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Elevated CO_2 Concentration and Air Temperature Impacts on Mangrove Plants (*Rhizophora apiculata*) Under Controlled Environment

Baseem Tamimia[1*], Wan Juliana W. A.[*2,1], Nizam M.S. [2,1], Che Radziah Che Mohd Zain [1]
[1]Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia
[2]Institute for Climate Change, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia

Abstract
Global climate change has shown to have a significant impact on critical ecosystems, that in turn has led to elevated CO_2 and temperatures that accompany changes in many abiotic factors, including mangrove forests, facing challenges in their habitat. This study conducted to investigate the morphological and physiological characteristic of the mangrove *Rhizophora apiculata* in response to elevated CO_2 concentration and air temperature for the selection of tree species that are able to adapt to climate change. The seedlings were grown in controlled growth chambers with two temperatures, 21 and 38°C, under elevated CO_2 at 650 ppm for three months. The plants watered with two liters of saline water of 28 ppt every 48 hours. Thus, after two weeks the mangrove recorded positive results for all parameters to high temperature. The differences in temperature resulted in significant differences and positive interaction between elevated CO_2 and decreased temperature that led to the samples survived for all parameters and the growth was very slow, but negative interaction and the samples almost perished under elevated CO_2 and increase the temperature for growth and photosynthesis response. These results suggested that the low level of photosynthetic capacity might be attributed to the decreased CO_2 fixative reaction system and photosynthetic pigment contents.

Keywords: Climate change, Elevated CO_2, Temperature, Photosynthetic, Growth.

ارتفاع تركيز (CO_2) ودرجة حرارة الهواء يؤثر على نباتات المنغروف (*Rhizophora apiculata*) في بيئة مراقبة

بسيم التميمي[1*]، وان جوليانا وان احمد[2،1*]، محمد نظام محمد سعد[2،1]، جيرازيا جي محمد زين[1]
[1] مدرسة العلوم البيئة والموارد الطبيعية، كلية العلوم والتكنولوجيا، الجامعة الوطنية الماليزيه، بانكي43600 –سلنكور، ماليزيا
[2] معهد تغير المناخ، الجامعة الوطنية الماليزية، بانكي43600 –سلنكور، ماليزيا

الخلاصة
أظهرالتغير المناخي العالمي تأثيرا كبيراً على النظم البيئية الحيوية، والذي ادى بدوره الى ارتفاع تركيز ثاني اوكسيد الكربون ودرجة الحرارة والتي تصاحب التغيرات في العديد من العوامل اللاأحيائية، بما في ذلك غابات المنغروف, التي تواجه تحديات في بيئتها. أجريت هذه الدراسة للتحقق من الصفات المرفولوجية والفسيولوجية لنبات المنغروف a*Rhizophora apiculat* استجابة لارتفاع تركيز ثاني اوكسيد الكربون ودرجة حرارة الهواء لاختيار انواع الاشجار القادرة على التكيف مع تغير المناخ. تمت زراعة الشتلات في غرف نمو مضبوطة مع درجتين حراريتين، 21و38 درجة مئوية، تحت تركيز ثاني أكسيد الكربون المرتفع عند 650جزء

*Email: ayie@ukm.edu.my

في المليون لمدة ثلاثة أشهر .النباتات سقيت مع لترين من المياه المالحة من 28جزء لكل الف كل 48 ساعة .وهكذا، بعد أسبوعين سجلت غابات المنغروف نتائج إيجابية لجميع المعلمات لدرجة حرارة عالية .نتج عن الاختلافات في درجة الحرارة اختلافات معنوية وتفاعل إيجابي بين ثاني أكسيد الكربون المرتفع وانخفاض درجة الحرارة التي أدت إلى بقاء العينات لجميع المعلمات وكان النمو بطيئًا جدًا، ولكن التفاعل السلبي وتأثر اغلب العينات تقريبًا تحت ثاني أكسيد الكربون المرتفع وارتفاع درجة الحرارة واستجابة لانخفاض النمو والتمثيل الضوئي. هذه النتائج تشير الى ان انخفاض مستوى القدرة على التمثيل الضوئي قد يعزى الى انخفاض نظام رد فعل تثبيت CO_2 ومحتوى الكلوروفيل.

1. Introduction

The global environment is changing due to the elevated atmospheric carbon dioxide concentration [CO_2], concomitant increasing temperatures and many abiotic factors interactions [1, 2], these factors being determinants in the photosynthetic rates and growth rates in plants [3,4], any changes they present in the atmospheric composition and climate will significantly affect planetary ecosystems [5]. Over the last century, atmospheric CO_2 concentration has increased from 280 to 400 ppm as previous studies have indicated making this an eminent and undeniable global environmental change (GEC), with the current rate of increase averaging at 1·5 μmol mol^{-1} $year^{-1}$ [6]. It expected that CO_2 concentrations could reach 700ppm (Figure-1), by the end of the century as global population and economic activity increases [7], leading to warmer global temperatures. The rise in CO_2 and other greenhouse gasses like methane, chlorofluorocarbons, and nitrous oxide predicted temperatures, late or early frosts), and (c) climatic variability can be obscured by these broad mean annual changes [2]. Its extent, however, is subject to the factors causing radiative forcing and the complex feedbacks between different elements and the climate system. Recent model projections suggest a global mean surface air temperature increase of 1 to 4.5°C by 2100 AD [8,9], making the 0.3 to 0.6°C rise of mean annual surface air temperature over the last century a clear effect of recent atmospheric changes [10]. However, important details in (a) diurnal and seasonal patterns, (b) frequency, timing and duration of extremes (e.g. high or low in temperature predictions [11]. One example is that recent scenarios predict most warming in mid- and high-northern latitudes in late autumn and winter, and little or none (or even cooling in mid-latitudes) in summer (Figure-2), which could affect growing season length. Indeed, there is already evidence of a change in growing season length [12]. Another example is the strong evidence that, over land, the increase in night time minimum temperature has been about twice the increase in the maximum [11]. Plant growth will greatly have affected by the continuing changed in diurnal cycles compared to an even change in temperature over 24 hours but these broad global mean temperature predictions obscure aspects critical to natural and managed ecosystems [1].

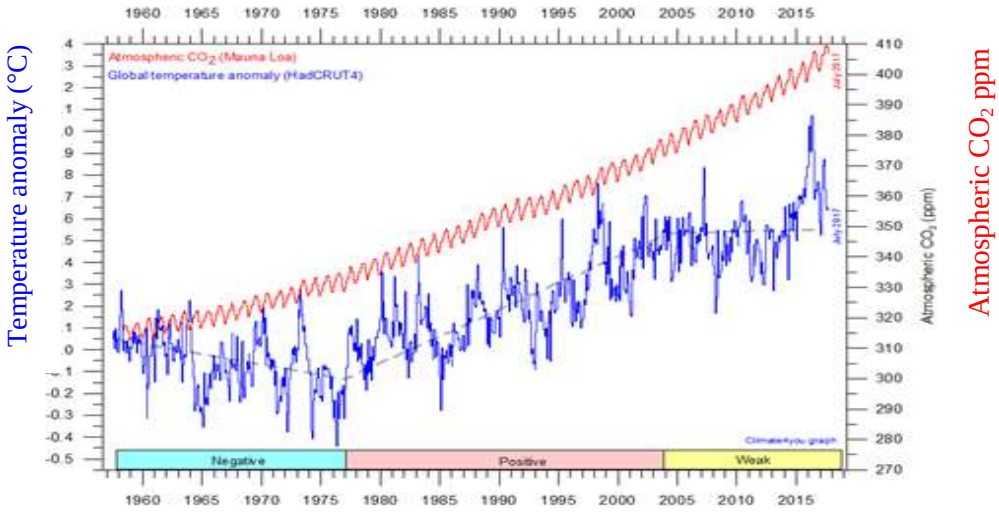

Figure 1- Diagram showing the HadCRUT4 monthly global surface air temperature estimate (blue) and the monthly atmospheric CO_2 content (red) according to the Mauna Loa Observatory, Hawaii. Last diagram update: 31 August 2017[13].

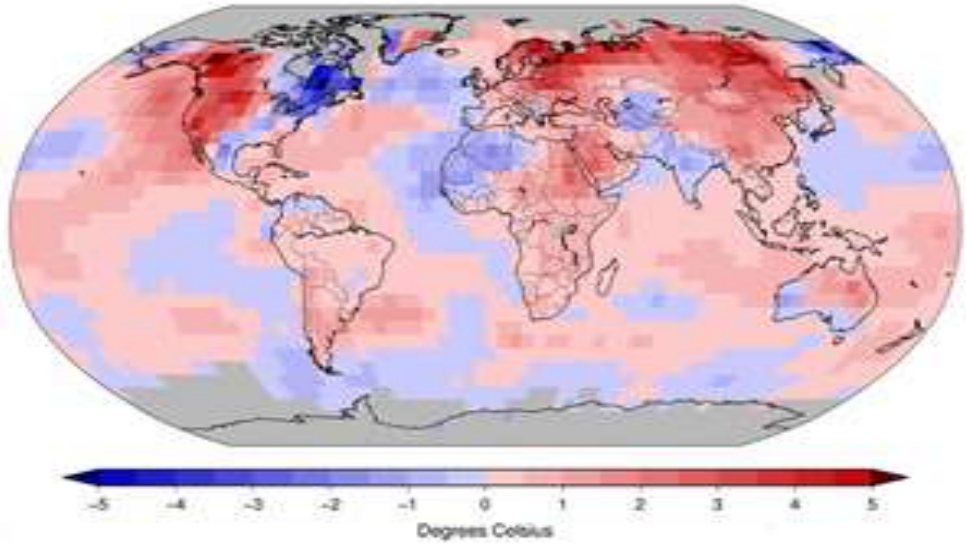

Figure 2- Land & Ocean temperature departure from average mar 2015 (with respect to a 1981-2010 period)[14].

Mangroves are remarkable ecosystems that are valuable economically and ecologically. They are located at the interface of land and sea and offer a considerable array of goods and services. Mangrove ecosystems are vital for food security and the protection of coastal communities. They provide a wide diversity of forest products, nurseries for aquatic species, fishing grounds, carbon sequestration, and crucial natural coastal defenses for mitigating the impacts of erosion and storms. Global climate change and the associated risks of sea level rise and extreme weather events have increased the importance of mangrove ecosystems. Calls for conservation have also increased in recent years with the increasing evidence that mangroves may have an important role as natural buffers in protecting coastlines from the impacts of storms and extreme waves. [15]. Climate change has a high probability to have a strong impact and exacerbate existing pressures on coastal ecosystems, including mangroves. At present, anthropogenic climate change is widely regarded as one of the greatest threats to natural ecosystems worldwide. Effect of anthropogenic climate change includes elevation of atmospheric carbon dioxide and rise in relative sea level and sea water temperature. These phenomena possibly increase with the frequency and magnitude of extreme weather events and associated elevated storm surges and wave height [16, 17].

The conservation and restoration of mangroves and associated coastal ecosystems play important roles in climate change adaptation strategies. Mangroves are not only valuable in climate change mitigation efforts but are also influential in adaptation strategies to changing climates [18]. Due to the affect mangroves have in adapting to climate change, more investments should be funnelled to its development plans, as climate change adaptation is a growing concern in most international development agendas [11]. Thus, the objective of this study is to determine the effects of elevated CO_2 that expected at the end of this century, to different temperature on the growth of the most dominant and commonly distributed mangrove forest from the Rhizophoraceae family found in Malaysia [19], Depending on the temperature recorded in Malaysia (25-34°C) (Figure-3), so the temperature was increased and decreased ±4°C according to Malaysian temperature that mentioned in earlier studies [20]. Wherefore the mangrove forests should be preserved, particularly due to their economic importance and their important role in preserving the ecosystem and diversity of organisms.

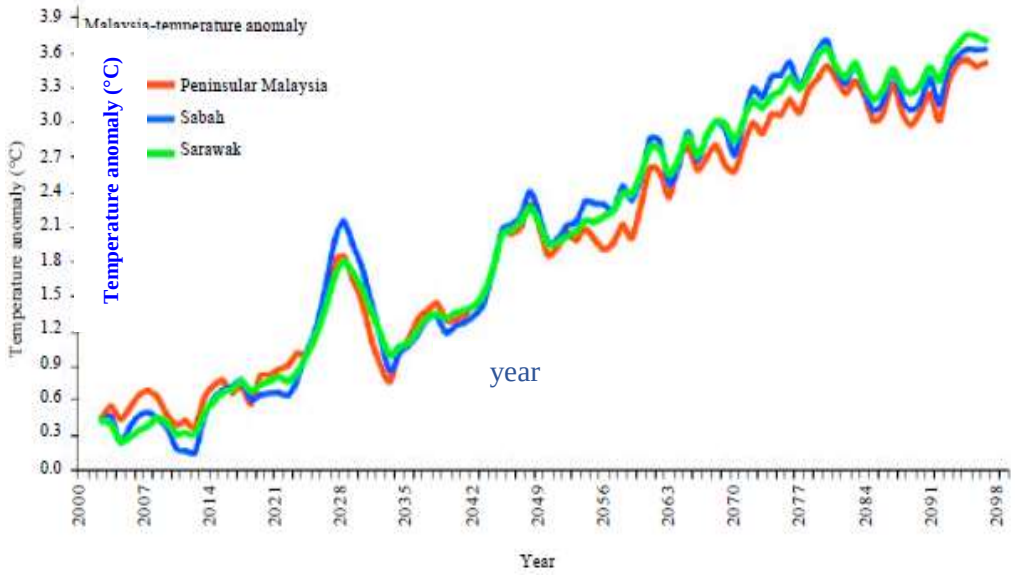

Figure 3- Meteorology Department, MMD, 2009) [20].

2. Materials and Methods

2.1 Growth Facility:

This research study was conducted at the "Tropical Ecophysiology Lab.", in UKM, Bangi, Malaysia (2° 55' 12.03"N, 101° 47' 2.99 E). The facility consists of Plant Growth Chamber model (GC-202C), the plant growth chamber monitored and controlled the relative humidity (± 1.0% at 80 %), lighting (1000 µmol m^{-2} s^{-1} PAR), temperature set at 21/18 °C and 38/35 °C (day/night) and CO_2 sensors (650 ± 30 ppm) for the whole project duration, which took three months. The mangrove plant seedlings with soil were collected at the age of three months from Kuala Gula in Perak (4.924012, 100.459581). These mangrove seedlings were transplanted inbox size (42-62cm). The mangrove seedlings were then planted in two groups with seven samples in each box. Two weeks later, the samples were checked in terms of physical growth and transferred to the plant growth chambers. The first group was exposed to levels of the plant growth chamber at temperature 21°C + CO_2 650 ppm and the second group was at temperature 38°C + CO_2 650 ppm, depending on the temperature recorded in Malaysia (24-35°C) (Figure-3), so the temperature was increased and decreased ±4°C, according to Malaysian temperature that mentioned in earlier studies [20]. Meanwhile, the plants were watered with two litres of saline water (28 ppt) every 48 hours and were not given any fertiliser. All dead or damaged plant material was removed from the mesocosms, and all visible fauna (e.g. snails and crabs) were removed to avoid confounding effects of soil burrowing, herbivory, and other activities. Each mangrove seedling was labelled according to groups and treatment. Any changes in the seedling health were also recorded qualitatively.

2.2 Experimental Design and Growth Measurement

The plant growth parameters were measured to study the response of the mangrove plants to an elevated CO_2 concentration and air temperature. The measurement of the number of leaves, plant height, number of branches, and Diameter of stems, all the morphological parameters, were done manually using the foot rule, and Log rule calliper, and the photosynthesis rate were measured by using a Li-cor 6400 at 11 am for all Li-cor measurements, then before each measurement, leaves were equilibrated in the cuvette at saturating PPFD (1000 µmol m^{-2} s^{-1} PAR), temperature set at 28 °C, CO_2 – 400µmol mol^{-1} and Flow rate – 300mmol s^{-1} for most systems, 200mmol if photosynthesis is low. Determination of chlorophyll concentration was conducted using standard procedure on the reduction of the acetone volume [21]. Where 0.1g of mangrove plants leaves were chopped into small pieces (about 2 mm), and the leaves were put into a test tube, after which 20ml 80% acetone was added to the test tube. The mixture was homogenized by a shaker and then incubated in the dark for 48 hours. Concentrations of chlorophyll a and chlorophyll b were analysed using a spectrophotometer at the wavelength of 663nm and 645nm, respectively. The chlorophyll concentrations were calculated using [22, 23] the following equations:

$C_{chl\text{-}a} = 12.7A_{663} - 2.69B_{645}$

$C_{chl-b} = 22.9\ A_{645} - 4.68\ B_{663}$

Total chlorophyll = $C_{chl-a}+C_{chl-b}$

Fresh and dry weights of the seedlings were measured using a digital scale, of which the dry weight was obtained after the samples were dried in the oven at 65°C for seven days. The measurement was done three times. The first quantitative measurement was made on the 1st of July 2015 and the second on 17th of August 2015 (after 45 days) and the measurements were made until the final measurements on 1st of October 2015 (after 90 days).

2.2Data Analysis

The experimental data were subjected to variance analysis (ANOVA) via SAS (Release 9.4) software and Duncan's multiple-range tests (DMRT) determined a significant difference at α=0.05 level [24].

3. Results and Discussion

3.1Seedlings Preparation and Growth Measurement

Seedlings growth parameters (plant height, the number of branches, and stem diameter) between treatments of elevated CO_2 and different temperature displayed various responses depending on the number of days of treatments. Observations on plant height, the number of branches, and stem diameter showed increased significant differences between the first treatments and after 45 days of exposure. Subsequent observation after 90 days of treatments revealed various responses depending on different temperature and number of days of treatments.

At 90 days of exposure, the mean height of plants under elevated CO_2 concentration and temperature 21°C increased, whereas the plants under elevated CO_2 concentration and temperature 38°C decreased. (Figure-4A). The difference in temperature resulted in a significant difference in the number of leaves in which of the plants under elevated CO_2 concentration and temperature 21°C at 1-45 days was increased but at 90 days was decreased. On another hand, the plants under elevated CO_2 concentration and temperature 38°C continued to decline until most samples died (Figure-4B). To illustrate, the result of Number of branches was not significant between 45-90 days for the plants under elevated CO_2 concentration and temperature 38°C, the increase in the number of branches for the plants under elevated CO_2 concentration and temperature 21°C at 90 days was slightly significant, (Figure-4C). At 90 days of exposure, the mean diameter of stems under elevated CO_2 concentration and temperature 21°C increased, whereas the plants under elevated CO_2 concentration and temperature 38 °C decreased. (Figure-4D).

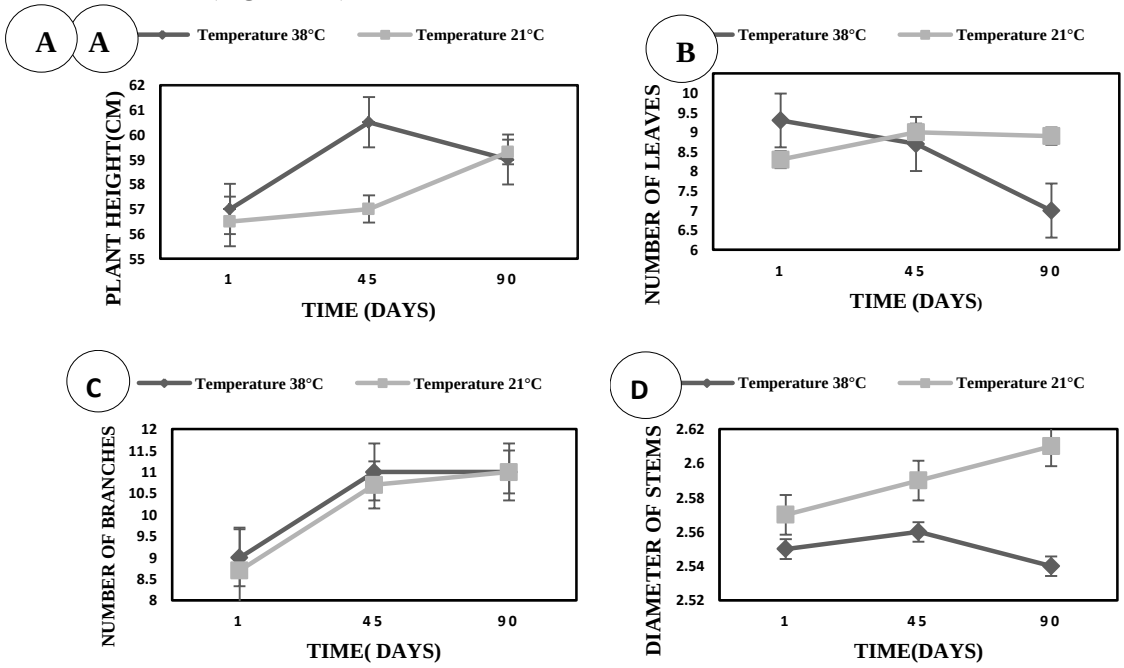

Figure 4- Comparative responses from elevated CO_2 (650ppm) and different temperature of (A) average height (cm) (B) Number of leaves (C) Number of branches, and (D) Diameter of stems of mangrove seedlings *R. apiculate.*

3.2 Photosynthetic Rate, Biomass and Chlorophyll Concentration Measurement

The result shows that the photosynthesis process was poor and inefficient under elevated CO_2 concentration and different temperature. Photosynthesis responses declined gradually and slowed down at 1-45 days depending on different temperature and the number of days of treatment. At 90 days of exposure, the Photosynthesis responses declined under elevated CO_2 concentration and temperature 38°C, whereas the plants under elevated CO_2 concentration and temperature 21 °C recovered in photosynthesis responses (Figure- 5A). The result found that the total chlorophyll under elevated CO_2 concentration and different temperature displayed various responses depending on the number of days of treatments. Total chlorophyll increased gradually at 1-45 days for all treatments. At 90 days of exposure, the total chlorophyll declined significantly under elevated CO_2 concentration and temperature 38°C, whereas the plants under elevated CO_2 concentration and temperature 21°C declined slightly. (Figure-5B, 6)

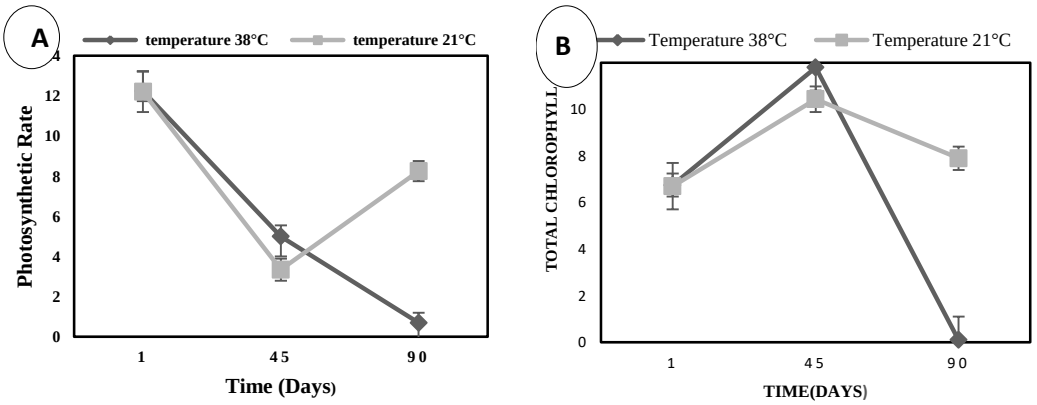

Figure 5- Comparative responses from elevated CO_2 (650ppm) and different temperature of (A) photosynthesis rate, and (B) Total Chlorophyll of mangrove seedlings *R. apiculate.*

Observation of the fresh and dry shoot weight and fresh and dry root weight of mangrove plant showed a significant difference in all parameters. The fresh mangrove shoot weight is higher for elevated CO_2 and temperature 21°C at 84.1g compared to 56.8g in elevated CO_2 and temperature 38°C (Table-1). The dry shoot weight of the plants is higher in elevated CO_2 and temperature 21°C, which is 14.7g compared to 6.1g at elevated CO_2 and temperature 38°C. The mean fresh mangrove root weight at elevated CO_2 and temperature 38°C averaged at 31.6 grams, while the elevated CO_2 and temperature 21°C at 73.9g. The dry root weight at elevated CO_2 and temperature 38°C averaged at 13.5g while in elevated CO_2 and temperature 21 °C the average was higher at 23.3g. The percentage increase in the growth of the mangrove plant in ascending order is fresh shoot weight, fresh root weight, dry root weight, and dry shoot weight.

Table 1- Responses of mangrove seedlings *R. apiculata* to elevated CO_2 conditions (650ppm) and air temperature.

	Shoot		**Roots**	
Treatment	Fresh weight (g)	Dry weight (g)	Fresh weigh (g)	Dry weight (g)
Temperature 38°C	56.8 ± 0.7^{b}	6.1 ± 1^{b}	31.6 ± 0.57^{b}	13.5 ± 0.4^{c}
Temperature 21°C	84.1 ± 0.1^{a}	14.7 ± 1.2^{a}	73.9 ± 0.3^{a}	23.3 ± 0.7^{a}

Note: Mean ± standard deviation (SD) followed by different letter of the same column of treatment is significantly tested using (DMRT) at $\alpha=0.05$

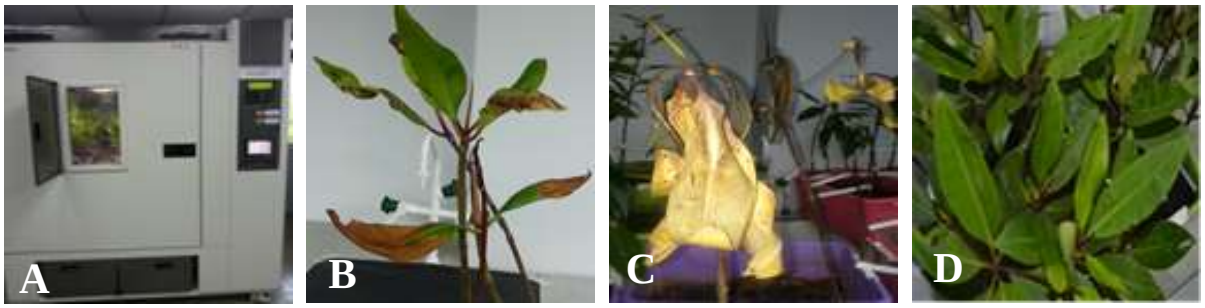

Figure 6- The impact of elevated CO_2 and high temperature on mangrove plants inside the growth chambers displayed various responses depending on a number of days of treatments (A) Plant growth chamber (B) mangrove plants response at 45 days in temperature 38°C (C) mangrove plants responses at 90 days in temperature 38°C, and (D) Mangrove plants responses at 90 days in temperature 21°C.

The results showed significant differences in the parameters studied and affected by elevated CO_2 and different temperature, where various responses were displayed depending on a number of days of treatments. There was an observed response to elevated CO_2 on the morphological parameters, especially on the number of leaves that saw a significant decrease, but the physiological parameters were affected the plant reaction increased the total chlorophyll Trying to increase the photosynthesis rate for adaptation and resistance to variable conditions, especially after the first 45 days. That means, the elevated atmosphere carbon dioxide is useful for the plant photosynthesis, but more than the optimum rate has become counterproductive [25]. The increase in temperature leads to an imbalance between the respiration and photosynthesis rate, it considered a toxic factor, that damage the protein components of the protoplast, the destruction of chlorophyll, yellowing of leaves and thus inhibiting growth, which affected the photosynthesis rate [26]. That means the high-temperature presence has a negative impact on mangrove growth that was clear at the end of the study (90 days). Most of the samples died in this treatment. This indicates that the increase in temperature has a physiological effect on the plant, through the effect on the biological activities within the plant, especially enzymes [5] (RubisCo enzyme responsible for CO_2 fixation in Calvin cycle). However, the RubisCo limits photosynthesis when electron transport limitations dominate and there can be a rapid fall-off of the photosynthetic rate at high temperatures. Therefore, the impact on the control of carbon fixation by the manipulation of one enzyme would differ depending on growth conditions [27]. As for the low temperature, its effect was very slow, leading to slow growth and the survival all the plants, which is why the studied morphological parameters did not show great differences compared to samples in high temperature, but there was a clear effect on photosynthesis at elevated CO_2 and low temperature. The results of this study were identical to [27].

Climate change on mangrove plants considered dangerous by interfering between biotic and abiotic factors in global warming, especially during the early phases of growth. Where these results provide confirmatory evidence that the effect of the interaction between the elevated CO_2 and temperature is negative and dangerous, which will not only affect the geographical distribution of mangrove plants but also their survival. Moreover, the interaction of the other factors may have a different effect, so the study should be increased in this field to improve the knowledge of the interaction between the factors, which could affect growing season length. Indeed, evidence of changes in growing season length exists [12], along with the effect time periods have on diurnal cycles, which have greatly affected plant growth compared to even temperature changes over 24 hours and the extent of heat stress [1].

4. Research Limitations

- It is not possible to provide an environment exactly like that expected in the future.
- The size of the growth chambers restricts the size and specific type of samples.
- The size of the growth chambers is specific, limiting the box sizes used to cultivate the samples as well as the amount of water inside the boxes.

5. Research Contributions

Gather the largest amount of information on the effect of CO_2 and temperature that may contribute to the conservation and greater utilization of mangrove forests. Along with the environment that it is characterized by and how to adapt them to environmental changes, which helps to know the appropriate places to increase mangrove forest and pay more attention to these forests of importance.

6. Potential Future Works

Studies and scientific knowledge about the growth of plants under natural conditions may be many and varied. However, there is a need to increase the scientific knowledge of extreme environmental conditions, which are often not considered, affecting the survival, vitality, and production of plants, especially in the context of frequent climate change and global warming conditions. Overall, the study of plant response to environmental stress has been a leading and important issue, which may help to understand the plant's response and explain the geographical distribution thus performance both in growth and production of the state of an environmental gradient. Understanding the plant's response to stress may be important as well as knowing the real impact of the interactions of environmental factors that lead to understanding the adaptation and acclimation in plants.

7. Conclusion

Generally, this research study showed that the rising CO_2 and temperature levels have a great impact on the growth rate. It is imperative to understand CO_2 responses in varying temperature ranges due to the history of GEC and its future, as well as the differing temperature ranges in different regions of the world. However, the impacts of Temperature and CO_2 are not the only factors affecting plants, light, water, and nutrient supply are equally critical in assessing and interpreting the effects of increased CO_2. Indeed, many of these interactions may be already included in the experiments reported. Nevertheless, the rapid responses to elevated carbon dioxide and temperature levels during the early phases of growth as in seedling establishment may be important determinants as regeneration of species.

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