# Agrosol and Brassinolide Applications Improve Growth and Physiological Responses of fig *(Ficus Carica* L.*)*

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**Abstract:** Agrosol and brassinolide applications had increased changes in the growth and physiology of fig. The experiment was arranged as an RCBD factorial with three replications. The Results of this research indicated that increasing agrosol (control, 2, 2.5, and 3 kg/ha) and brassinolide concentration (control and 200 ml/L) caused some differences in growth and physiology of fig. However, the differences were not consistent and most of the changes happened only in the first or fourth months. Agrosol 2 kg/ha and brassinolide 200 ml/L showed higher growth and physiology than the other concentrations after receiving agrosol and brassinolide treatment. There was no effective treatment of brassinolide alone but it had a significant effect when combining with agrosol except in chlorophyll fluorescence.

**Keywords** : Agrosol, Biomass production, Brassinolide, Fig, and Leaf gas exchange.

## 1. INTRODUCTION

Fig *(Ficus carica* L.*)* is classified as a member of the family Moraceae. It is originated from the Mediterranean region. Even though figs are typically deserted and underutilized but it is an important tree in many rural and marginal areas. Figs fruits contain high nutritional and pharmacological value also produce rubber [1].

Agrosol is a water-soluble compound composed of finely ground minerals that are sprayed onto a plant leaf surface where it enters the leaf and discharges $CO_2$. Agrosol has contents Calcium and Magnesium. It is developed to improve plant growth and photosynthetic rate, better yields, a reduction in water usage, and a more resilient plant [2]. Immediately after agrosol has been sprayed in a fine mist onto the leaf surface, agrosol will enter the plant through a pore, known as stomata. Minerals inside agrosol will discharge $CO_2$ to the plant. This encouragement the production of proteins and glucose and severely improves many oxygens discharged into the environment [3].

Generally, plants are provided from the outside with foliar and soil fertilizers, supplying for losing nutrients. In arid conditions, groundwater supply will scarce and farmers need to apply artificial irrigation. Many farmers using nutrients supply and water from alternative sources to solve this problem. Anyway, $CO_2$ fertilization is something that still mostly unknown in open land cultivation. To achieve ideal growth, plants need a certain $CO_2$ balance. The optimum photosynthetic rate will achieve if $CO_2$ content Ranging from 0.1-1.0% v/v. Unfortunately, air usually has a $CO_2$ contain solely 0.03% v/v. It means, plants are performing under their full potential [2, 3].

Plant growth regulators (PGR) are widely used in modern agriculture at low dosages and typically applied via foliar sprays with water as a carrier that affects developmental or metabolic processes in higher plants such as fig [4]. Brassinolide (BL) is one of the phytohormones essential for plant growth and development as well as important for cell division and expansion which may increase crop yield and stress tolerance. Furthermore, BL is also able to alleviate various biotic and abiotic stress effects [5]. Zulkarnaini *et al*. [6] reported that increasing BL concentration on fig caused some differences in physiological changes and growth of fig, but the differences were not consistent and most of the changes happened only in the first or second month. BL stimulates physiological changes, improved water relations, and metabolic processes such as photosynthesis in the wheat plant under drought stress conditions [7]. Our other research resulted that BL application on fig increased leaves, shoots and roots of fig at 16.93%, 26.31%, and 16.93%, respectively [8].

Meanwhile, agrosol application on pea plants decreased soil pH and increased the availability of phosphorus and micronutrients [2]. In other research showed that the application of agrosol 3-6 g/L increased the vegetative growth of cherry tomato plants [9].

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In Indonesia, there are at least 21 known cultivars of fig and most of them are from Improved Brown Turkey (IBT) variety [10]. There is limited information on the exogenous application of brassinolide and agrosol on this variety. So, this research objective was to probe the outcome of different concentrations of exogenous application of BL and agrosol on growth and physiological responses in Improved Brown Turkey (IBT) of fig.

## 2. MATERIALS AND METHODS

### 2.1. Plant Material and Experimental Design

Sources of fig were propagated using cutting methods taken from mature two- to three-year-old figs and transferred into media containing 3:2:1 mixed soil (top soil: organic matters :sand). Fig sources were set as an RCBD (Randomized Complete Block Design) in three replications. Study was performed in the experimental field from May until August 2018, school of graduate studies, Islamic University of Riau, Indonesia, located at 0°26'46.8"N 101°27'21.8"E in Marpoyan, Pekanbaru, Indonesia. Data were recorded weekly and monthly after treatment.

### 2.2. Treatments

Three-week-old of fig IBT variety was used in this study. The experiment was comprising two factors which were two concentrations (0 and 200 ml/L) of BL and four levels (0, 2, 2.5 and 3 kg/ha) of agrosol. Following the treatment, the plant was labelled as B0, B1, F0, F1, F2 and F3, respectively. Treatment B0 and F0 was served as a control. For the application of BL treatment, the fig variety IBT was sprayed without (0 ml/L) or with (200 ml/L) BL solution. The 200 ml/L BL solution was prepared earlier by diluting 200 ml A-Grow BPB in 0.1% aqueous ethanol at a constant water temperature of 50–60°C. Then add 52 ml MPC with 20 L water. The 200 ml/L BL was sprayed onto the surface and bottom part of the leaves in the morning at 0900-1100. Before the application of the treatment, the weather was in good condition and not rainy to ensure the optimum absorption of the treatment to the plant [11].

Agrosol treatments were adjusted in the form of a fine spray onto the leaf surface and sprayed onto the leaf surface in the form of a fine mist. The spraying nozzles with a diameter 150-300µm, at pressures ranging between 3 and 4 bar was used to obtain a fine layer agrosol treatment to the fig leaves of plant. While filling the tank of the sprayer with water, the agrosol at a different amounts representing different four concentrations (0, 2, 2.5 and 3 kg/ha) were slowly and evenly added. The stirrer should not be deactivated in order to prevent the materials settlement after an extended standstill, otherwise, the material must be properly mixed again before spraying started. The treatment was applied at the right weather condition in the morning at 0900-1100. It took 2–4 hrs approximately to be sucked by the plant. The agrosol was applied every 14 days during the vegetative period [2].

### 2.3. Measurements

#### *2.3.1. Growth Measurements*

- Plant height (PH) determination.

The distance from soil to the shoot tip was measured using a ruler.

- Total leaf area (TLA) determination.

Fig sample leaves were put in plastic bags and saved in a freezer with temperature 6°C in darkness for maximum 12 hrs [6]. After that, quantifying the TLA was quantified using a leaf area meter (Model LI – 3100A Lincoln Inc, Nebraska, USA). The device should be calibrated using standard calibration plate with a 100 cm$^2$ area before used. Leaves of fig were passed through between a light sensor array. Separated leaves were then passed through the device and arranged within the field of view and overlapping of adjacent leaves was avoided. Each experimental unit was measured from mean value of three sample plants.

- Total dry biomass (TDB) determination.

Prior to drying, the plants were separated into leaves, stems and roots and placed in paper bags. Sample leaves dried in an oven at 45$^0$C for 3 days in order to get constant weight. A sensitive electronic weighing scale (Model CDS 125, Mitutoyo Inc, Japan) was used to measure total dry weight of plant.

- Specific leaf area (SLA) determination.

The SLA of fig leaves was calculated based on a dry weight basis [12].

$$SLA = \frac{Seedling\ leaf\ area\left(cm^2\right)}{Seedling\ total\ weight\left(g\right)} \quad (1)$$

- Shoot to root ratio (S/R) determination.

S/R of fig was estimated by using equation as reported by Zulkarnaini *et al*. [6] on dry weight basis:

$$S/R = \frac{Sw}{Rw} \quad (2)$$

Where Sw=Total shoot dry weight (g) and Rw = Total root dry weight (g).

- Net assimilation rate (NAR) determination.

NAR values were measured using a formula as described by Zulkarnaini *et al*. [12].

$$NAR = \frac{(W_2 - W_1)(\ln A_2 - \ln A_1)}{(A_2 - A_1)(t_2 - t_1)} \quad (3)$$

Where W represents dry weight of whole plant (g), A represents leaf area per plant ($cm^2$), and t=time (month).

- Relative growth rate (RGR) determination.

Relative growth rate (RGR) was the net gain in total dry matter per unit leaf area per unit time.

$$RGR = \frac{Ln\,W_2 - Ln\,W_1}{(t_2 - t_1)} \quad (4)$$

Where $W_1$ represents dry weight of plant/$m^2$ recorded at time $t_1$ (g), $W_2$ represents dry weight of plant/$m^2$ recorded at time $t_2$ (g), and $t_{1\text{-}2}$ represents interval time (month).

#### 2.3.2. Physiology Measurements

- Leaf gas exchange and Intercellular $CO_2$ (Ci) determination.

A portable photosynthesis system (LICOR–6400, Inc., USA) was used to measure photosynthesis, stomatal conductance, transpiration rate and intercellular $CO_2$ of fully expanded fig leaves. The device was warmed and calibrated for half hour with ZERO IRGA mode. Gas exchange measurements were carried out 0900 – 1100 approximately.

- Chlorophyll content (CC) measurement.

All of fig leaves with different greenness (pale yellow, light green and dark green) were carried back to laboratory. Then, use a hole puncher to make leaf discs 3mm in diameter and immediately soaked in 20 ml of 80% acetone in an aluminium foil covered glass bottle and left in the dark for approximately 7 days until all the green colour had bleached out. Lastly, 3.5 ml solution was transferred to determine chlorophyll-a and chlorophyll-b at 664nm and 647nm wavelengths using a light spectrophotometer (UV-3101P, Labomed Inc, USA). Chlorophyll a and chlorophyll b content were determined based on the method as described by Zulkarnaini *et al*. [13]. Data were collected monthly.

$$\text{Chlorophyll a (mg/cm}^2\text{ FW)} = 13.19\,(A_{664}) - 2.57\,(A_{647}) \quad (5)$$

$$\text{Chlorophyll b (mg/cm}^2\text{ FW)} = 22.1\,(A_{647}) - 5.26\,(A_{664}) \quad (6)$$

$$\text{Total Chlorophyll (mg/cm}^2\text{ FW)} = \frac{3.5x(chl\ a + chl\ b)}{4} \quad (7)$$

Where: $A_{647}$ and $A_{664}$ was absorbances of the solution at 647 and 664nm, respectively. 13.19, 2.57, 22.1 and 5.26 were the absorption coefficients. 3.5 was the total volume used in the analysis taken from the original solution (mL) and 4 was the total discs area ($cm^2$).

- Chlorophyll fluorescence ($f_V/f_m$) determination.

The measurement were obtained from 0900 – 1200. Fully expanded fig young leaves were darkened for 15 minutes by attaching light-exclusion clips to the central region of the leaf surface. After that, use a portable chlorophyll fluorescence meter (Model Handy-PEA, Hansatech Instruments Ltd, Kings, Lynn, UK) to measure chlorophyll fluorescence for 5 seconds [14]. Values of initial fluorescence ($f_o$), maximum fluorescence ($f_m$) and variable fluorescence ($f_v$) were obtained from this procedure. $F_v$ was derived as the differences between $f_m$ and $f_o$. The mean value of three representative plants was used to represent each block.

- Instantaneous water use efficiency (WUE) determination.

Photosynthetic water-use efficiency (known as intrinsic or instantaneous WUE) is the ratio of the rate of carbon assimilation (photosynthesis) to the rate of transpiration [15].

$$WUE = \frac{A}{E} \quad (8)$$

Where A = Photosynthetic rate (μmol.$m^{-2}s^{-1}$) and E = Transpiration rate (mol $m^{-2}s^{-1}$).

### 2.4. Statistical Analysis

All the data acquired were analyzed using SAS 9.4 software (Statistic Analysis System). Significant difference of mean values was determined and analyzed using two-way ANOVA and the mean differences were compared and tested using Least Significant Different (LSD) at P ≤ 0.05 level of significance

## 3. RESULTS AND DISCUSSION

### 3.1. Fig Growth

The growth of the fig plants (Figure **1-7**) was influenced by interaction between agrosol levels and brassinolide. Treatment with different concentration of agrosol (control, 2, 2.5 and 3 kg/ha) and brassinolide (control and 200 ml/L) led to an augmentation in total leaf area, plant height, specific leaf area, total dry biomass, net assimilation rate, shoot-to-root-ratio and relative growth rate.

#### *3.1.1. Plant Height*

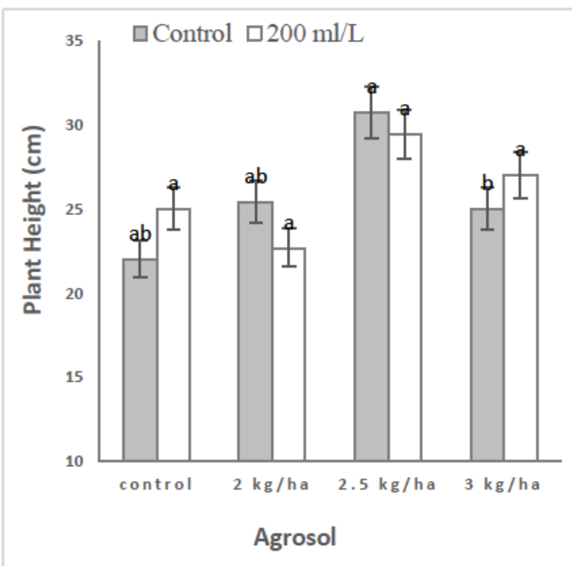



**Figure 1:** Significant interaction between BL and agrosol on plant height of fig at 3rd WAT. Bars followed by the different small letters significant at P≤0.05.

Plant heights of fig (Figure **1**) were affected by interaction between BL and agrosol. No significant effect was found on plant height at every week of observation when agrosol and BL alone was applied. But it has an effect when combined between BL and agrosol at 3rd week after treatment (WAT). Increasing concentration of BL from 0 to 200 ml $L^{-1}$) and agrosol from 0, 2 to 2.5 kg $ha^{-1}$) caused an increment of plant height of fig and decreased when concentration of agrosol at 3 kg $ha^{-1}$.

#### *3.1.2. Total Leaf Area*

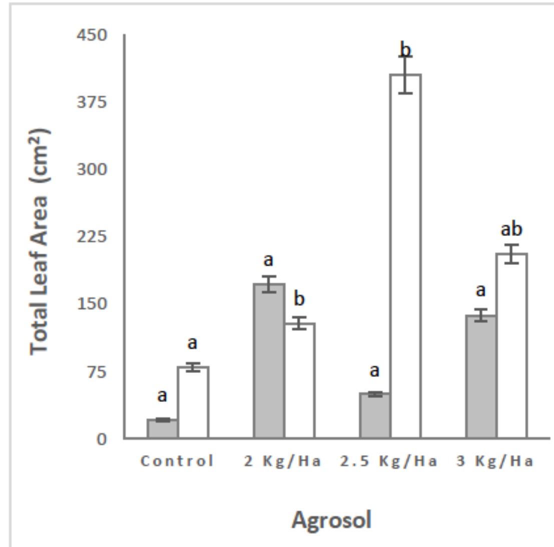



**Figure 2:** Significant interaction between BL and agrosol on total leaf area of fig at 2nd MAT. Bars followed by the different small letters significant at P ≤ 0.05.

Total leaf area (TLA) of fig (Figure **2**) was affected by interaction between BL levels and agrosol. The interaction between BL levels and agrosol gave significant effect on TLA at 1st and 2nd MAT. The highest TLA value of interaction between BL and agrosol was 404.92 $cm^2$ when fig plant treated with 200 ml $L^{-1}$ BL and 2.5 kg $ha^{-1}$ agrosol. While, the lowest value (20.60 $cm^2$) of TLA was determined when the plant treated with 200 ml $L^{-1}$ BL without application of agrosol.

#### *3.1.3. Total Dry Biomass*

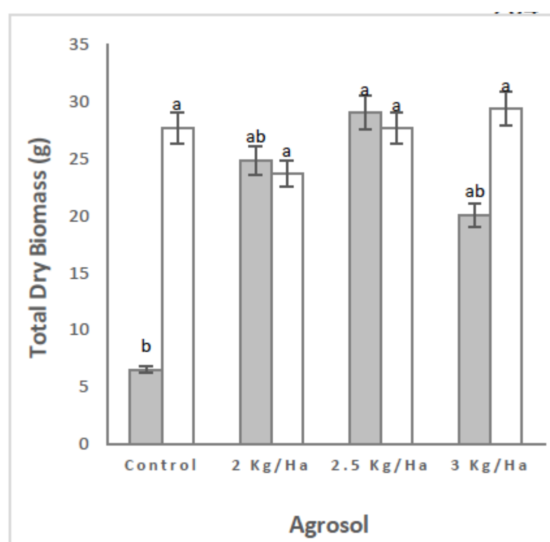



**Figure 3:** Significant interaction between BL and agrosol on total dry biomass (TDB) of fig at 3rd MAT. Bars followed by the different small letters significant at P≤0.05.

The total dry biomass (TDB) (Figure **3**) was significantly affected by interaction between agrosol and BL application. However, neither BL application nor anti-transpiration alone gave the significance response to TDB. The interaction between agrosol and BL was exhibited the significant effect on TDB at 3rd MAT. The application of 200 ml $L^{-1}$ BL showed higher TDB of fig plant than control at every month of observation regardless of agrosol treatment.

### 3.1.4. Specific Leaf Area

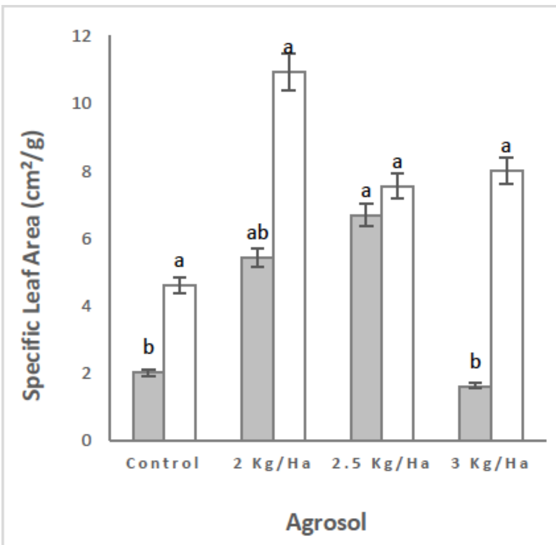



**Figure 4:** Significant interaction between BL and agrosol application on specific leaf area (SLA) of fig at 2nd MAT. Bars followed by the different small letters significant at P≤0.05.

The specific leaf area (SLA) of fig was affected by interaction between BL and agrosol application during all time of observation (2nd, 3rd and 4th MAT) except at the 1st MAT (Figure **4**). The effect on SLA was more pronounced when the treatment was combined with BL application than agrosol alone. Agrosol treatment at 2.5 kg $ha^{-1}$ with 200 ml $L^{-1}$ BL showed the highest SLA throughout the experimental period and the lowest SLA was found at control agrosol and BL. The low SLA in the elevated treatment clearly indicated the enhanced on leaves thickness under elevated BL.

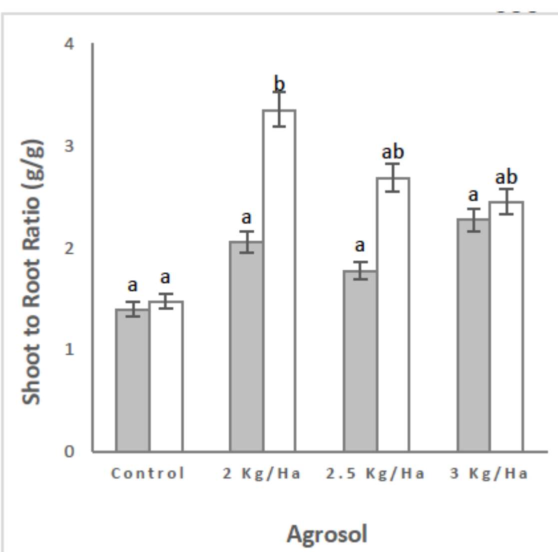



**Figure 5:** Significant interaction between BL and agrosol on S: R of fig at 4th MAT. Bars followed by the different small letters significant at P≤0.05.

### 3.1.5. Shoot to Root Ratio

The rate of S: R depends on the leaf morphology and biomass allocation to specific organs. The segregation between shoot and root growth can be derived from S:R. Analysis of variance showed a significant difference (P<0.05) at the 2nd and 4th MAT treatment of interaction between agrosol and BL. Treatment of 200 ml $L^{-1}$ BL and 2 kg $ha^{-1}$ agrosol exhibited the highest of S:R value (Figure **5**). The highest S: R was 3.35 and the lowest value was 1.39 throughout the 0 – 4th MAT periods.

### 3.1.6. Net Assimilation Rate

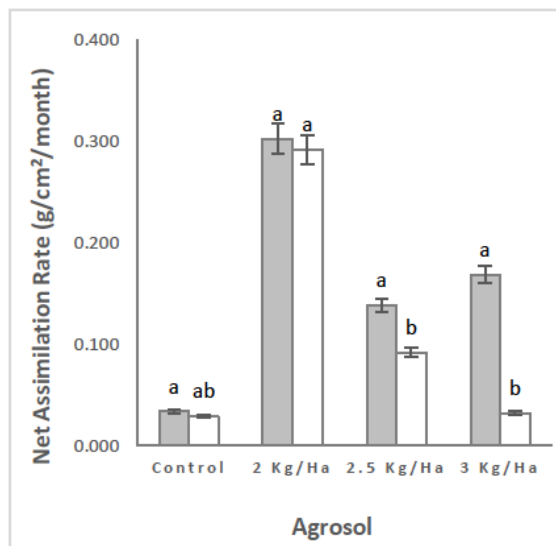



**Figure 6:** Significant interaction between BL and agrosol net assimilation rate (NAR) of fig at 1st to 2nd MAT. Bars followed by the different small letters significant at P≤0.05.

Figure **6**, presented the net assimilation rates (NAR) of fig exposed to control (without BL) to four levels (0, 2.0, 2.5 and 3.0 kg $ha^{-1}$) of elevated agrosol. NAR of fig was affected by interaction between BL and agrosol levels. NAR showed increased strongly when treated with 2 kg $ha^{-1}$ of agrosol and decreased when increasing the concentration from without to 2.5 and 3.0 kg $ha^{-1}$ of agrosol. Application of 200 ml $L^{-1}$ BL showed higher NAR than control.

#### 3.1.7. Relative Growth Rate

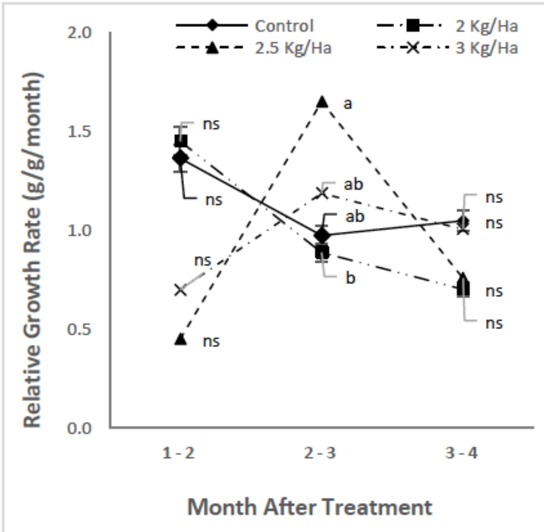



**Figure 7:** Changes in RGR as influenced by agrosol of fig throughout 4th MAT. Curves followed by the different small letters significant at P≤0.05.

The relative growth rate (RGR) of fig (Figure **7**) was only affected by agrosol levels. No interaction was detected between BL and agrosol treatment. Application of agrosol showed inconsistent RGR result on fig. Obviously, from the result it shows that RGR from 2rd to 3rd MAT was higher when the plant was treated with 0 > 2.0 > 3.0 > 2.5 kg $ha^{-1}$ agrosol.

All of parameters have an effect at every five-weekly and monthly observations. Increasing agrosol concentration (2 and 2.5 kg/ha) and brassinolide concentration (200 ml/L) led to an augmentation in TLA, PH, SLA, TDB, NAR and S/R but there was a decrease when agrosol concentration was 3 kg/ha when compared with the control samples. Whilst at parameter RGR fluctuated over a period of study and only agrosol treatment alone affected the RGR. There wasn't an effect treatment of brassinolide alone but it had a significant effect when combining with agrosol application on growth of fig. Agrosol concentration at 2 kg/ha and brassinolide concentration at 200 ml/L showed higher growth than the other concentrations at every five-weekly and monthly observation.

As like morphological parameters, physiological traits such as photosynthesis, transpiration rate, stomatal conductance, water use efficiency, intercellular $CO_2$ and chlorophyll have shown some differences with brassinolide application, but the differences were not consistent and most of the changes happened only in first or fourth month. Both the agrosol and the brassinolide treatments were effective on the physiological responses of fig. Brassinolide treatments (control and 200 ml/L) was significant only at parameter chlorophyll fluorescence. Agrosol concentration at 2 kg/ha and brassinolide concentration at 200 ml/L showed higher physiological responses than the other concentrations at monthly observation.

We studied the effect of exogenous agrosol and brassinolide application on some growth and physiological traits of fig. The main functions of agrosol and brassinolide were to promote the plant growth by improved PH value of the plant resulting in a better utilization, increased intensity of green coloring and improve physiological responses of plants by improved the $CO_2$ supply to the plants [3] and to promote the plant growth especially for cell elongation and division [16]. Zulkarnaini *et al*. [6] reported that the best concentration of brassinolide application to promote growth and physiological changes of fig was 200 ml/L therefore we use this suggested concentration to this research.

As levels of agrosol (control, 2, 2.5 and 3 kg/ha) and brassinolide increased (control and 200 ml/L), plant height, total leaf area, total dry biomass, specific leaf area, shoot-to-root-ratio and net assimilation rate parameters also linearly improved at 9%, 47%, 13%, 34%, 22% and 58%, respectively, higher than recorded for the control treatment.

Same results were described by other researchers for other plants *i.e*. Kandil [2] for pea plant; Gaj *et al*. [17] for potato; and Brataševec *et al*. [18] for rebula. The stimulation of growth was more pronounced on above-ground biomass than below-ground biomass, showing a high shoot-to-root ratio [19]. The increment of growth in this research might have been caused increasing in carboxylation rate after using the agrosol and BL treatment, which increased carbon assimilation, increased plant sink strength, channelling it to stimulate increase in total leaf area, plant height, specific leaf area, total dry biomass, net assimilation rate and shoot-to-root-ratio [20].

Specific leaf area (SLA) was one growth parameter that characterized the thickness of the leaves. Generally, high SLA plant had the thinnest leaves. Specific leaf area was found to be greater than the control (p≤0.05) under increasing concentration agrosol and brassinolide. The result indicates that plants have thinner leaves. The thinner leaf might have been due to decrease in the mesophyll layer after receiving agrosol and brassinolide [21]. The decrease in leaf thickness could also have been due to lower leaf weight ratio at second and fourth MAT compared with at first to third MAT. The leaf area was maintained at lowest SLA. That indicated that leaves of fig were thickest at control of agrosol and brassinolide. This indicated that increase in SLA is caused by decrease in leaf weight compared with increase in leaf area [22, 23].

The net assimilation rate (NAR) of plants were growth characteristics that best describe plant growth performance under specified conditions [24]. It was evident that plants under elevated agrosol and BL have high NAR. Increase in plant growth grown under different density and ontogeny on size of Pinus strobus has also been reported by Boyden *et al*. [25] who reported that increase in total biomass by 30% in Pinus had increased NAR by 4% compared with the control. The reduction in NAR was due to the ontogenical development of fig [26, 27].

The present results were in agreement with growth differentiation model theory proposed by Shipley [24] which stated that growth (RGR) would increase by enhancement of resource availability (agrosol and brassinolide) owing to more assimilate production being stimulated by high agrosol and brassinolide-enriched above and below-ground biomass of fig [28]. The enhancement of RGR in fig under high agrosol and brassinolide was particularly caused by the enhancement of above-ground biomass rather than below-ground biomass as translated by the negative relationship between RGR and NAR ($R^2$ = 0.31; P≤0.05; Figure **15.I**) as observed by Huo *et al*. [29].

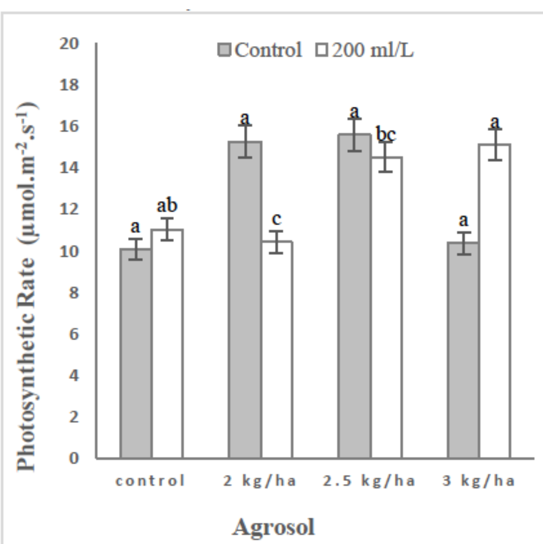



**Figure 8:** Significant interaction between BL and agrosol on photosynthesis rate (A) of fig at 1st MAT. Bars followed by the different small letters significant at P≤0.05.

The general reduction in RGR with time was attributed to ontogenical effect of the plant as observed by Takatani *et al*. [30] where rapid growth occurred with young plants treated with high $CO_2$ and nitrogen levels but decreased as plants aged.

### 3.2. Fig physiological Responses

#### *3.2.1. Photosynthesis Rate*

Photosynthesis (A) is a process used by plants and other organisms to convert light energy into chemical energy that can later be released to fuel the organisms' activities. The A of fig was affected by interaction between BL and agrosol levels (Figure **8**). Combination treatment between BL and agrosol levels had significant effect on A at 1st MAT. Increasing concentration from 0 to 200 ml $L^{-1}$ of BLs and from 0 to 2, 2.5 and 3 kg $ha^{-1}$ of agrosol levels caused an increment of A value. The application of agrosol gave the pronounced result to highly increase the A to 15.58 µmol $m^{-2}$ $s^{-1}$ as compared to other treatment under without application of BL. While, the lowest (10.07 µmol $m^{-2}$ $s^{-1}$) A value was obtained when the plant was not applied with any BL and agrosol treatment.

#### *3.2.2. Stomatal Conductance*

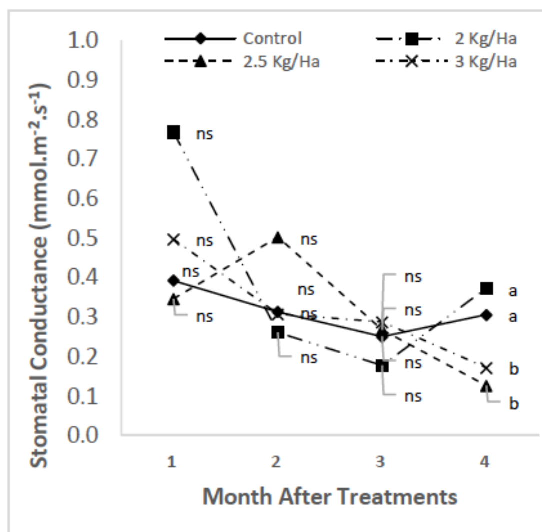



**Figure 9:** Changes in stomatal conductance (gs) as influenced by agrosol of fig throughout 4th MAT. Curves followed by the different small letters significant at P≤0.05.

All over the experiment, those plants elevated with agrosol were found to have higher stomatal conductance (gs) than the control plants (Figure **9**). At 1st MAT, gs value of plant treated with 2 and 3 kg $ha^{-1}$ agrosol had higher value than 2.5 kg $ha^{-1}$ agrosol and control. At 2nd to 4th MAT, gs value tend to show decreased except for 2.5 kg $ha^{-1}$ agrosol. The gs of fig was found most affected by the agrosol alone but not with the BL application.

### *3.2.3. Transpiration Rate*

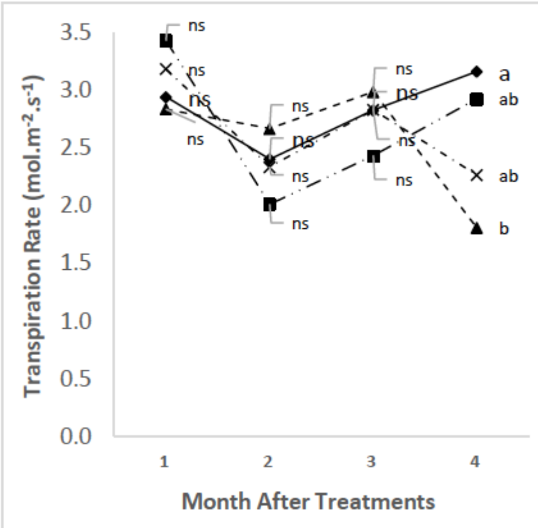



**Figure 10:** Changes in E as influenced by agrosol of fig throughout 4th MAT. Curves followed by the different small letters significant at P≤0.05.

The transpiration rate (E) of fig was affected by agrosol levels (Figure **10**). Treatment agrosol levels had significant effect on E at 4th MAT. Increasing concentration of BLs from 0 to 200 ml $L^{-1}$ had no effect on E of fig. In contrast, increasing concentration of agrosol caused the inconsistent increment of E value. From the result of study it shows that at 4th MAT, mean of E was higher when the plant was treated with 0 > 2.0 > 3.0 > 2.5 kg $ha^{-1}$ of agrosol.

### *3.2.4. Intercellular $CO_2$*

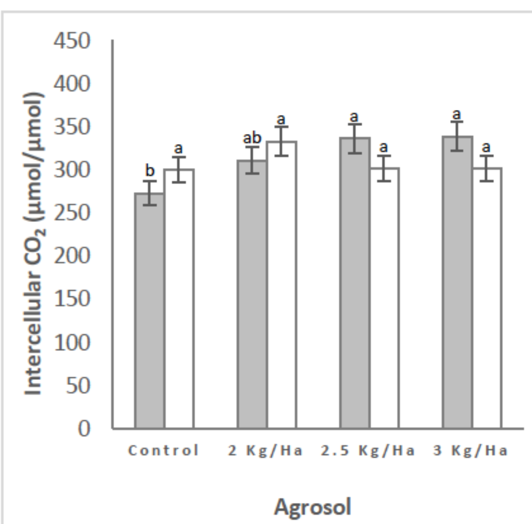



**Figure 11:** Significant interaction between BL and agrosol on intercellular $CO_2$ (Ci) of fig at 1st MAT. Bars followed by the different small letters significant at P≤0.05.

Intercellular $CO_2$ (Ci) is the relationship between existing of water vapor and entering of $CO_2$ through stomata. The Ci of fig was affected by interaction between BL and agrosol levels (Figure **11**). Treatment interaction between BL and agrosol levels had significant effect on Ci at 1st MAT. Increasing concentration of BLs (0 and 200 ml $L^{-1}$) and agrosol levels (0, 2, 2.5 and 3 kg $ha^{-1}$) caused an increment of Ci value. The Ci value of plant treated with 200 ml $L^{-1}$ BL was greater than control (0 ml $L^{-1}$ BL). The Ci value was higher when the plant was treated with 2>3>2.5>0 kg $ha^{-1}$ of agrosol at 1st MAT.

### *3.2.5. Chlorophyll Content*

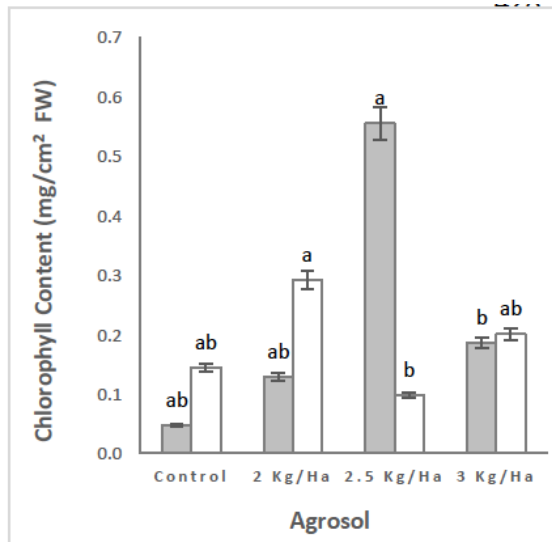



**Figure 12**: Significant interaction between BL and agrosol on total CC of fig at 1st MAT. Bars followed by the different small letters significant at P≤0.05.

It was analyzed, that production of chlorophyll content (CC) was influenced by interaction between BL levels and agrosol (Figure **12**). Treatment interaction between BL and agrosol levels had significant effect on CC at first MAT. From the graph, we can look that CC value of BL 200 ml $L^{-1}$ was greater than BL control at every increment of agrosol levels except at agrosol 2.5 kg $ha^{-1}$. The highest CC value of interaction between BL and agrosol was 0.56 mg/$cm^2$ FW on BL control + agrosol 2.5 kg $ha^{-1}$. The lowest CC value of interaction between BL and agrosol was 0.05 mg/$cm^2$ FW on treatment of BL control + agrosol control too.

### *3.2.6. Chlorophyll Fluorescence*

The maximum quantum efficiency of PSII ($f_v/f_m$) between the agrosol and BL treatments was as shown in Figure **13**. It was observed that plant applied with higher BL levels had higher maximum $f_v/f_m$. The maximum $f_v/f_m$ or chlorophyll fluorescence of fig was affected by BL levels. Whereas, treatment of agrosol levels had no significant effect on $f_v/f_m$. At 1st MAT, the $f_v/f_m$ of control and 200 ml $L^{-1}$ BL were 0.28 and 0.59 µmol $mol^{-1}$, respectively.

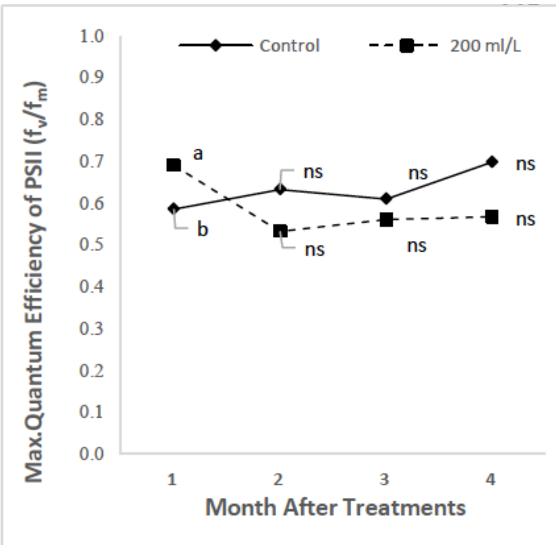



**Figure 13:** Change in chlorophyll fluorescence ($f_v/f_m$) as influenced by BL of fig throughout 4th MAT. Curves followed by the different small letters significant at P≤0.05.

### 3.2.7. Water use Efficiency

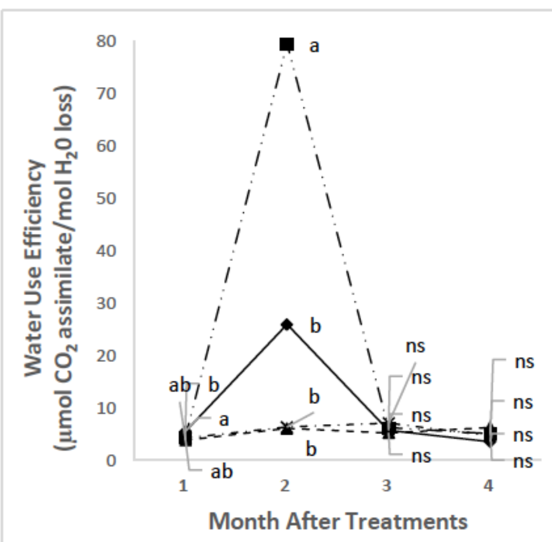


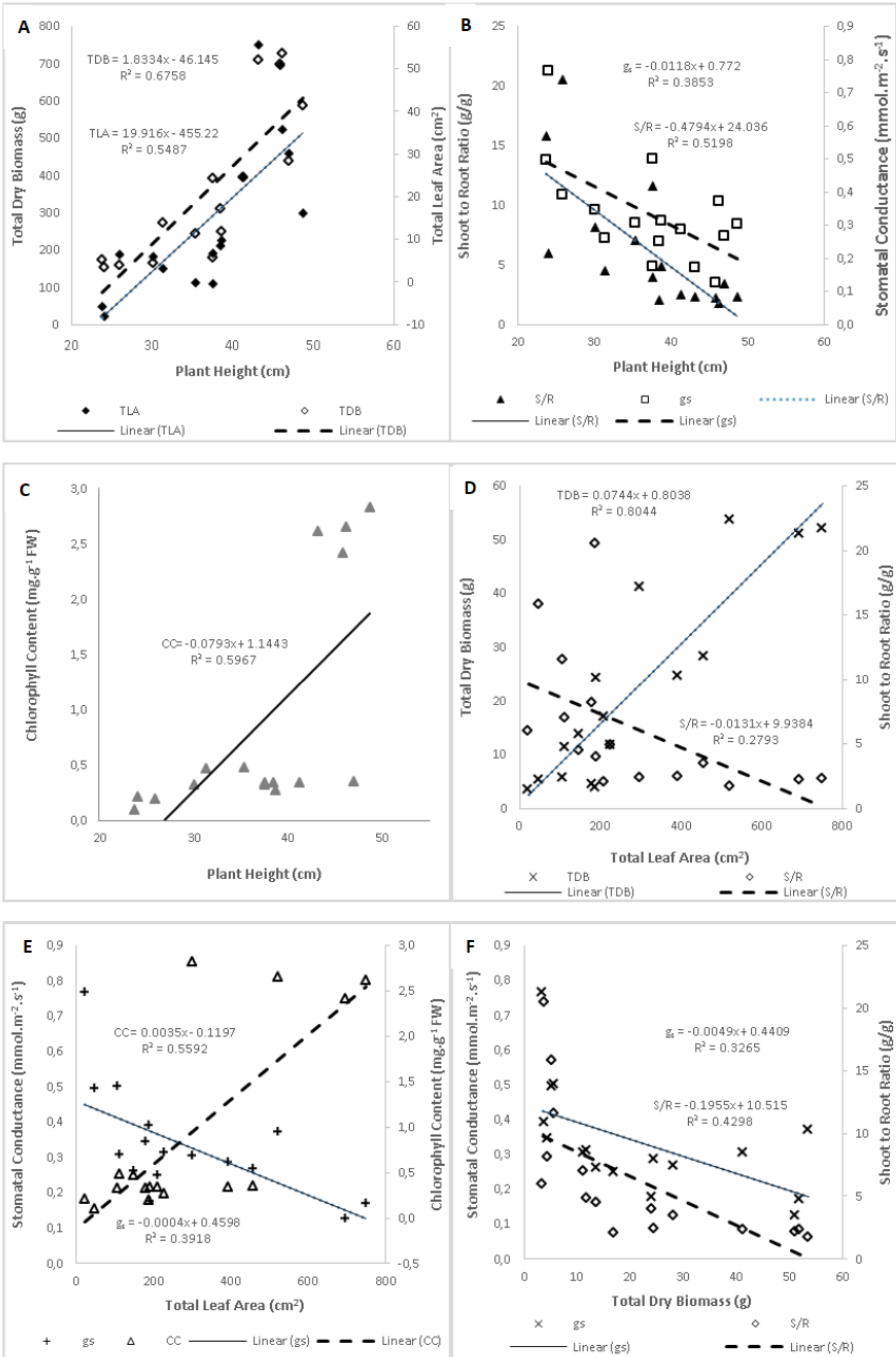

**Figure 14:** Change in water use efficiency (WUE) as influenced by agrosol of fig throughout 4th MAT. Curves followed by the different small letters significant at P≤0.05.

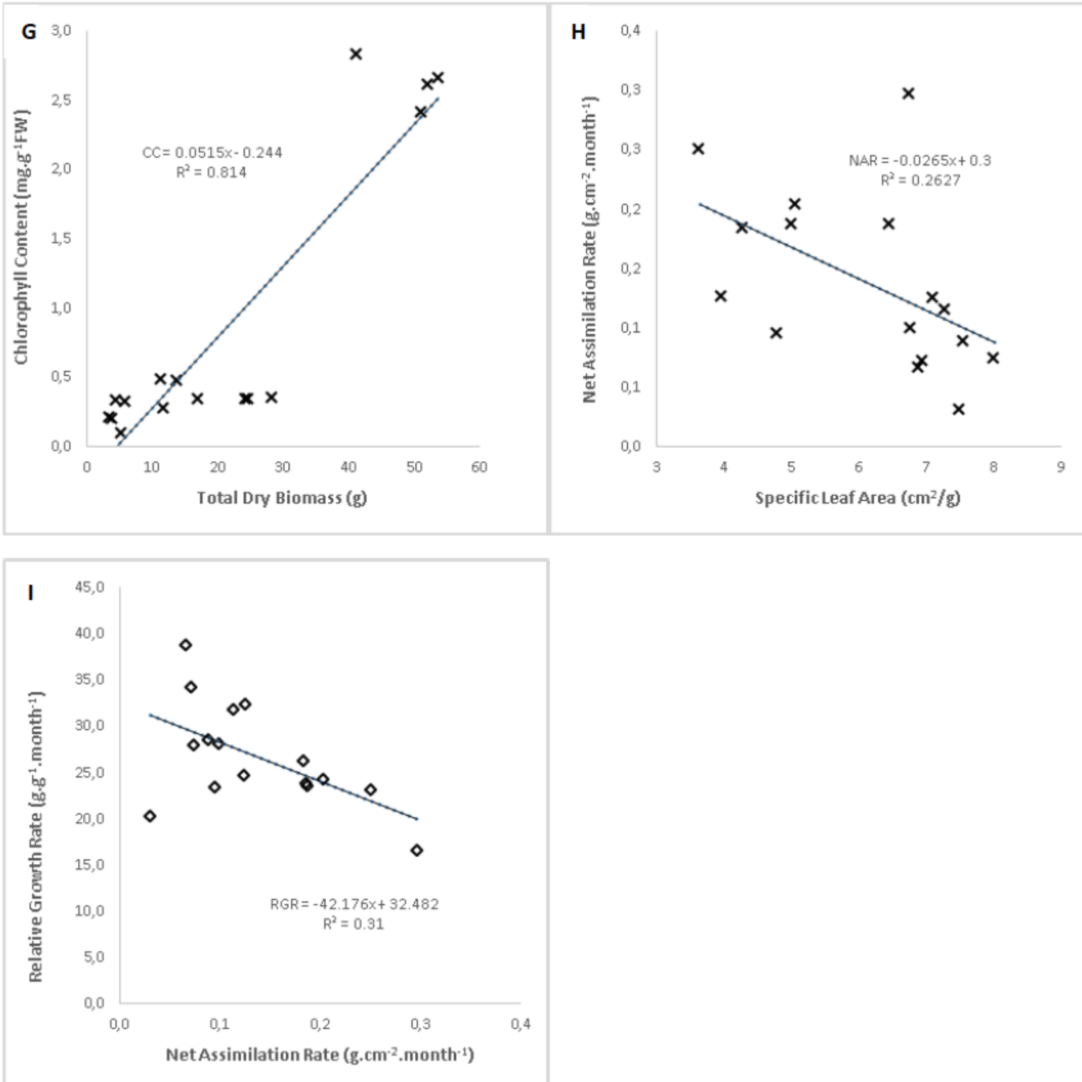
The instantaneous water use efficiency (WUE) of fig was affected by different concentrations of agrosol and brassinolide applied (Figure **14**). Whilst, there wasn't significant effect of interaction between BL and agrosol on WUE. Increasing concentration of agrosol from 0 to 2 kg $ha^{-1}$) caused increasing WUE slightly in four month observations. Meanwhile, increasing concentration of agrosol from 2.5 to 3 kg $ha^{-1}$ strongly increased WUE until 2nd MAT and then strongly decreased WUE at 3rd MAT. Commonly, the best concentration of agrosol treatment was 2 kg $ha^{-1}$ and the lowest agrosol concentration was 2.5 kg $ha^{-1}$.

Figure **8-14** shows that the physiological responses of fig were influenced by the brassinolide levels and the agrosol. Brassinolide and agrosol interaction in fig was significant on photosynthetic rate (A), intercellular $CO_2$ (Ci) and chlorophyll content (CC). Agrosol alone was significant on stomatal conductance (gs), transpiration rate (E) and water use efficiency (WUE). Treatment of brassinolide alone was significant on chlorophyll fluorescence or maximum quantum efficiency of photosystem II ($f_v/f_m$), Increasing combination agrosol concentration (control, 2 and 2.5 kg/ha) and brassinolide concentrations (control and 200 ml/L) led to an increment of photosynthetic rate, intercellular $CO_2$ and content of chlorophyll but there was a decrement when agrosol concentration was 3 kg/ha. Increasing treatment of agrosol concentrations alone (control, 2, 2.5 and 3 kg/ha) had decreased the stomatal conductance but its fluctuated on transpiration rate and water use efficiency over a period of study.

Interaction-treatment between agrosol and brassinolide enhanced photosynthesis (4.86%), intercellular $CO_2$ (16.78%) and chlorophyll content (5.66%). Brassinolide (BL) had profound impact on leaf photosynthesis and plant performance. BL improved leaf carbon assimilation rate, which is the light harvesting machine of plant photosynthesis (A). It was investigated in the current research that increasing agrosol and brassinolide concentration can increase A in fig seedlings (Figure 8) as showed by improved $f_v/f_m$ which was a measure of the maximum efficiency of PSII (*i.e*. the quantum efficiency if all PSII centres were open [31]. The low $f_v/f_m$ in elevated treatment indicates low efficiency of plants under elevated brassinolide, in utilizing their photons, and explaining why A was high in elevated treatments.

It was convinced that reduced stomatal conductance (gs) might contribute to plant acclimation to high intercellular $CO_2$ (Ci) [32]. In the present study, it was observed that the Ci of fig started to drop after 2 MAT (data weren't be shown), the drop of Ci in the present study might be due to reduced leaf thickness as the plant aged [33]. There was less mesophyll layer under this condition, so less $CO_2$ were assimilated per unit leaf area. This phenomenon was also observed by Henson and Chang [20] in oil palm leaves.


A
TDB = 1.8334x - 46.145
R² = 0.6758
TLA = 19.916x - 455.22
R² = 0.5487
Total Dry Biomass (g)
Total Leaf Area (cm²)
Plant Height (cm)
TLA
TDB
Linear (TLA)
Linear (TDB)
B
gs = -0.0118x + 0.772
R² = 0.3853
S/R = -0.4794x + 24.036
R² = 0.5198
Shoot to Root Ratio (g/g)
Stomatal Conductance (mmol.m⁻².s⁻¹)
Plant Height (cm)
S/R
gs
Linear (S/R)
Linear (S/R)
Linear (gs)
C
CC= -0.0793x + 1.1443
R² = 0.5967
Chlorophyll Content (mg.g⁻¹ FW)
Plant Height (cm)
D
TDB = 0.0744x + 0.8038
R² = 0.8044
S/R = -0.0131x + 9.9384
R² = 0.2793
Total Dry Biomass (g)
Shoot to Root Ratio (g/g)
Total Leaf Area (cm²)
TDB
S/R
Linear (TDB)
Linear (S/R)
E
CC= 0.0035x - 0.1197
R² = 0.5592
gs = -0.0004x + 0.4598
R² = 0.3918
Stomatal Conductance (mmol.m⁻².s⁻¹)
Chlorophyll Content (mg.g⁻¹ FW)
Total Leaf Area (cm²)
gs
CC
Linear (gs)
Linear (CC)
F
gs = -0.0049x + 0.4409
R² = 0.3265
S/R = -0.1955x + 10.515
R² = 0.4298
Stomatal Conductance (mmol.m⁻².s⁻¹)
Shoot to Root Ratio (g/g)
Total Dry Biomass (g)
gs
S/R
Linear (gs)
Linear (S/R)




**Figue 15:** Significant relationships between PH with (**a**): TDB and TLA; (**b**): S/R and $g_s$; (**c**): CC; between TLA with (**d**): TDB and S/R; (**e**): $g_s$ between TDB with; (**f**): $g_s$ and S/R; (**g**): CC; (**h**): between SLA with NAR; and (**i**): between NAR with RGR at p≤ 0.05, n=96.

Up-regulation of A may as represented by an increases in leaf intercellular $CO_2$ concentration (Ci), which was also related to increase in the thickness of the leaves (high SLA) under elevated agrosol and brassinolide that contain high photosynthetic protein especially Rubisco [34]. The latter might up–regulate several enzyme related to carbon metabolism that simultaneously increase the Ci [34]. This data implies that high A under elevated agrosol and brassinolide could be attributed to more efficient net assimilation due to extra carbon fixation exhibited by high Ci per unit area, which was related to increased thickness of mesophyll layer, mainly caused by increased palisade layer of fig [35].

Agrosol-treatment alone decreased stomatal conductance (16.08%), transpiration rate (4.14%) and water use efficiency (11.16%). Stomata are the windows that admit water and $CO_2$ in and out of the plant. Stomatal conductance, transpiration rate and water use efficiency were found to have declined. Agrosol improves the $CO_2$ supply to the plants ($CO_2$ fertilization effect), by increasing $CO_2$ saturation in the intercellular spaces beneath the stomata. The increased levels of $CO_2$ lead to an increased plant growth. This intensified growth is based on an increased photosynthesis rate of the plants [36].

Instantaneous water use efficiency (WUE) of fig seedlings was decreased under elevated agrosol except at agrosol 2 kg/ha (Figure **14**). Increasing agrosol concentration up to 3 kg/ha had demonstrated substantial increase in WUE until 2 MAT and decreased for next observations. The deterioration of WUE in elevated plants was due to increase in A alone rather than reduction in E [37, 38]. Zhang *et al*. [39]

attributed the reduction of WUE to high turgor pressure in plants enriched with agrosol, thus explaining the increased rates of leaf expansion under elevated agrosol.

Brassinolide-treatment alone increased chlorophyll fluorescence (12.86%). The BL-induced improvement in chlorophyll fluorescence might be caused by enhancement in leaf-water balance as indicated by increased water potential [40] and increased content of chlorophyll and higher leaf area in BL-treated plants [11].

### 3.3. Correlation Analysis

Correlation analysis was conducted to find the correlation between the parameters. Figure **3** shows that a significant positive inter-correlation among parameters such as total dry biomass, total leaf area and content of chlorophyll. Increase in total dry biomass, total leaf area and chlorophyll content was associated with an increment in plant height, total leaf area and total dry biomass with an r value of 67.58%, 54.87%, 3.97%, 59.67%, 80.44%, 55.92% and 81.40%, respectively.

Significant negative correlation was noted between shoot-to-root-ratio with plant height; stomatal conductance with plant height; shoot to root ratio with total leaf area; stomatal conductance with total leaf area; stomatal conductance with total dry biomass; shoot-to-root-ratio with total dry biomass; net assimilation rate with specific leaf area and relative growth rate with net assimilation rate. Increase in shoot-to-root-ratio, stomatal conductance, net assimilation rate and relative growth rate was associated with a decrement in plant height, total leaf area, total dry biomass, specific leaf area and net assimilation rate with an r value of 38.53%, 51.98%, 27.93%, 39,18%, 32.65%, 42.98%, 26.27% and 31.00%, respectively.

## 4. CONCLUSIONS

Agrosol and brassinolide applications had brought notable changes in growth and physiology of fig. Though increasing agrosol (control, 2, 2.5 and 3 kg/ha) and BL concentration (control and 200 ml/L) caused some differences in fig growth and their physiology, but the differences were not consistent and most of the changes happened only in first or fourth month. Agrosol 2 kg/ha and brassinolide 200 ml/L showed higher growth and physiology than the other concentrations after receiving agrosol and brassinolide treatment. There wasn’t an effective treatment of brassinolide alone but it had a significant effect when combining with agrosol application on growth and physiology of fig except in chlorophyll fluorescence. Treatment of interaction between agrosol and brassinolide was the most affected on fig. Treatment of agrosol alone was affected on few parameter on fig.

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