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Coleus cultivars (*Solenostemon scutellarioides* (L.) Codd.) as potential bioindicators of chronic ozone exposure

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Abstract. Sensitivity of plant under ozone exposure can indicate its potency of being important bioindicator. Early studies have found that coleus plant (*Solenostemon scutellarioides* (L.) Codd.) has a potency to be ozone bio-indicator. This study aims to investigate the effect of chronic ozone exposure on root and leaf biomass and to quantify any change based on the plant appearance. Four different cultivars of coleus plant with different colour namely fully green (FG), green purple (GP), yellow purple (YP), and reddish (RD) had been selected. These four cultivars were fumigated with three different concentrations of ozone gas (≤ 10 ppb, 40 ppb, and 150 ppb) for 8 hours fumigation during 30-day period of experiment. All cultivars showed a stable leaf biomass after 30-day period of ozone exposure. Similarly, root biomass of all cultivars was not significant changed after fumigation period. Nonetheless, magnitude of ozone symptoms on leaf showed variation in certain cultivars. FG cultivar showed a significant number of chlorosis leaves under 40 and 150 ppb ozone exposure. The exposure of 150 ppb ozone also caused a noticeable percentage of curling leaves on RD cultivar. Moreover, the purple area in YP and GP showed a larger ratio under exposure of 150 ppb ozone. Consequently, coleus plants displayed resistance responds in terms of biomass. On the other hand, the plants also revealed variation in leaf symptom magnitudes and colour patterns under ozone exposure. However, these cultivars are potential to be bio-indicator due to their sensitivity in terms of appearance.

1. Introduction

Tropospheric ozone level tends to increase in current years. Regionally, ozone was observed to increase in many places [1]. Massive growth of human population and severe managed environment are among the causes of the increase. It is important to note that in some places ozone level can be very dangerous and over the air quality standards during daytime [2]. This pollutant is shortly being the third most important greenhouse gas occurs in the ambient air [3].

This pollutant regards as cause of many decreases in cropping plants productivity [4]. Chronic symptoms and diseases after inhaling specific amount of ozone ambient have been reported in soybean [5], mug bean [6], rice [7], and many other cropping plants [8]. Mitigation about this problem is hardly conducted to its state as a secondary pollutant in which it can be sourced from many sites far from its high-level occurrences [9] unless there is the great strategies on the cropping plants to reduce its impacts [10]. The ozone exposure also affects human health [3, 11]. It can affects skin lipid conditions [12], increasing the prevalence of premature death [13] and other harmful effects [14]. Peñuelas, Ribas

[15] assumed that bioindicator can be the indication of the increase to draw comprehensive picture of this airborne problem.

Among the specific purpose of bio indicator is to use the biota to be indicator for change and/or specific case of detrimental condition in the environment or certain occasions [16]. Several plants have been proven good bio-indicators while others only showed clear sign of ozone increase after acute exposure [17-19]. The bioindicator of ozone should be sensitive of the ozone exposure and can show clear specific symptoms of high concentration of ozone exposure [20]. Moreover, the bioindicator plants are also expected to increase people awareness about the climate change and health impacts regarding this pollutant [21]

Previous researches had been conducted to assess potential plant for bio indicator purposes. Cutleaf coneflower (*Rudbeckia laciniata*) plants was assessed to obtain more comprehensive result of the plant regarding its visible foliar injury occurrences in which it was found to be relatively slow to occur [22] although the plant showed stippling chlorosis and necrosis and recognized as a bioindicator [23]. *Ipomoea nil* (L.) Roth. cv. 'Scarlett O'Hara' was also evaluated and it showed the noticeable visible leaf injuries while haziness in carbon assimilation and stomatal conductance parameters under the exposure of above 40 ppb ozone (AOT40) as the threshold [24].

However, it is rare to found some species with different cultivars can have such a major difference in terms of responses. Among those species *Phaseolus vulgaris* showed different responses within cultivars because of its different physiological process in their metabolisms [25].

Coleus has been recognized as the ornamental plant for many years. This plant has wide range of colors in its leaf. It is easy to find and cultivate so that this species is among the favorite ornamental plants in tropical and subtropical areas. Because of its fast growing in cultivation and captivating colors, this plant has been assessed under many stresses to obtain the most suitable cultivars in different conditions. Several studies conducted showed that this plant has fast and obvious responses to the environment changes although those responses do not lead to the death of the plant. Based on these sensitivities, several studies applied the stress for many purposes such as bioactive compound production from this plant [26] and quest for plant model for better understanding of this plant responses under environmental stresses [27-29].

Although many studies had been done to find suitable and susceptible plant for bioindicator, lac of investigation has been done on ornamental plant. Coleus has been chosen for this study as a plant model for ozone bioindicator. Thus, the aims of this study were effect of chronic ozone exposure on root and leaf biomass and to quantify any change based on the plant appearance. However, the sensitivity level and susceptibility of several cultivars of coleus was investigated and the plant can be beneficial for indicating ozone level and tis severity. Moreover, result of this study can initiate related studies especially on physiological and molecular mechanism of plant under ozone exposure.

2. Materials and Methods

2.1. Plant preparation and ozone exposure

Four cultivars of Coleus (*Solenostemon scutellarioides* (L.) Codd) which are Coleus Kong Green (fully green/FG), Coleus Kong Scarlet (green and purple/GP), Coleus Wizard Pastel (yellow and purple/YP), and Coleus Wizard Scarlet (reddish/RD) were chosen to be the experimental plants in this study (Figure 1). Plants were grown and treated as described in [30].

2.2. Ozone exposure

Chronic episode of ozone exposure was fumed into chambers of ozone fumigation as shown in Figure 2. Briefly the mechanism of the chambers is described. An ozone generator)Oz model 3020 from Ebase corp. ltd.(was used to generate ozone gas into fumigation chambers. The ozone gas produced by the ozone generator went through a hose to be combined with air coming from an air compressor. The air passed through a charcoal filter. A valve near the end of the hose adjusts the amount of ozone that was combined with the filtered air in a plastic pipe leading to a fumigation chamber, to regulate the ozone concentration entering the fumigation chamber. The valve was controlled by an Ozone Monitor Model 1008-PC from Dasibi Environmental Corp. to control the ozone concentration in the fumigation chambers.

Figure 1. Experimental plants (a) yellow purple cultivar (b) green cultivar (c) reddish cultivar (d) green purple cultivar.

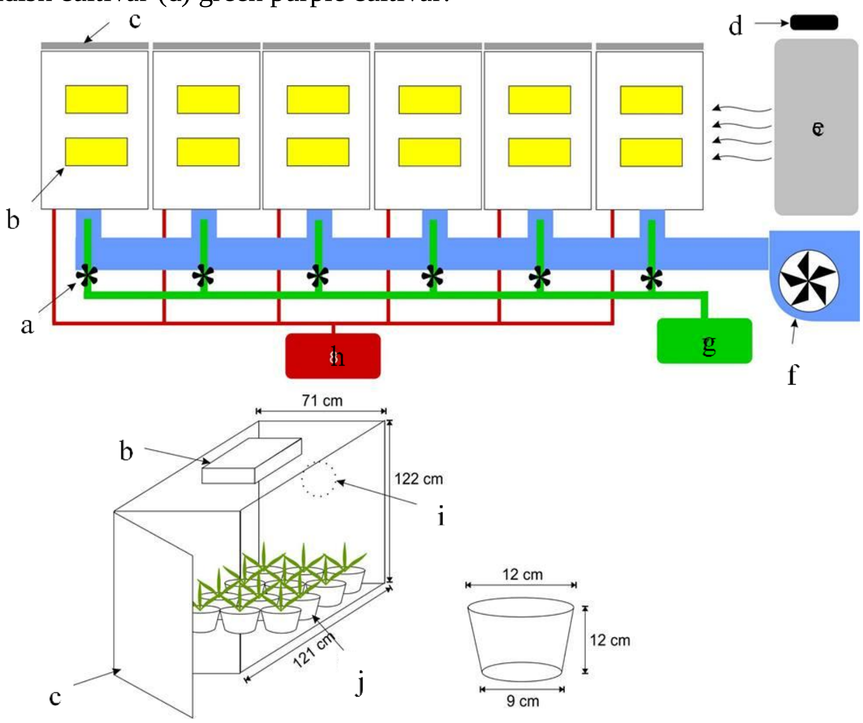

Figure 2. Installation of ozone fumigation chambers and plant placement. (a) valve, (b) lamp, (c) door, (d) controller, (e) air Conditioner, (f) air compressor, (g) ozone generator, (h) ozone monitor, (i) air intake, (j) plants pot.

The fumigation chambers which were used in this experiment have the proportions 121 cm in length, 71 cm width and 122 cm in height. All six chambers was fumed to have three different ozone concentrations. Two chambers had charcoal-filtered air only with ozone concentration lower than 10 part per billion (ppb) (<10 ppb ozone), two chambers contained air with ozone concentration about 40 ppb)40 ppb ozone(, and two chambers contained air with ozone concentration of about 150 ppb)150 ppb ozone(. Before fumigation occurs, each chamber was filled with air at the required ozone concentration, which was monitored by the ozone monitor and continued to measure during the fumigation. Ozone concentration in the chamber tended to stable during the fumigation period as shown in Figure 3.

All the other factors namely temperature and light intensity were adjusted to be similar. Temperature inside the chambers was ranging in 28-32˚C from 06.00 to 18.00 and 25-28˚C 18.00 to 06.00. Photosynthesis photon flux density)PPFD(inside the chamber was 400 to 500 µmol s^{-1} m^{-2} at the shortest coleus plants height for 12 hours per day. Fumigation period was 28 days with chronic ozone regime in the chamber atmosphere for 8 hours from 08.00 to 6.00.

2.3. Plant evaluations and data analysis

2.3.1. Specific leaf area. Specific leaf area was measured by following the method from Wilson, Thompson [31]. Leaf area the upper fully expanded leaves of each plant was measured using Portable Leaf Area Meter model AM350 from Opti-Science. After measuring the leaf area, the leaf was dried in oven at 70˚ C for 72 hours. Dry weight was measured after drying process and the Specific Leaf Area (SLA) was calculated by dividing total area of leaf (mm^2) by total weight of the leaf (gr).

2.3.2. Root biomass. Root biomass was calculated by measuring the dry root mass. All the plants were gently pulled out with all the soil from the pots. The soil was gradually removed from the root in the running tap water. After all the materials were run out from the root the root then dried using tissue. The roots were then put into the envelope and sealed with label. Roots were dried at 70^o C for 72 hours. The roots were taken one by one from the oven to get rid of gaining any water absorption. After that the root were measured in weight in order.

2.3.3. Symptom and purple area quantification. Every symptom was assessed quantitatively. Previous study was reported that curling leaf in RD cultivar and chlorosis in FG cultivar [30]. In this analysis, the result was an individual percentage of visible symptoms obtained from Equation (1).

$$p = n(N^{-1})\cdot 100\% \quad (1)$$

p, *n*, and *N* are percentage of symptoms, number of fully expanded damage leaves, and total number of fully expanded leaves, respectively. Percentage of number damage in each plant in the three different ozone regime thus collectively compared.

Table 1. Classification of purple area in percentage and unit of purple area.

Broad level	Percentage	Unit of Purple Area
Little area	)0-5 %(	1
Small area	)5-20 %(	2
Medium area	)21-40 %(	3
Medium Large area	)41-60 %(	4
Large area	)61-80 %(	5
Extra Large area	)81-100 %(	6

As purple area of GP and YP cultivars (Figure 4) dramatically expanded, a quantitative investigation was conducted to draw the effect of the exposure in quantitative way using the unit proposed by Ladd, Skelly, Pippin, & Fishman (2011). Every fully expanded leaf was classified into Purple area Unit based on the percentage of the purple area appeared in the leaves (Table 1). The data from each cultivar under three different ozone concentration were analyzed to draw the effect.

3. Result and discussion

3.1. Ozone concentration

The production of ozone gas had been monitored through 30-day period of fumigation. Fluctuation of Gas concentration was detected in every concentration (Figure 3). In the <10 ppb ozone chambers, the fluctuation was detected with minimum concentration 2 and maximum 15 ppb. Similarly, high range of fluctuation was found in 40 ppb ozone chambers, where the lowest was 15 ppb and the highest concentration was 58 ppb. In the highest fumigation concentration, 150 ppb ozone, the lowest concentration in this third concentration was 134 ppb whereas the ozone level was once monitored up to 173 ppb in a day. However means of all chambers, <10 ppb, 40 ppb, and 150 ppb were relatively close to the desired concentration, 7.7 ppb, 40.4 ppb, and 150.8 ppb, respectively.

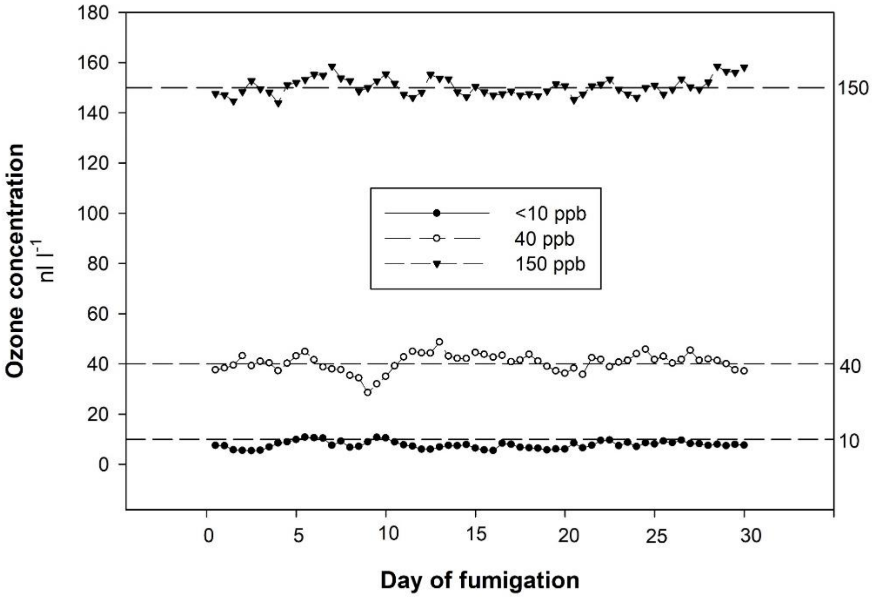

Figure 3. Ozone concentration inside the chamber during 30-day fumigation using MA 0.3.

3.2. Biomass

3.2.1. Root biomass. The assessment showed there was no significant change among the treatments)Figure 5(. It was also found that YP and RD are lighter than GP and GP. In line with that, the strucutre of the leaves also showed a similar pattern where the former cultivars showed ticker leaves whereas the latter two had thinner leaves (Figure 6). Overall, the ozone did not affect the root weight in all cultivars after 30-day period of ozone exposure. Several previous studies showed similar result. In *Calluna vulgaris*, there was no response in term of root biomass or length toward ozone exposure [32]. However, cropping plant such as rice responded the exposure differently as Ariyaphanphitak, Chidthaisong [33] revealed that root biomass of rice decreased when ozone exposure increased.

As ozone damage the plants in photosynthesis pigments, the reactive oxygen species derived from ozone are possible to interfere the transportation of photosynthate to belowground biomass [34].

However, this result is still premature to be widely accepted in many related species. Pregitzer, Burton [35] had emphasized that chronic exposure on perennial plants, the effect is not significant on plants root biomass. The mechanism was further discussed by Grantz, Gunn [36] where an increase of ozone is potential to induce a loss in plants' root mostly but since there is a separation effect occasionally of ozone to alter the allocation of substance to root without suppressing the productivity or photosynthesis substrate availability. Moreover, they also explained that there will be always specific cases where the effect can occurs without suppressing the translocation of photosynthesis product distribution.

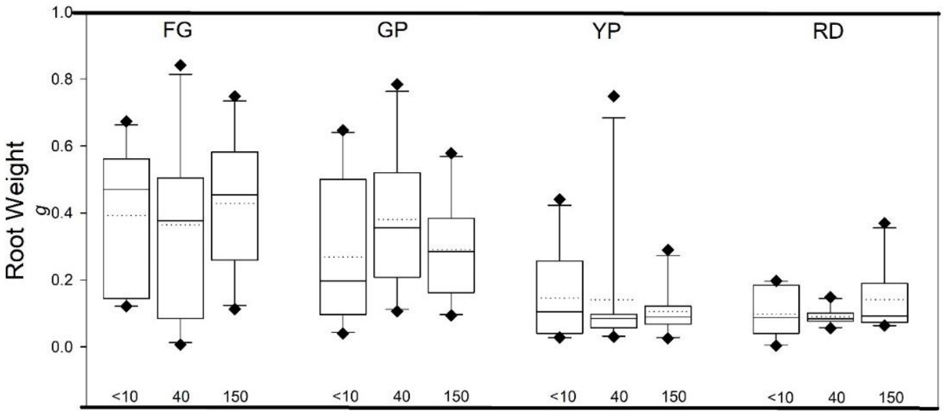

Figure 4. Root Biomass of four coleus cultivars after 30-day period of fumigation.

3.2.2. Specific leaf area. This parameter aimed to describe the biomass of the leaf. Thus, it has been assessed in order to detect the change in the leaf weight per area as a response to ozone exposure. There were two cultivars with thin leaves while the other two were thicker. Thick leaf cultivars consisted of FG and GP though thin leaf cultivars consisted of YP and RD. In other parameters, these two groups did not make any significant different in terms of responses except FG which showed an increase of specific leaf area after the exposure.

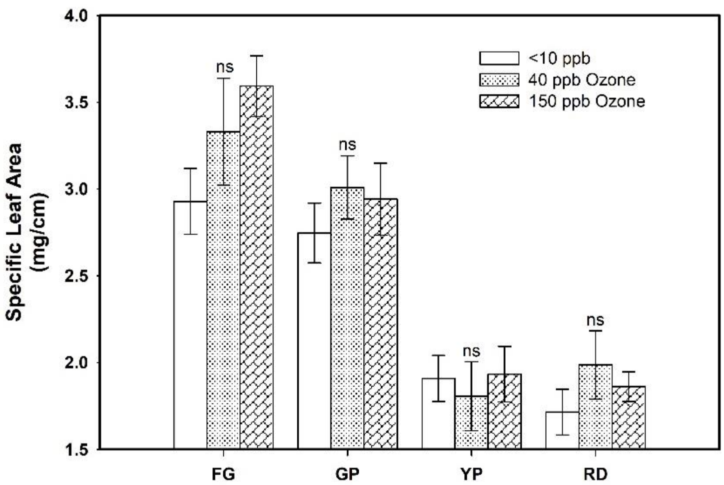

Figure 5. Specific leaf area of four coleus cultivars after 30-day period of fumigation. ns: there is no significant difference among treatments.

Thickness of leaves in specific leaf area in GP, YP, and FG seems to be similar with result of *Calluna vulgaris* that shows a stable net photosynthesis in leaves after fumigation after ozone exposure [32] while several thin-layer leaves grasses showed similar results with FG cultivar [37]. However, it is rare to observe same species can show such noticeable different among its cultivars. The different response in FG can be described as an alternative response to the ozone since it does not contain significant amount of anthocyanin in its leaves [30] since anthocyanin acts as antioxidant to reduce detrimental effects of ozone intake [38].

3.3. Plant symptoms, appearances and their quantification

3.3.1. Purple area percentage in purple-area-containing cultivar. Prior study of Padri and Umponstira [30] discovered an increase of the purple area in terms of percentage per total leaf area. In this study, the purple area were quantitatively measured and further compared. It has been found that a significant increase of purple area occurred in 150 ppb ozone *(*Figure 6*)* in the cultivar where the purple area could be observed (GP and YP).

Figure 6. YG (upper) and PG (lower) cultivars after 30-day fumigation of ozone left side is the <10 ppb ozone and right side 150 ppb ozone. P indicates the purple area.

Wider purple areas in two cultivars YP and GP were suspected to appear because of an increase of several pigments contained in the leaves as it was prior described by Padri and Umponstira [30]. Even though percentage purple area is strongly related to the complicated genetic materials which can be induced by several stress such as light [39], anther, gibberellin, sugar content [40] and gene availability [41], ozone currently becomes among the considered causes of the change [42].

A wider purple area in leaves is caused by coloration of anthocyanin, carotenoid, and betalains [43, 44]. Moreover, it has been emphasized that there is a relation between the colours of leaves especially in the red-purple area as well as its width with the anthocyanin content [44]. Thus, the width of such colours is the units that determined by the pigments composition. As it is well-known that the pigments affects directly the colour of leaves [45], the ozone exposure in this result was the sole source that can cause the expansion of green area. In these two specific cultivars, it has been observed that there were a significant increase of purple area in the 150 ppb ozone exposure. Thus, this result can comply the prior research regarding these two cultivars in which the contents of purple pigments was increasing after ozone exposure [30].

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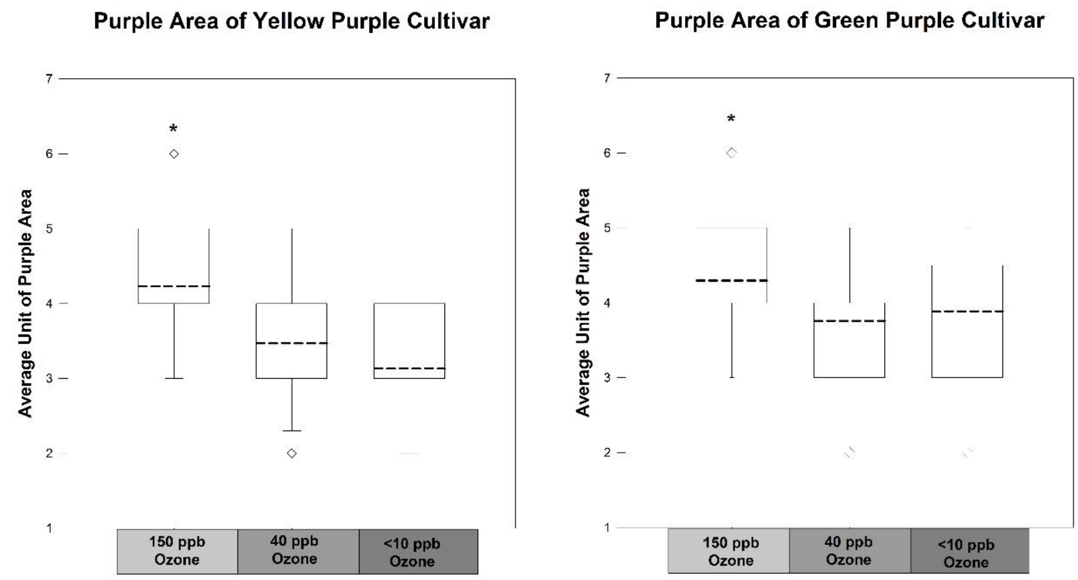

Figure 7. Ratio unit of purple area on the leaf of four coleus cultivars after 30-days fumigation.

3.3.2. Curling and chlorotic area. Statistically, there was no different between <10 ppb ozone and 40 ppb ozone on curling leaf symptom in RD where the curling leaf symptom was observed. Nevertheless, 150 ppb ozone was showing a significant different result from other treatments (Figure 8). Nevertheless, FG showed a different response in 40 ppb ozone and 150 ppb ozone compared with CF (Figure 8). It is necessary to note that curling leaves showed an increase in each additional ozone concentration. The percentage of chlorotic leaves was starting to increase at 40 ppb ozone although the statistical analysis does not show any significant difference. Nonetheless, the change had large amount of chlorotic leaves in 150 ppb ozone The chlorotic and curling leaf are common symptoms of leaf under ozone exposure caused by the rupture of the plastid containing chlorophyll and excess production of ethylene under oxidative stresses, respectively [46-48].

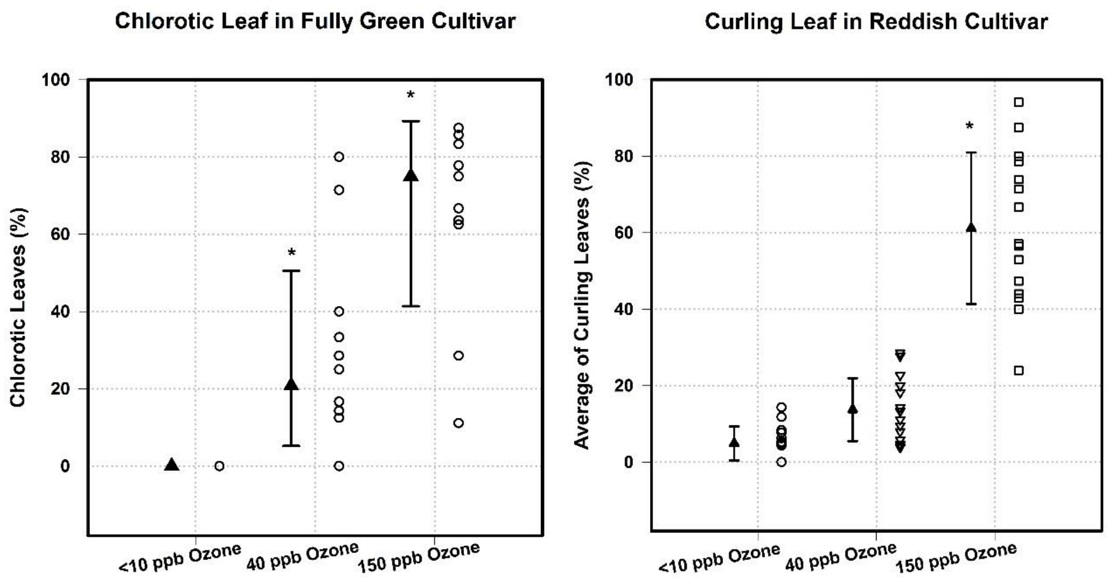

Figure 8. Quantification of symptoms on leaf of FG and RD coleus cultivars after 30-days period of ozone exposure.

As the most famous method to assess the air pollution effect on plants, percentage of leaf area that affected have been estimated in many plants [49] even though to some extent it can be no reliable due to the high probability of bias [50]. Even so, a clear pattern of different percentage of both symptoms can rise the reliability of this estimation method. the only warning toward this method is when it comes to the lage survey and the bias can occur from many surveyors [51]. Lastly, this method which called Horsfall–Barratt rating system from Barratt and Horsfall [52] is still realible in the laboratory and specific field assessment for ozone symptoms.

3.4. Bioindicator potential

Bioindicators are organisms or communities in which the response of certain change and/or occurrence of novel process observed to comprehensively draw the condition of the ecosystem and or environment [53]. In ozone, it can range from forbs to trees [54]. Nevertheless, the term of bioindicator nowadays is not limited only to draw the ecosystem condition but also to specifically point the change of environmental state at defined time [55]. In the nature, plants respond elevation ozone in many different physiological and thus affecting the morphological changes such as stipples, patches, discolored, and many more in which some of them are specific and unspecific symptoms [17]. However, apart from the curling and chlorotic leaf symptoms, the change of morphological appearance specifically the purple area shows state of the art in symptoms of the leaf where most of the passive bioindicator of ozone show leaf injuries as the main symptoms [17, 22, 23, 55].

Since the novel and valid change of the plants in terms of ozone responses occurred, the degree of responses at efficient level of ozone exposure is also an important part of bioindicator plant [20]. According to Sandermann Jr [56] who proposed the level of sensitivity, all of the cultivars showed in intermediate sensitivity to ozone exposure. Yet, for bioindicator purpose, FG and RD are the potential cultivars. For bio indicator purposes, Manning, Godzik [57] stated that bio-indicator of ozone can be chosen by considering the visible symptoms that easily recognized and thus this requirement was also demostrated in the results.

Several plant species have been showing a sensitivity to ozone exposure and later on it is called ozone-sensitive plants [58, 59]. The plants sometimes make a big different and show several symptoms and loose of biomass after or during ozone exposure. On the other hand, even in the most sensitive plants, the effects of ozone sometimes are very nebulous and the symptoms end up being neglected due to its low appearance. However, performance of this plants shows a vast response under moderate exposure of ozone and thus it can be categorize as potential bioindicator plant [54]. Further, physiochemical activities of this plant under such stress condition will be able to reveal more in terms of metabolic pathway that affected.

4. Conclusion

Biomass parameters namely root biomass and specific leaf area showed a stable numbers after the assessment whereas the scale of ozone symptoms and its occurrence on leaf showed different quantity in specific cultivars. FG cultivar displayed chlorosis leaves under 40 and 150 ppb ozone exposure while RD presented the same significant change in terms of curling leaf under 150pb ozone exposure. Purple area of GP and YP were increasing as the ozone did at 150 ppb ozone level. To conclude, FG and YP cultivars are potential plants for bio-indicator based on their performance in response to the ozone exposure. Correspondingly, as this result reveals a very promising respond to ozone stress, further study is necessary to confirm this result in terms of molecular effect on this plant.

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