours. Afterward, the crude extracts were filtered using Whatman No. 1 filter paper.

Both extracts were then diluted with distilled water in the following ratios: 0:100, 25:75, 50:50, 75:25, and 100:0 (extract: distilled water) to achieve concentrations of 0%, 25%, 50%, 75%, and 100%, with distilled water (0%) serving as the control. The diluted extracts were placed in properly labeled containers and stored in the refrigerator at 4°C.

Seed Germination in pots

Thirty plastic pots each with dimension 30 × 13 cm were prepared by perforating the base of the pots and filled with top humus soil. Seeds of test plants were sterilized by washing them in 5% sodium hypochlorite to avoid the effect of microorganism contamination. Sterilized seeds were then washed thoroughly three times in distilled water before planting. Ten seeds were planted in the pot. Pots were treated with different concentrations of leaf and root extracts while the pots for the controls were treated with distilled water. Each treatment had three replicates. After 60 days, the experiment was terminated and measurements were taken.

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Antioxidant Enzyme Analysis

The Allelopathic effect of *Nypa fructican* on *Rhizophora mangle*, *Tridax procunbens* and *Cyperus rotundus* was determined through the enzymatic activities of catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD). All analyses were performed using a spectrophotometer. Fifty milligrams of the sample were macerated with liquid nitrogen and then homogenized in 50 mM phosphate buffer (pH 7.0). The homogenate was centrifuged at 4°C for 20 minutes at 10,000 g. The supernatant was collected as the enzyme extract and was subsequently used for antioxidant enzyme assays. The activities of various enzymes in response to allelopathic stress were measured as follows:

Catalase Activity

Catalase (CAT) activity was determined spectrophotometrically by measuring the rate of H_2O_2 disappearance at 240 nm (Aebi 1984). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0) and 10.5 mM H_2O_2. The reaction was run at 25°C for 2 min, after adding the enzyme extract and rate of decrease in absorbance at 240 nm ($E = 39.4 mM^{-1} cm^{-1}$) was used to calculate the enzyme activity.

Superoxide Dismutase Activity

The supernatant was also used for the assay of SOD following the method of Beauchamp and Fridovich (1971). Reaction mixture was prepared by mixing 27 ml of 50 mM potassium phosphate, (pH 7.8), 1.5 ml of L-methionine (300 mg/10 ml) and 1 ml of nitroblue tetrazolium salt (NBT) (14.4 mg/10 ml). Aliquots (2 ml) of this mixture were delivered into small glass tubes, followed by 20 µl of enzyme extract and 10 µl of riboflavin (4.4 mg/100 ml). The mixture was illuminated for 15 min in an aluminum foil-lined box, containing two 20 W fluorescent tubes. A control tube in which the sample was replaced by 20 µl of buffer was run in parallel and the absorbance at 560 nm was measured in all tubes. Under the described conditions, the increase in absorbance without the enzyme extract was taken as 100% and the enzyme activity was calculated by determining the percent inhibition per minute. 50% inhibition was taken as equivalent to 1 unit of SOD activity.

Peroxidase activity

Peroxidase activity was analyzed by determining the absorbance at 470nm caused by oxidation of guaicol to tetraguaiacol as described by Mandal et al. (2008), with some modifications. 3ml of reaction mixture was constituted by adding 20mM of (0.1ml) of guaiacol, 0.1mM of potassium phosphate buffer (pH 5.0) (2.5ml), 0.2ml of 40mM of H_2O_2, and the enzyme extract (0.2ml). The extinction coefficient of POD enzyme is 26.6mM cm. POD unit was taken as the amount of protein required to oxidize 1mM of tetraguaiacol per minute.

Results

Table 1 shows the Superoxide dismutase (SOD) activity (units/ml) of *R mangle*, *T. procumbens*, *C. rutundus* exposed to the leaf and root extract of *N. fructican*.

The result shows that the SOD activity of the plants treated with the control (0%) for *R. mangle* (leaf, 16.94 ± 0.06 and root, 12.53 ± 0.04), *T. procumbens* (leaf, 11.46 ± 0.03 and root, 9.83± 0.02) and *C. rotundus* (leaf, 20.22± 0.11 and root, 13.83± 0.12) were slightly lower than those of the groups treated with 25-100% extract.

There were observed extract concentration dependent increases in SOD in *R. mangle* ranging from 23.10± 0.10 to 51.48± 0.12 for plants treated with 25-100% leaf extract of *N. fructican*, while the root extract increased the SOD from 18.77± 0.03 - 33.68± 0.09 respectively.

There were observed extract concentration dependent increases in SOD in *T. procumbens* ranging from 15.11± 0.12 to 35.51 ± 0.11 for plants treated with 25-100% leaf extract of *N. fructican*, while the root extract increased the SOD from 11.69 ± 0.06- 24.21 ± 0.09 respectively.

There were observed extract concentration dependent increase in *C. rotundus* ranging from 23.64 ± 0.38 to 44.60 ± 0.12 for plants treated with 25-100% leaf extract of *N. fructican*, while the root extract increased the SOD from 15.65± 0.05 - 38.53± 0.13.

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Table 1: Superoxide dismutase activity (units/ml) of *Rhizophora mangle*, *Tridex procumbens* and *Cyperus rotundus* exposed to root and leaf extracts of *Nypa fructican*

Extract conc (%)	*Rhizophora mangle*		*Tridex procumbens*		*Cyperus rotundus*	
	Leaf	**Root**	**Leaf**	**Root**	**Leaf**	**Root**
Control (0)	16.94±0.06^{a}	12.53±0.04^{a}	11.46±0.03^{b}	9.83±0.02^{b}	20.22±0.11^{c}	13.83±0.12^{c}
25	23.10±0.10^{a}	18.77±0.03^{a}	15.11±0.12^{b}	11.69±0.06^{b}	33.64±0.38^{a}	15.65±0.05^{c}
50	29.72±0.09^{a}	22.82±0.01^{a}	21.85±0.10^{b}	17.37±0.07^{b}	31.26±0.11^{c}	23.81±0.09^{a}
75	38.19±0.04^{a}	29.32±0.06^{a}	26.72±0.08^{b}	20.78±0.05^{b}	38.13±0.12^{a}	32.08±0.11^{c}
100	51.48±0.12^{a}	33.68±0.09^{a}	35.51±0.11^{b}	24.21±0.09^{b}	44.60±0.12^{c}	38.53±0.13^{c}

Key: Values are mean ± standard deviation of triplicate determinations. Values with different superscript letters per row are statistically significant (P<0.05).

Table 2 shows the Peroxidase (POD) activity (units/ml) of *R mangle*, *T. procumbens*, *C. rutundus* exposed to the leaf and root extract of *N. fructican*. The result show that the POD activity of the plants treated with the control (0%) for *R. mangle* (leaf, 48.12 ± 0.21 and root, 33.42 ± 0.24), *T. procumbens* (leaf, 41.85 ± 0.19 and root, 28.42± 0.13) and *C. rotundus* (leaf, 52.87± 0.27 and root, 33.83± 0.18) were slightly lower than those of the groups treated with 25-100% extract.

There were observed extract concentration dependent increases in POD in *R. mangle* ranging from 67.24± 0.21 to 115.58± 0.31 for plants treated with 25-100% leaf extract of *N. fructican*, while the root extract increased the POD from 58.93± 0.11 - 98.10± 0.23 respectively.

There were observed extract concentration dependent increases in POD in *T. procumbens* ranging from 62.17± 0.31 to 102.94 ± 0.42 for plants treated with 25-100% leaf extract of *N. fructican*, while the root extract increased the POD from 42.87 ± 0.21- 92.74 ± 0.27 respectively. There were observed extract concentration dependent increase in *C. rotundus* ranging from 73.18± 0.42 to 118.57 ± 0.31 for plants treated with 25-100% leaf extract of *N. fructican*, while the root extract increased the POD from 48.24± 0.33 -96.51± 0.25.

Table 2: Peroxidase activity (units/ml) of *Rhizophora mangle*, *Tridex procumbens* and *Cyperus rotundus* exposed to root and leaf extracts of *Nypa fructican*

Extract conc (%)	*Rhizophora mangle*		*Tridex procumbens*		*Cyperus rotundus*	
	Leaf	**Root**	**Leaf**	**Root**	**Leaf**	**Root**
Control (0)	48.12±0.21^{a}	33.42±0.24^{a}	41.85±0.19^{b}	28.42±0.13^{b}	52.87±0.27^{c}	33.83±0.18^{a}
25	67.24±0.21^{a}	58.93±0.11^{a}	62.17±0.31^{b}	42.87±0.21^{b}	73.18±0.42^{c}	48.24±0.33^{c}
50	83.95±0.13^{a}	71.81±0.15^{a}	74.68±0.23^{b}	64.73±0.33^{b}	86.03±0.19^{c}	66.93±0.21^{c}
75	94.20±0.16^{a}	86.42±0.28^{a}	96.70±0.41^{b}	80.42±0.31^{b}	93.14±0.23^{a}	88.16±0.19^{c}
100	115.58±0.31^{a}	98.10±0.23^{a}	102.94±0.42^{b}	92.74±0.21^{b}	118.57±0.31^{c}	96.51±0.25^{c}

Key: Values are mean ± standard deviation of triplicate determinations. Values with different superscript letters per row are statistically significant (P<0.05).

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Table 3 shows the Catalase activity (units/ml) of *R mangle, T. procumbens, C. rutundus* exposed to the leaf and root extract of *N. fructican*. The result show that the CAT activity of the plants treated with the control (0%) for *R. mangle* (leaf, 24.85 ± 0.11 and root, 19.22 ± 0.10), *T. procumbens* (leaf, 23.79 ± 0.13 and root, 15.04± 0.09) and *C. rotundus* (leaf, 17.29± 0.08 and root, 30.88± 0.15) were slightly lower than those of the groups treated with 25-100% extract. There were observed extract concentration dependent increases in CAT in *R. mangle* ranging from 31.76± 0.12 to 63.86 ± 0.21 for plants treated with 25-100% leaf extract of *N. fructican*, while the root extract increased the CAT from 26.01 ± 0.1-48.35± 0.18 respectively. There were observed extract concentration dependent increases in CAT in *T. procumbens* ranging from 28.63± 0.15 to 52.86± 0.15 for plants treated with 25-100% leaf extract of *N. fructican*, while the root extract increased the CAT from 25.88± 0.13- 46.38± 0.23 respectively. There were observed extract concentration dependent increase in *C. rotundus* ranging from 28.18± 0.19 to 58.49± 0.30 for plants treated with 25-100% leaf extract of *N. fructican*, while the root extract increased the CAT from 35.72± 0.16-65.11± 0.24.

Table 3: Catalase activity (units/ml) of *R. mangle*, *T. procumbens* and *C. rotundus* exposed to root and leaf extracts of *N. fructican*

Extract conc (%)	*Rhizophora mangle*		*Tridex procumbens*		*Cyperus rotundus*	
	Leaf	**Root**	**Leaf**	**Root**	**Leaf**	**Root**
Control (0)	24.85±0.11^{a}	19.22±0.10^{a}	23.79±0.13^{a}	15.04±0.09^{b}	17.29±0.08^{b}	30.88±0.15^{c}
25	31.76±0.12^{a}	26.01±0.10^{a}	28.63±0.15^{b}	25.88±0.13^{a}	28.18±0.19^{b}	35.72±0.16^{b}
50	38.43±0.31^{a}	32.44±0.14^{a}	33.21±0.16^{b}	34.02±0.11^{b}	43.95±0.24^{c}	41.63±0.21^{c}
75	47.19±0.19^{a}	39.63±0.16^{a}	44.92±0.17^{b}	40.57±0.21^{a}	51.84±0.26^{c}	57.94±0.21^{b}
100	63.86±0.21^{a}	48.35±0.18^{a}	52.86±0.15^{b}	46.38±0.23^{b}	58.49±0.30^{c}	65.11±0.24^{c}

Key: Values are mean ± standard deviation of triplicate determinations. Values with different superscript letters per row are statistically significant (P<0.05).

Discussion

Superoxide dismutase (SOD) is a primary antioxidant enzyme that converts superoxide radicals into hydrogen peroxide and oxygen, thereby protecting cells from oxidative damage. The increased SOD activity observed in treated plants indicates that *N. fructicans* extracts may have induced oxidative stress, leading to an upregulation of antioxidant enzymes. This finding is consistent with research by Del Rio et al, (2018), which showed that plant SODs are upregulated in response to abiotic stresses such as allelopathic exposure, salinity, or heavy metals. Leaf tissues consistently exhibited higher SOD activity compared to root tissues, likely because leaves, as active sites of photosynthesis, have greater electron transport activity, making them more susceptible to reactive oxygen species (ROS) accumulation (Akbari et al.,2020). As a result, plants may preferentially direct their antioxidant defenses to the leaves to protect the essential photosynthetic machinery. Peroxidase (POD) is another important antioxidant enzyme that helps scavenge reactive oxygen species and strengthen cell walls during stress. The elevated POD activity in treated plants suggests that *N. fructicans* extracts may induce oxidative stress, triggering a defensive enzymatic response. POD activity significantly increased with higher extract concentrations, reaching peak levels at 100% concentration for the test plants. Amongst the three antioxidant enzymes POD had the highest activity (118.57±0.31) at 100% for the leaf extract, followed by CAT (58.49±0.26). This aligns with previous studies linking increased peroxidase activity to oxidative stress conditions caused by allelochemical exposure (Kausar *et al.,* 2012). Catalase is a crucial antioxidant enzyme responsible for breaking down hydrogen peroxide into water and oxygen. Its activity is often used as a biomarker for oxidative stress and the plant's defense response. *C. rotundus* appears particularly responsive, likely due to its robust root systems and adaptive physiology.

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The observed increase in catalase activity supports findings that allelopathic compounds from *N. fructicans* can induce oxidative stress in neighboring plants, activating their antioxidant defenses (Numbere, 2018).

Conclusion

The investigation revealed that both the leaf and root extract of *Nypa fructican* have a significant impact on antioxidant enzyme (SOD, CAT, and POD) activities of *Rhizophora mangle*, *Tridax procumbens* and *Cyperus rotundus*. This study highlights the potential of *Nypa fructican* as a source of natural compounds for sustainable weed management.

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