*Journal of Advances in Biology & Biotechnology*

*15(1): 1-6, 2017; Article no.JABB.36300*
*ISSN: 2394-1081*

# Effects of Simulated Drought Stress on Secondary Metabolite Production in Red Mangrove (*Rhizophora mangle* L.; Rhizophoraceae)

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Author ENI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AA managed the analyses of the study and managed the literature searches. Both authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/JABB/2017/36300
Editor(s):
(1) Maria Serrano, Department of Applied Biology, EPSO, University Miguel Hernandez, Orihuela, Alicante, Spain.
Reviewers:
(1) Sauren Das, Indian Statistical Institute, India.
(2) Jnandabhiram Chutia, Gauhati University, India.
(3) Guillermo Castañón Nájera, Universidad Juárez Autónoma de Tabasco, México.
Complete Peer review History: http://www.sciencedomain.org/review-history/21050

***Original Research Article***

***Received 22nd August 2017***
***Accepted 18th September 2017***
***Published 19th September 2017***

## ABSTRACT

An experiment was performed to determine the response of drought on the phytochemical production in *Rhizophora mangle* L. Seedlings raised from the plant body (propagule) were exposed to water deficit condition for 10 days. Growth and quantitative analysis of the phytochemical compound were analysed after the drought treatments. It was observed that *R. mangle* was susceptible to drought stress. Drought treatment resulted in the reduction of phytochemical content and Relative water content (RWC). The results showed that drought affects the secondary metabolite contents along with damages caused in *R. mangle*.

*Keywords: Drought; growth; propagules; secondary metabolites; Rhizophora mangle.*

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## 1. INTRODUCTION

Mangroves form unique communities in tropical and subtropical coastal and low tidal lowlands. Nigeria has the largest mangrove forest in Africa, occupying the lower stretches of the southern limit of the Niger Delta [1]. Mangroves are groups of tropical plants that grow along mean sea level in stable shorelines where cluster to form a distinct assemblage of communities known as mangrove forests [2]. This mangrove vegetation provides shoreline protection to coastal communities and habitat for flora and fauna. However, the healthy existence of mangrove forests in Nigeria is being threatened by change in climate such as drought [3]. *Rhizophora mangle* commonly known as red mangrove which inhabited in estuarine ecosystems, it grows on viviparously germinating propagule. It is considered an invasive species where it forms dense, monospecific thickets [4]. The are habitat for organisms like crocodiles and nesting for birds [5].

It has been reported that the bark and root of *Rhizophora mangle* are used in folk remedies to treat different sickness includes asthma, convulsion, diarrhoea, dysentery, stomach ache and haemorrhage [6].

Plant secondary metabolites are organic compounds synthesized by plants as part of the defence system against herbivores, pathogens and also to overcome abiotic stress [7]. They have also been implicated in conferring protection against environmental stresses in plants [8]. Secondary metabolites from *Sesame* sp. serve as important sources for food additives, flavours, fragrances, dyes, insecticides and pharmaceuticals [9]. The plant like *Jatropha curcas* L., have proven to be the most reliable hub in the cure of diseases due to their contents of the secondary metabolites [10]. The efficient groups of these bioactive compounds are alkaloids, tannins, steroids, terpenoids, flavonoids, glycosides, saponins and carotenoids [11]. Certain metabolites like elicitors or molecules often accumulate in plants when subjected to stress. However, the production of these compounds depends on the developmental phase of the plant. These phytochemicals have been reported to exhibit hemolytic, antifungal, antibacterial, anti-inflammatory properties [12].

Therefore, this paper presents the effects of drought assessed on the growth and production of phytochemical in *R. mangle*, a well-known species (red mangrove) found in coastal areas of Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Plant Growth and Treatments

Mature propagules of *R. mangle* were collected from Majidun estuary (06° 36' 20.2" N, 03° 26' 46.3" E) in Ikorodu area of Lagos state, Nigeria. For acclimation purpose, propagules were planted at the botanical garden University of Lagos, Nigeria and grown for 2 weeks in a large plastic container containing sediment from the estuary. Selected seedlings of the same equal height (12 cm) were hand picked and was transplanted into each nursery sowing bag for 3 weeks representing treatments and replicated thrice. At the end of the acclimation period, two treatment factor was considered, each representing a treatment (well watered and drought condition). Drought stress was imposed in bags for 10 days after which parameters were evaluated.

### 2.2 Determination of Whole Plant Biomass

Harvested plants were washed thoroughly in running tap water to remove attached soil particles and rinsed twice with distilled water. They were then placed in labelled paper bags and weighed after oven dried at 65°C for 72 h. The dried samples were weighed on a digital table-top digital weighing balance (Mettler PM 34-K Delta) to determine the biomass accumulation [13].

### 2.3 Relative Water Content (RWC)

The second leaf on each plant was harvested for the determination of Relative water content (RWC) was calculated using [14] formula, i.e.,

$$RWC\ (\%) = [(\text{Fresh Weight} - \text{Dry Weight}) / (\text{Turgid Weight} - \text{Dry Weight})] \times 100$$

FW: Fresh weight; DW: Dry weight; TW: Turgid weight.

### 2.4 Phytochemical Analysis

The leaves of freshly harvested plants were collected from both the control and the treated plants for phytochemical analyses. Samples were rinsed in a running tap water and air dried at room temperature in the laboratory. The qualitative and quantitative analyses of alkaloid, flavonoids, glycosides, phenols, saponins and tannins present in the plant were conducted using standard procedures as described by [15-17].

### 2.5 Statistical Analysis

The results are expressed as arithmetic means (n =3) of three replicates. All experimental data were analyzed by SPSS program version 16.0 (SPSS Inc., Chicago, IL, USA). One way analysis of variance (ANOVA) $P \leq 0.05$ were performed between genotypes using Tukey's test. All values are presented as means ±standard error.

## 3. RESULTS

On the effect of drought on plant biomass, it was observed that drought reduced the biomass weight. The control well watered plants had a corresponding mean data of 7.91±0.06 g as compared to the treated which had a data of 6.03±0.04 g. The relative water content of *R. mangle* showed that drought caused a notable ($P \leq 0.05$) reduction in the treated plant with value of 15.33 ±0.22% in contrast with the well watered plants with a mean data of 36.44±0.07% respectively (Fig. 1).

Drought had a significant effect on phytochemical production in *R.mangle* leaves. After 10 days of treatment well watered plants recorded a mean alkaloid data of 42.13±1.32 mg $g^{-1}$ dry weight in comparison to 28.41±2.11 mg $g^{-1}$ dry weight in drought treated plants respectively (Fig. 2). Cardiac glycoside concentration in the control plants was 23.47±0.32 mg $g^{-1}$ dry weight, which was significantly higher than 18.55±0.35 mg $g^{-1}$ dry weight of treated plants (Fig. 2). The effect of drought lowered significantly ($P \leq 0.05$) the level of flavonoid concentration in plant exposed to drought. Seedlings treated with drought had a mean data of 18.14±0.37 mg as against $g^{-1}$ dry weight 24.62±1.22 mg $g^{-1}$ dry weight of control plants (Fig. 2). Total phenol showed the same trend after 10 days of drought stress. The content of phenol in drought treated plant was significantly reduced to a mean data value of 3.22±0.17 mg $g^{-1}$ dry weight in contrast to well watered control with a value of 5.33±0.04 mg $g^{-1}$ dry weight (Fig. 2). Drought effect regarding saponins production showed that drought reduced its concentration in the leaves of *R. mangle*. After drought treatment, the control well watered plants had a mean data value of 23.14±0.23 mg $g^{-1}$ dry weight as compared to 17.26±0.31 mg $g^{-1}$ dry weight of not well watered plants (Fig. 2). The content of tannin in *R. mangle* leaves after 10 days of drought treatment showed less significant differences in its concentration. The control plants had a mean data of 43.47±2.43 mg $g^{-1}$ dry weight unlike 41.17±2.33 mg $g^{-1}$ dry weight were observed in plants treated with drought respectively (Fig. 2).

## 4. DISCUSSION

This study examined the drought stress effects on *R. mangle* seedlings when the propagules were grown in nylon bags a simulated drought condition. The growth index represented by whole plant biomass was significantly reduced during drought treated seedlings Iwuala et al. [18] reported that drought adversely affects growth and productivity as a result of changes in plants.

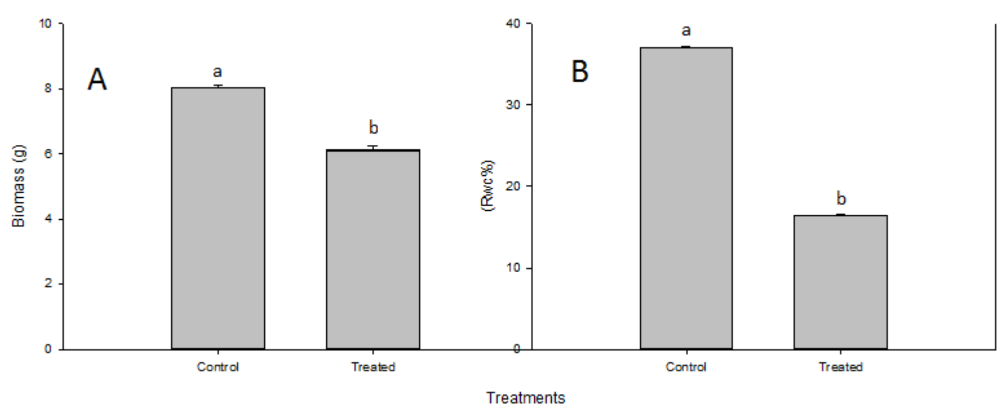


**Fig. 1. Changes in (A) Biomass of seedlings (B) Relative water contents of *R. mangle* leaves as affected by drought**

*Data represents means ±SE (n=3)*

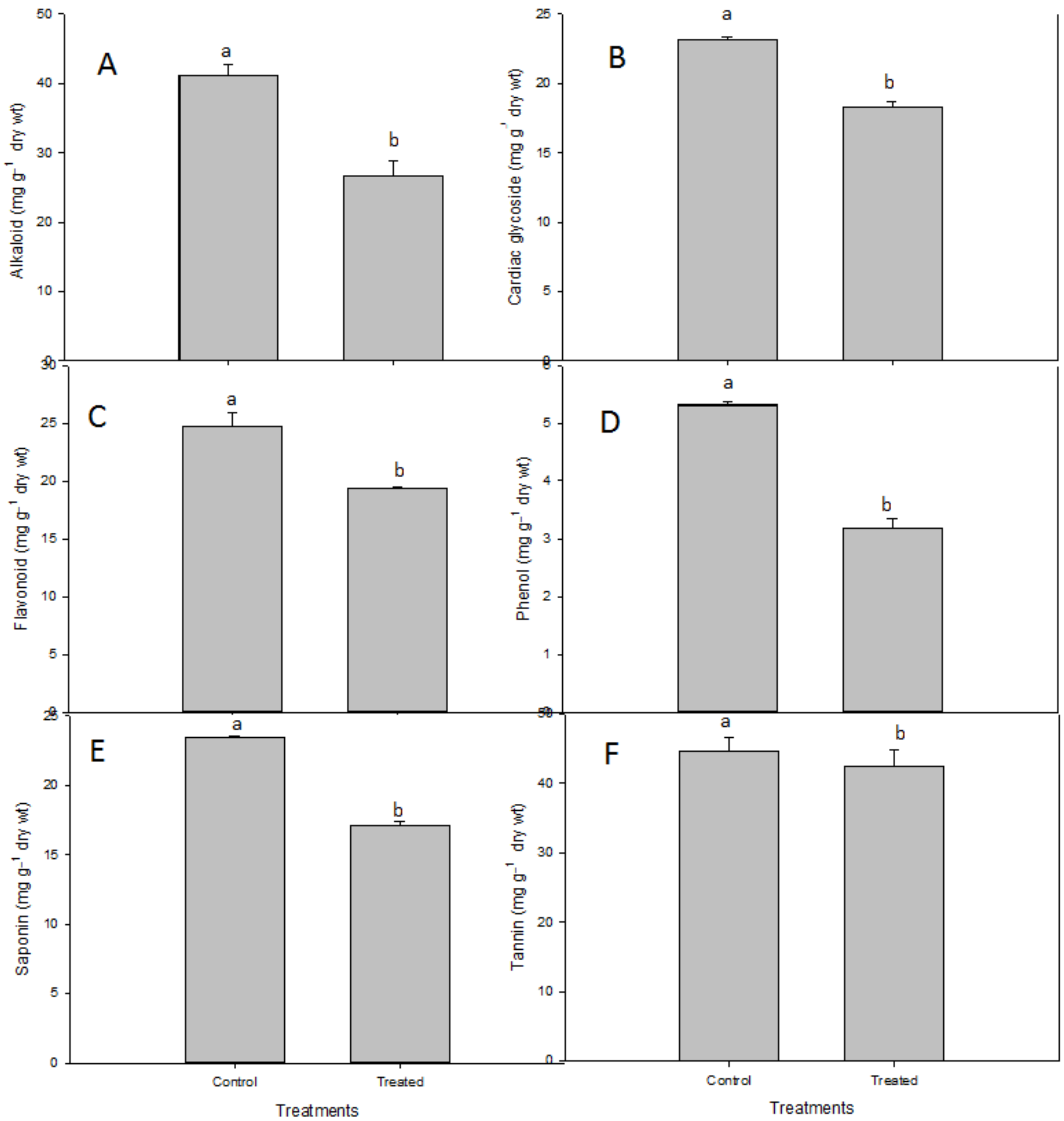


**Fig. 2. Changes on (A) Total alkaloid (B) Cardiac glycosides (C) Flavonoids (D) Phenols (E) Saponins (F) Tannin content in *R. mangle* leaves after drought treatment**

*The data represent means± SE (n=3)*

The growth inhibition by drought might be due to the alterations and improper nutrients accumulation. The low relative water content observed in the leaves of drought treated plants of *R. mangle* could be attributed to the disruption of the normal plant water relations caused by drought application, thus affecting water supply and absorption within the plant roots. Similar observations were reported by [19] that drought application significantly reduced the relative water content in *Celosia argentae*. It has been reported that under environmental stresses free radicals are produced in excess such as drought, heavy metals and this cause lipid degradation and oxidative damage [20]. The phytochemical quantification as well as the quantitative evaluation of the control plants revealed high concentrations of alkaloids, tannins, cardiac glycosides, flavonoids, phenol, as well as saponins were observed. Previous works also showed similar findings where alkaloids is known to inhibit pain, flavonoids due to its anti-inflammatory is involved in the activation of inflammation in *Jatropha curcas* [21,22,23,14,24]. However, a decrease in the quantitative values of the phytochemicals due to drought treatment was observed. Zobayed et al. [24] stated that the signalling of secondary metabolites is dependent on the assimilated carbon observed during photosynthesis. The net photosynthesis rates decreased when plants are under stress [14]. The low concentrations of phytochemical observed in drought treated seedlings could be related to limited carbon assimilation that may consequently affect carbon allocation for secondary metabolite production.

## 5. CONCLUSION

The obtained results showed elevated levels of phytochemicals, which suggest the efficacy of *R. mangle* to produce medicinally important chemicals under well watered conditions. The data generated in this study provide the detrimental effects of drought on the phytochemical production in *R. mangle.* This property is of great interest in Pharmacognosy as a useful ethnomedicinal plant. The outcome of this work though preliminary, however, would be a strong base for advance and refine research on this important plant especially for pharmaceutics purposes.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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*The peer review history for this paper can be accessed here:*
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