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Unravelling Synergistic Effects of Palm Bunch Ash and Glutathione on Plant Growth †

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Abstract: Palm bunch ash (PBA), a waste biomass from the palm oil industry, has been widely regarded as an alternative source of fertilizer to improve soil health, plant growth, and yield. Glutathione (GSH), a bioactive tripeptide with potent antioxidant properties, has been proposed as a plant growth regulator that improves stress tolerance in plants. However, the use of PBA in combination with GSH has yet to be explored and remains a gap in the literature. Herein, we aimed to assess the individual and the combinatory effects of PBA and GSH on vegetative plant growth, whereby okra was selected as the model plant and cultivated under well-watered, outdoor conditions. Plant growth parameters such as plant height, stem girth, number of leaves, and leaf surface area were measured over a period of two months. The results showed that the application of PBA and GSH significantly influenced the plant growth parameters. The GSH-treated group recorded the tallest plant height (47.19 cm) as compared to the control group, PBA-treated group, and combination group of PBA and GSH. The combination group of PBA and GSH recorded the best parameters in terms of stem girth (4.45 mm), number of leaves (6.35), leaf surface area (118.38 cm^2) with improved resistance towards diseases. These results implied that the combined application of PBA and GSH may have led to a synergistic effect on okra plant growth. Our findings suggest that the combined application of PBA and GSH is indeed recommended to improve plant growth and development.

Keywords: Palm bunch ash; glutathione; fertilizer; plant growth regulator

1. Introduction

Palm bunch ash (PBA) has become increasingly popular as an organic fertilizer for various plant crops, especially for countries heavily involved in cultivation of oil palms such as Indonesia, Malaysia, and Nigeria [1,2]. PBA is produced as a result of incinerated empty fruit bunches (EFB), which is deemed one of the major waste products of oil palm cultivation [3]. PBA have pH values of more than 7 and are rich in potassium, along with varying amount of nutrients such as phosphorous, calcium and magnesium [3]. These properties allow PBA to reduce the acidity and improve the soil nutrient levels, which result in improvement of vegetative growth and the yield of several plant crops such as maize, ginger, and okra [2,4,5].

Looking beyond soil conditions, abiotic stresses such as drought, salinity, and metal toxicity still pose a threat during the growth of plants. Abiotic stresses typically cause an excess production of reactive oxygen species (ROS), which increase the likelihood of enzyme inhibition, and eventually damage to the plant cells [6]. In response, the antioxidant defense system regulates the ROS in plants, protecting them from abiotic stresses. Among the non-enzymatic antioxidants, glutathione (GSH) is proven to be one of the most abundant water-soluble thiol compounds found throughout plant tissues [7]. The biosynthesis of GSH is usually induced by abiotic stresses but could be possibly inhibited under extreme stress conditions. Hence, recent studies have been focused on the exogenous application of GSH on plants via foliar spray or seed soaking, to overcome the deficiency of endogenous GSH during extreme stresses, and this has proven successful [6–10]. Under stressed conditions, exogenous GSH play key roles in increasing the reduced antioxidant levels, enzymatic activities, and photosynthetic activities, effectively reducing the oxidative damage, and alleviating any toxicity imposed on the plants [7,11]. Evidently, exogenous application of GSH have been proven to increase the stress tolerance of plants that ultimately led to improved growth and development of plants.

In view of the benefits that might be conferred by PBA and GSH on plants, there has yet to be a systematic assessment done on their combinatory effects on the vegetative growth of plants. Hence, this study hypothesizes that the co-treatment of PBA and GSH could potentially result in synergistic effects on plant growth. Okra (*Abelmoschus esculentus* (L.) Moench) plants were chosen to be assessed in this study due to their suitability to be grown in tropical regions like Malaysia and relatively shorter maturity period of 60–70 days as compared to other crops [12,13]. A comprehensive design of experimental groups was employed for PBA and GSH application, with both in individual and combined treatments on the vegetative growth of okra plants, observed through parameters such as plant height, stem girth, number of leaves, and leaf surface area.

2. Methods

The experiment was conducted in Penang, Malaysia, from March 2021 to July 2021. Plastic pots (25 cm × 20 cm) were used to plant the sterilized okra seeds that were subjected to 4 different treatments, namely: (A) Control group; (B) PBA group; (C) GSH group; (D) PBA-GSH group. For the control group, 6 water-soaked okra seeds were sown into a pot of black soil (3 kg). For the PBA group, 6 water-soaked okra seeds were sown into a pot of PBA-soil mix (200 g:3 kg). For the GSH group, 6 GSH-soaked okra seeds were sown into a pot of black soil (3 kg). For the PBA-GSH group, 6 GSH-soaked okra seeds were sown into a pot of PBA-soil mix (200 g:3 kg). Each treatment group consisted of 4 replicate pots where 6 pre-treated okra seeds were sown at 2 cm depth and equally distanced from one another in each pot, respectively. All pots were watered daily throughout the different growth stages.

Soil pH and nitrogen-phosphorus-potassium (NPK) tests were performed to analyze the new black soil, PBA and spent soil from each treatment group at day 35. Soil samples were added into test tubes, followed by their respective test reagents, gently shaken, and left to settle. The color obtained was compared against the respective color charts to obtain the pH and NPK nutrient level readings. Data was recorded based on various growth-related parameters of the okra plants such as plant height, stem girth, number of leaves per plant, area of leaves, and visual assessment. Measuring tape and digital vernier calipers were used to measure shoots and girth of every plant and recorded as mean values for each group weekly. The number of leaves on each plant in every pot was counted and recorded as mean values on a weekly basis. For the area of leaves, 6 leaves were randomly selected from each pot where the widths and lengths of each leaf were measured with measuring tape, calculated using Equation (1) in the below, and recorded as mean leaf area. For visual assessment, observable plant conditions such as chlorosis, necrosis, or wilting were noted and recorded. The following shows the equation used to calculate area of okra leaves:

$$\text{Area of okra leaves} = \text{Lamina length} \times \text{Maximum width} \times k, \quad (1)$$

where k is the coefficient using ratios of linear measurement to graph determination, which was found to be 0.75 for okra plants [14].

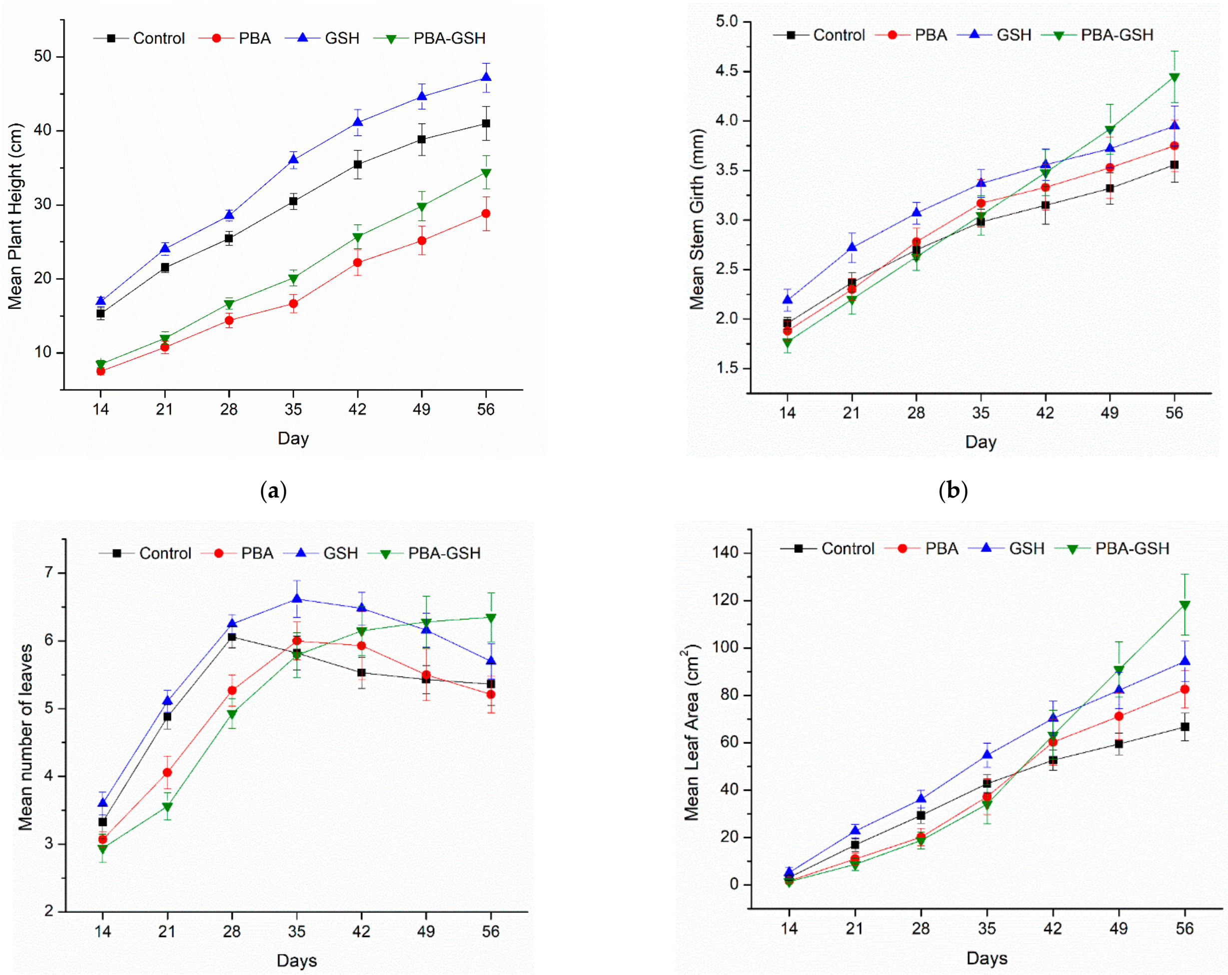
The data obtained were recorded as mean values and analyzed with one-way Analysis of Variance (ANOVA) test and Least Significance Difference (LSD) test to determine the significance of differences between the treatment groups at $p \leq 0.05$. Both tests were conducted with IBM SPSS Statistics software v26.0 (IBM corp, Armonk, NY, USA) for all parameters.

3. Results and Discussion

The pH of pure black soil and PBA-black soil mix at day 35 remained the same as day 0 at pH 6.5 and 7, respectively, lying between the suitable range of pH of okra plants. Phosphorus levels were sufficient for all soil samples, but nitrogen levels were found to be deficient for all soil samples. Potassium levels were adequate for black soil samples including control and GSH group soil samples, whereas potassium levels were in surplus for PBA-soil mix samples including PBA and PBA-GSH groups.

From Figure 1a, a consistent trend was observed where the highest mean height of the okra plant was recorded from the GSH group significantly, followed by the control group, PBA-GSH group, and PBA group throughout the experiment. The application of GSH improved shoot height significantly due to an increase in various enzymatic activities and improvement in total photosynthetic pigment [8,11]. Notably, the mean height recorded from the PBA and PBA-GSH groups were significantly lower than the control and GSH groups. This is possibly due to the surplus in potassium as recorded from the soil test. The interaction of potassium and other macronutrients, especially nitrogen, is essential to maintain efficient nutrient transport and promote shoot growth of plants [15–17]. Hence, the surplus of potassium may have caused a reduction in nitrogen-metabolizing enzymatic activities, decreasing nitrogen uptake, which led to an overall shorter plant height [2,18].

From day 14 till day 42, a similar trend was observed for mean stem girth, mean number of leaves per plant, and mean leaf area, where the GSH group recorded the best-improved parameters followed by the control group, PBA group, and PBA-GSH group as shown in Figure 1b–d, respectively. This may be best explained by the role of GSH in increasing total photosynthetic pigments by up-regulation of gene expressions for photosynthesis. The light energy is captured by chlorophyll, found in chloroplasts, that are highly concentrated in the leaves of a plant and the outer parts of stems [19]. Hence, the application of GSH that increased the net photosynthetic rate correlates to the positive impacts on mean stem girth, number of leaves per plant, and area of leaves.



(**a**) (**b**)

(**c**) (**d**)

Figure 1. Graphs of plant growth parameters from different treatment groups with standard errors (n = 12) from day 14 till day 56. (**a**) Mean plant height; (**b**) Mean stem girth; (**c**) Mean number of leaves per plant; (**d**) Mean leaf surface area.

On day 35, the number of leaves in the control group decreased significantly due to several conditions observed through visual assessment, such as white spots as shown in Figure 2a and chlorosis. The white spots are indicative of powdery mildew, a fungal disease that favors hot and humid weathers [12]. The main cause of chlorosis is the lack of chlorophyll, which is often related to a deficiency in nutrients like magnesium and nitrogen as recorded from the soil test [20–22]. Chlorosis is often followed by wilting of the leaves, leading to a significant decrease in the mean number of leaves in the control group. Plants in the other groups were less susceptible to the fungal disease due to sufficient nutrients supplemented by PBA and the improvement in resistance of GSH.

From day 42 onwards, both PBA and PBA-GSH groups surpassed the mean stem girth and mean leaf area compared to the control group as shown in Figure 1b,d. As opposed to the adverse effect in plant height, the surplus of potassium resulted in a positive effect on the stem girth due to the significantly lower potassium accumulation in stems among the other organs in the plant [16]. The presence of sufficient potassium in the stem cell differentiation area increased cell elongation, leading to the increase in stem girth [23]. Similarly, the accumulation of potassium in the leaves effectively contributed to an increased rate of photosynthesis and gas exchange rate in leaves, which led to an increase in cell expansion in the leaves, increasing the area of leaves [16,24].

(**a**) (**b**) (**c**) (**d**)

Figure 2. Pictures of leaf conditions observed from different treatment groups. (**a**) White spots on leaves or *Powdery mildew* from control group; (**b**) Whitefly eggs spotted behind one of the curled leaves from PBA group; (**c**) Big green leaves with minimal white spots in some leaves from GSH group; (**d**) Bigger and darker green leaves from PBA-GSH group.

Unlike the other growth parameters, the mean number of leaves recorded an overall decrease after day 42 from all groups except the PBA-GSH group. The mean number of leaves from the PBA group decreased significantly, mainly due to the attack of whiteflies, which was observed through the white eggs behind several leaves of the PBA group as shown in Figure 2b. Notably, some leaves were curled up, crinkly, and had a shrunken leaf area. This observation fits the symptoms of the okra enation leaf curl virus caused by a virus named *Begomovirus* and transmitted via vectors like whiteflies [25]. This disease will essentially lead to decreased number of leaves, smaller leaf area, and even reduced okra yield. Interestingly, the leaves from GSH and PBA-GSH group were less susceptible to the attacks as seen in Figure 2c,d.

From day 49 onwards, the PBA-GSH group surpassed the GSH group and recorded the best results in terms of mean steam girth, the mean number of leaves, and mean leaf area. PBA fulfilled the increasing demand for potassium intake due to the increased rate of transpiration, and stomatal conductance, while the application of GSH enhanced the increase in photosynthetic activities [26]. Furthermore, the plants from the PBA-GSH group had healthier leaves with a darker shade of green as compared to leaves from the GSH and the control group as shown in Figure 2d. This is because the PBA-GSH application resulted in better resistance to diseases and enhanced chlorophyll b, an accessory pigment responsible for reflecting the yellow-green color in the leaves [27,28].

4. Conclusions

This study showed that the combined application of PBA-GSH brings about the best improvement in terms of the mean stem girth, the mean number of leaves per plant, the mean leaf area, and the resistance of plants to diseases. The synergistic effects of the combined PBA-GSH applications were increasingly evident in the later growth stages as the PBA fulfilled the increasing demand of nutrients of the plants, coupled with the enhancement of GSH in increasing photosynthetic activities in the plants. For future works, encapsulation could be proposed to produce controlled release for the application of PBA and GSH to cater to the varying demands of supplements or nutrients at different plant growth stages. In short, the study suggests that the combined application of PBA and GSH is indeed the best treatment for the vegetative growth and development of okra plants.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/IOCAG2022-12192/s1.

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