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Effects of Foliar Application of Oil Palm Empty Fruit Bunch Ash Nanoparticles on Stomatal Anatomy of Potato Leaf Plants (*Solanum tuberosum* L.)

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ABSTRACT

The productivity of potatoes (*Solanum tuberosum* L.) in Indonesia is still low. Fertilization needs to be done to increase potato productivity. This study aimed to examine the effects of applying oil palm empty fruit bunch (OPEFB) ash nanoparticles on the anatomy of potato stomatal and leaf cells (*Solanum tuberosum* L.). The research was carried out from March to June 2021 in the Sumberejo Village, Ngablak District, Magelang Regency, Central Java, and at the Agrobiotechnology Laboratory, Faculty of Agriculture, University of Muhammadiyah Yogyakarta. The study used a single-factor treatment design with a Randomized Completely Block Design (RCBD). The treatments tested included the foliar application of OPEFB ash nanoparticles at several concentration, consisting of 0% (control), 0.1%, 0.2%, 0.3%, and 0.4%. The results showed that foliar application of nanoparticles OPEFB ash affected stomatal anatomy, namely guard cell width, stomatal aperture, and density. The application of OPEFB ash nanoparticles with a concentration of 0.3% was most effective in increasing the opening of stomata because it affects the activity of the photosynthetic process.

Keywords: Nano fertilizer, Oil palm empty fruit bunch ash, Potassium, Stomata

ABSTRAK

Tingkat produktivitas tanaman kentang (Solanum tuberosum L.) di Indonesia masih rendah. Pemupukan perlu dilakukan untuk meningkatkan produktivitas kentang. Penelitian ini bertujuan untuk menguji pengaruh penyemprotan nano partikel abu tandan kosong kelapa sawit (TKKS) terhadap anatomi stomatal dan sel daun tanaman kentang (Solanum Tuberosum L.). Penelitian dilaksanakan pada bulan Maret hingga Juni 2021 di lahan desa Sumberejo, kecamatan Ngablak, kabupaten Magelang, Jawa Tengah dan di Laboratorium Agrobioteknologi Fakultas Pertanian, Universitas Muhammadiyah Yogyakarta. Penelitian menggunakan Rancangan Acak Kelompok Lengkap (RAKL) faktor tungga terdiri dari 5 perlakuanl. Perlakuan yang diuji meliputi penyemprotan foliar partikel nano abu TKKS dengan konsentrasi 0% (kontrol); nano TKKS konsentrasi 0,1%; konsentrasi 0,2%; konsentrasi 0,3%; konsentrasi 0,4%. Hasil penelitian menunjukkan bahwa aplikasi foliar partikel abu TKKS berpengaruh terhadap anatomi stomata yaitu lebar sel penjaga, bukaan stomata dan kerapatan stomata. Aplikasi partikel nano abu TKKS dengan konsentrasi 0,3% paling efektif dalam pembukaan stomata yang mempengaruhi proses fotosintesis.

Kata kunci: *Pupuk nano, Abu tandan kosong kelapa sawit, Kalium, Stomata*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the important food ingredients for humans and the main vegetable crop, after rice, wheat, and corn. In Indonesia, potato plants have become one of the priority foods to be developed as a source of carbohydrates to support food diversification. The demand for potatoes is increasing yearly, along with changes in lifestyle and the development of the potato processing industry (Isra, 2020). However, potato production in Indonesia has not been able to meet the demand for potatoes due to the increasing demand for potatoes. In contrast, potato production in Indonesia fluctuates from year to year. National potato production in 2019 (1.31 million tons/ha) has increased compared to 2018 (1.28 million tons/ha). However, potato production in 2020 decreased by 1.28 million tons/ha (Badan Pusat Statistik, 2020). The decrease in production will impact potato productivity, which is also low. One factor that leads to low potato production is

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the lack of nutrients that potato plants need.

Potato production is greatly affected by nitrogen, phosphorus, and potassium nutrients. The major functions of K in plants are controlling enzyme activity, cation-anion homeostasis, and membrane polarization. These are based on its osmotic nature, which is why it is needed for cell extension, turgor regulation, and stomatal movement (Warnita, 2019). One important role of K in the stomatal functions is stimulating enzyme to starch synthase for starch synthesis. Potassium also plays a role in the the stomatal aperture to meet the needs of CO_2 and water vapor for photosynthesis and in the stomatal closing to prevent excessive water loss from plant tissues. Suppose the function of the stomatal guard cells is not optimal. In that case, drought stress can occur in plants because K-deficient plants can significantly reduce the net CO_2 assimilation rate (Naumann et al., 2020).

The type of stomata of potato leaves belongs to the Amphistomatic type, which is located on both leaf surfaces, but stomata are mostly found on the underside of the leaves (abaxial). The anatomical structure of stomata is closely related to the ontogeny of the epidermis or the type of epidermis because stomata are from the modification of some of the epidermal cells. There are several factors that affect the opening and closing of stomata, including sunlight, potassium, availability of CO_2, water and temperature (Driesen et al., 2020). If these components are met, it will affect plant physiological processes such as transpiration, photosynthesis, and respiration that occur in leaf stomata.

The need for potassium (K) in potato plants can be fulfilled by the application of inorganic (synthetic) fertilizers or organic fertilizers (fertilizers derived from organic waste). One of the organic wastes that can be used as a potassium (K) fertilizer source is oil palm empty fruit bunch ash. According to the results of research by Efendi et al., (2020), OPEFB ash contains nutrients such as total N of 0.05%, P_2O of 54.79%, K_2O of 36.48%, MgO of 2.63%, CaO of 5.46%, Mn of 1,230 ppm, Fe of 3450 ppm, Cu of 183 ppm, and Zn of 28 ppm, with pH ranging from 11.9 to 12.0. Based on the research by Azizah (2019), the application of OPEFB ash nanoparticles with a concentration of 0.4% increased the shallots' productivity.

The fertilization for potato plants can be done through the soil or the leaves (foliar application). Leaf fertilization will be effective if the particle size of the fertilizer material is smaller than the leaf stomatal pore size. The effective absorption of nutrients in OPEFB ash through the leaves requires technological innovation by reducing particle size through nano-fication.

Nanotechnology is a technique for creating materials, functional structures, and devices at the nanometer scale. Fertilizers with -size has properties and abilities far superior to the starting material, such as being easily absorbed by plants with slow-release fertilizers (Ratih et al., 2021). For this reason, nanoparticles of OPEFB ash are needed as a source of potassium fertilizer that can be used to meet the K needs in the stomata of potato plants. In addition, research on the application of OPEFB ash nanoparticles on potato plants has never been carried out. Thus, this study aimed to determine the effects of foliar application of OPEFB ash nanoparticles on the stomatal anatomy of potato plants.

MATERIALS AND METHODS

Study area

Field research was carried out in Sumberejo Village, Ngablak Sub-district, Magelang District, Central Java, with coordinates of -7.4018090 LS 110.3908880 east longitude starting from March to June 2021. Observations were made in the field and in the Agrobiotechnology Laboratory, Faculty of Agriculture, University of Muhammadiyah Yogyakarta.

Experimental design

Experimental research was conducted with a single factor treatment arranged in a Randomized Completely Block Design (RCBD), consisting of five concentrations of OPEFB nanoparticles (0% (control), 0.1%, 0.2%, 0.3%, and 0.4%. Each treatment consisted of five replications, in which there were 25 plants in each unit. Thus, there were 125 potato plants. Each experimental unit contained a physiological plot consisting of three physiological plants. OPEFB ash nanoparticles were applied 20 days after planting, and the next application was carried out once every 10 days.

The data collected include stomatal length, stomatal guard cell width, stomatal aperture, stomatal density, cell wall thickness, and leaf cell area. Observations were made at each phase of potato plant growth at 40, 65, and 75 days after planting. Sampling was carried out directly in the sun without picking the leaves to keep the stomatal cells open using the replica method. As for the cross-section of the leaf, thin slices were made with a transverse direction in the thickness of the leaf, and the incisions were observed through an Olympus CX-22LEDRFS1 computer microscope with a magnification of 400x.

Statistical Analysis

The data obtained from this study were analyzed using Statistic Analysis System (SAS) 9.0 applications. Analytical method with Analysis of Variance (ANOVA) significance level of 5%. Means comparison between treatments was tested using Duncan Multiple Range Test (DMRT) at 5%.

RESULTS AND DISCUSSIONS

The results showed that foliar application concentration of OPEFB nanoparticles did not significantly affect the stomatal length during the vegetative phase and tuber ripening phase, but there was a significant difference observed in the tuber initiation phase (Table 1). Further test results showed that foliar application of OPEFB ash nanoparticles at a concentration of 0% produced the longest stomata of 50.78 µm. It was significantly different compared to that in the 0.4% concentration treatment.

Based on these results (table 1), the stomatal length is included in the very long category >25 µm (Makin et al., 2022). Foliar application of OPEFB nanoparticles at a concentration of 0% resulted in the longest stomatal length compared to that at a concentration of 0.4%. OPEFB ash nanoparticles contain potassium, which can maintain cell turgidity and cause guard cells to expand, resulting in the elongation of cellulose microfibrils outward. In the process of stomatal opening and closing, stomatal elongation occurs only in the cellulose microfibrils or cellulose fine fibers contained in the guard cell walls in a radial shape; this arrangement pattern is referred to as radial mycelation. The shape of the pattern allows only the long stretching of the cellulose microfibrils, but the two ends of the guard cells stick together so that when the cellulose microfibrils elongate, the thick abdominal wall limits the stretching. As a result, guard cells will bend and open, which affects the stomatal width instead of the stomatal length (Pautov et al., 2018). The stomatal length is in a fixed state when the stomatal aperture is based on the hardening of the stomatal poles, and polar clamping occurs (Carter et al., 2017). Thus, the stomatal length is related to the stomatal width, where the process becomes a single entity that affects the size of the stomatal porous. The longer cellulose microfibril

Table 1. Stomatal length (μm) as affected by various concentrations of OPEFB ash nanoparticles under different phases

Foliar application concentration	Stomatal Length (μm)		
	Vegetative Phase (40 day)	Bulb Initiation Phase (65 day)	Tubers Ripening Phase (75 day)
0 %	44.66 ± 5.55 a	50.78 ± 8.80 a	47.03 ± 5.66 a
0.1 %	40.40 ± 4.89 a	40.83 ± 3.97 b	42.85 ± 4.09 a
0.2 %	40.34 ± 6.34 a	39.69 ± 3.56 b	42.44 ± 8.70 a
0.3 %	38.76 ± 4.23 a	47.00 ± 7.15 ab	51.63 ± 7.73 a
0.4 %	41.40 ± 6.12 a	40.83 ± 4.20 b	50.08 ± 5.35 a
CV	12.80	11.95	13.93

Remarks: Means followed by the same letters are not significantly different based on the DMRT test at the level of 5%

Table 2. Stomatal aperture (μm) as affected by various concentrations of OPEFB ash nanoparticles under different phases

Foliar application concentration	Aperture Stomatal (μm)		
	Vegetative Phase (40 day)	Bulb Initiation Phase (65 day)	Tubers Ripening Phase (75 day)
0 %	2.86 ± 0.61 a	2.76 ± 0.33 a	2.54 ± 0.41 ab
0.1 %	2.76 ± 0.43 a	2.77 ± 0.59 a	2.23 ± 0.46 b
0.2 %	2.50 ± 0.42 a	2.24 ± 0.59 a	2.37 ± 0.45 b
0.3 %	3.12 ± 0.10 a	2.76 ± 0.48 a	3.03 ± 0.75 a
0.4 %	2.71 ± 0.50 a	2.45 ± 0.65 a	2.82 ± 0.32 ab
CV	15.70	21.58	16.60

Remarks: Means followed by the same letters are not significantly different based on the DMRT test at the level of 5%

will experience withdrawal due to the widening of the guard cells outwards.

There was no significant effect of OPEFB ash nanoparticle concentration on the stomatal length because the higher the concentration of OPEFB nanoparticle ash, the smaller the stomatal length. Lu et al., (2017) reported that K deficiency decreased stomatal length.

Based on Table 2, the concentration of OPEFB ash nanoparticles did not significantly affect the stomatal aperture in the vegetative and tuber initiation phase, but there was significant effect in the tuber ripening phase. Further test results showed that foliar application of OPEFB ash nanoparticles at a concentration of 0.3% had the largest stomatal aperture of 3.03 μm, but it was not significantly different from the stomatal aperture at a concentration of 0%.

Based on the results of the analysis, it was shown that the foliar treatment of OPEFB ash nanoparticles had an effect on the stomatal aperture. This was because the stomatal aperture occurred as a result of activity in guard cells that require potassium to maintain turgor pressure so that the stomatal are open. According to Barita et al., (2018), potassium plays a role in stimulating water absorption, thereby affecting the increase in cell turgor pressure; if the high cell turgor pressure is maintained, the stomata can be maximally open wider and longer. Potassium has a role in the process of opening and closing of stomata, which is influenced by several factors, namely the mechanism of turgor, the presence of osmotic pressure, accumulation of potassium ions, accumulation of abscisic acid, and environmental factors, such as sunlight, temperature, humidity and CO_2 concentration (Ratnasari et al., 2020). The opening of stomata results from activity in the stomatal guard cells, where there are cell organelles

Table 3. Guard cell width (μm) as affected by concentrations of OPEFB ash nanoparticles under different phases

Foliar application concentration	Guard Cell Width (*μ*m)		
	Vegetative Phase (40 day)	Bulb Initiation Phase (65 day)	Tubers Ripening Phase (75 day)
0 %	11.30 ± 1.54 a	10.75 ± 1.86 a	8.03 ± 1.74 b
0.1 %	11.46 ± 1.40 a	9.51 ± 2.04 a	8.79 ± 1.80 b
0.2 %	10.75 ± 2.33 a	9.65 ± 1.27 a	8.97 ± 2.12 b
0.3 %	10.81 ± 1.80 a	10.66 ± 0.57 a	8.26 ± 1.24 b
0.4 %	12.66 ± 1.88 a	9.47 ± 1.74 a	11.26 ± 1.51 a
CV	15.81	15.57	14.65

Remarks: Means followed by the same letters are not significantly different based on the DMRT test at the level of 5%

in these guard cells.

Cell organelles that play an active role in stomatal opening are vacuoles, which play a role in cell turgidity and shape. Stomata will open if the two guard cells experience increased cell turgor pressure. Turgor pressure is a condition where the cell expands because water from the surrounding cells enters it. Water movement is influenced by water potential, in which high water potential will go to cells with lower water potential. For stimulating water to enter the guard cell, the solute in the cell must be increased. According to Abidin (2022), the main solutes that mediate cell osmoregulation are K^+ and sucrose because of their high mobility of K^+ and solubility. The guard cells accumulate large amounts of K+ in the vacuole. Accumulating K+ in the vacuole against the electrochemical gradient (Lu et al., 2017) produces sufficient turgor for stomatal opening.

Foliar application of OPEFB ash nanoparticles at a concentration of 0.3% significantly affected the stomatal pore opening of potato plants. Likewise, according to Lu et al., (2017, an increase in potassium concentration to 0.12% showed a significant effect on the stomatal opening of *Brassica napus* leaves.

Foliar application of OPEFB ash nanoparticles did not significantly affect the width of guard cells in the vegetative and tuber initiation phase, but a significant effect was observed in the tuber ripening phase (Table 3). Further test results showed that foliar application of OPEFB ash nanoparticles at a concentration of 0.4% had the highest guard cell width of 11.2660 μm compared to other OPEFB ash nanoparticle concentrations.

The results showed that the foliar application of OPEFB ash nanoparticles affected the width of the guard cells. This is because the content of OPEFB ash nanoparticles in the form of potassium can maintain the turgidity of the vacuole cells in the guard cells, where the guard cells can change shape and size, which is reversible. The mechanism of guard cell dilation occurs due to changes or regulation of turgor, which is influenced by the theory of K ion movement or pump. The leaves absorb potassium by diffusion through ion exchange. According to Jasmi (2018), the main function of K is to activate enzymes and maintain cell water. K^+ ions support the activity of phosphorylase enzymes, which play a role in converting starch into glucose. Glucose plays a role in the osmotic potential of cells, which will move water to guard cells. As a result, the turgor pressure of the guard cells increases, and the stomata open by dilating the guard cells. Thus, when K^+ ions increase in guard cells, the activity of converting starch to glucose also increases (Advinda, 2018). Guard cells will also increase the osmotic potential of their cells, thereby increasing

Table 4. Stomatal density (mm^{-2}) as affected by various concentrations of OPEFB ash nanoparticles under different phases

Foliar application concentration	Stomatal density (mm^{-2})		
	Vegetative Phase (40 day)	Bulb Initiation Phase (65 day)	Tubers Ripening Phase (75 day)
0 %	486.8 ± 97.31a	308.89 ± 44.02 b	411.11 ± 82.03 a
0.1 %	442.2 ± 89.72a	331.11 ± 69.12 ab	468.89 ± 70.89 a
0.2 %	431.2 ± 187.81a	428.89 ± 63.64 a	382.22 ± 94.80 a
0.3 %	419.8 ± 136.40a	362.22 ± 64.12 ab	364.44 ± 101.95 a
0.4 %	406.8 ± 150.43a	426.66 ± 90.13 a	326.66 ± 51.88 a
CV	5.61	18.61	21.95

Remarks: Means followed by the same letters are not significantly different based on the DMRT test at the level of 5%

Table 5. Cell wall thickness (μm) as affected by various concentrations of OPEFB ash nanoparticles under different phases

Foliar application concentration	Leaf Cell Wall Thickness (μm)		
	Vegetative Phase (40 day)	Bulb Initiation Phase (65 day)	Tubers Ripening Phase (75 day)
0 %	5.23 ± 0.38 a	5.54 ± 0.06 a	4.98 ± 0.51 a
0.1 %	5.17 ± 0.21 a	5.33 ± 0.32 a	5.04 ± 0.32 a
0.2 %	5.19 ± 0.31 a	5.47 ± 0.10 a	5.32 ± 0.15 a
0.3 %	5.11 ± 0.12 a	5.37 ± 0.28 a	5.41 ± 0.16 a
0.4 %	5.53 ± 0.62 a	5.63 ± 0.32 a	5.50 ± 0.13 a
CV	7.54	4.82	5.77

Remarks: Means followed by the same letters are not significantly different based on the DMRT test at the level of 5%

turgor pressure and forming guard cell dilation.

This change in the shape of the guard cells occurs because the back cell wall is thin and elastic, protruding away from the opening, while the front cell wall will be straight or concave, the entire cell will appear bent, and openings with an increased size are formed (Roux & Leonhardt, 2018). When the guard cells widen, the metabolic activity in the cell will also be easy with the accumulation of ions or materials needed in the process. Thus, this mechanism proves that it can affect cell activities such as photosynthesis, respiration, transpiration, and another cell metabolism.

Foliar application of OPEFB ash nanoparticles at a concentration of 0.4% significantly affected the width of the stomatal guard cells in potato leaves. Research by Lu et al., (2017) showed that the application of potassium fertilizer significantly affected the width of the stomatal guard cells on *Brassica napus* leaves.

In table 4, the foliar application of OPEFB ash nanoparticles has not a significant effect on the stomatal density in vegetative and ripening phases of tubers, but a significant effect was found in tuber initiation phase. Further test results showed that the foliar application of OPEFB ash nanoparticles at a concentration of 0.2% had the highest stomatal density of 428.89 mm^{-2}, but was not significantly different compared to that at the concentrations of 0.4%, 0.3% and 0.1 %. Meanwhile, the 0.4% concentration differed significantly from the 0% concentration treatment.

Based on the results, concentration of 0% and 0.4% had significantly different results, and the

Table 6. Leaf cell area (μm^2) as affected by various concentrations of OPEFB ash nanoparticles under different phases

Foliar application concentration	Leaf Cell Area (μm^2)		
	Vegetative Phase (40 day)	Bulb Initiation Phase (65 day)	Tubers Ripening Phase (75 day)
0 %	3.05 ± 0.15 a	3.25 ± 0.08 a	3.22 ± 0.41 a
0.1 %	3.32 ± 0.16 a	3.30 ± 0.18 a	3.18 ± 0.21 a
0.2 %	3.32 ± 0.09 a	3.68 ± 0.82 a	3.27 ± 0.26 a
0.3 %	3.36 ± 0.26 a	3.44 ± 0.14 a	3.05 ± 0.32 a
0.4 %	3.15 ± 0.09 a	3.11 ± 0.23 a	3.29 ± 0.23 a
CV	5.33	12.27	7.91

Remarks: Means followed by the same letters are not significantly different based on the DMRT test at the level of 5%

average stomatal density was classified as moderate, which was in the range of 300 – 500/mm^2 (Claudia et al., 2020). This is because in general, stomatal density is related to stomatal size. High potassium concentrations can widen stomata and cause stomatal density to be quite high. According to Sihotang (2017), if the size of the stomatal is larger, the distance between the stomatal gets further by 20 times its diameter so that the evaporation process can take place optimally. This is evidenced by the width of the stomatal guard cells resulting from the foliar application of OPEFB ash nanoparticles at a concentration of 0.4%, showing the highest value compared to other concentrations (Table 4). Stomatal density affects two important processes in plants, namely transpiration and photosynthesis. Plants with high stomatal density have a higher transpiration rate than plants with low density. Because more stomata per unit area mean more CO_2 can be taken in and more water can be released. (Mercyana et al., 2021). Foliar application of OPEFB ash nanoparticles at various concentrations of potassium significantly affected the stomatal density of potato plants. Pratama et al., (2020) reported that the application of potassium fertilizer at a concentration of 0.3% significantly affected the stomatal density of oil palm plants experiencing drought stress.

Foliar application of OPEFB ash nanoparticles had no significant effect on cell wall thickness (Table 5). Based on the analysis results, the responses of leaf cells to the foliar application of OPEFB ash nanoparticles were not significantly different. This result is because potassium only affects the activity of the phosphorylase enzyme in stomatal guard cells that are not related to leaf cells. However, Widiyawati (2019) mentioned that thickening of the epidermal tissue was a structural defense response of plants against pathogen attacks. Epidermal cells are the outermost cell network as a place of penetration of pathogens. Structural defenses when attacked by pathogens include thickened epidermal cell structures that affect the stomatal surface and thickened cell walls to inhibit pathogen penetration so that pathogens do not damage deeper cell layers. However, in this case, the foliar application of OPEFB ash nanoparticles did not affect the cell walls' thickness, so pathogens would easily attack potato plants due to lack of protection. The results also showed that the foliar application of OPEFB ash nanoparticles on potato plants showed no significant effect on the thickness of the leaf epidermal cells. Likewise, research of Lu et al., (2016) reported that cell wall thickness was not affected by K nutrition.

Based on Table 6, foliar application of OPEFB ash nanoparticles had no significant effect on potato leaf cell area. This is because the leaf cells had enough water when taking sample in the morning. According to Saragih dan Ardian (2017), the content of OPEFB in the form of potassium can affect the optimal leaf cell area if the condition of the plant lacks water. Cell enlargement will also be hampered due to a decrease in the rate of photosynthesis because in these conditions, there is a decrease in the availability of nutrients, inhibition of protein synthesis so that the leaf area also decreases. Potassium will play a role in regulating the availability of sufficient water for cell enlargement. Enlargement of leaf cells becomes inhibited if the water content is low due to the need for turgor pressure for cell enlargement. The results of photosynthesis support the work of plant tissue cells in differentiation so that it will accelerate the growth and development of the plant, forming parts such as leaves. But in this case, the high potassium treatment did not affect the leaf area of potato plants. Foliar application of OPEFB ash nanoparticles on potato plants did not significantly affect the leaf cell area. However, Lu et al., (2016) reported that the leaf area was significantly down-regulated under K deficiency conditions.

CONCLUSIONS

Based on this research, it can be concluded that foliar application of oil palm empty fruit bunch ash nanoparticles on potato plants can affect stomatal anatomy, including the width of stomatal guard cells, stomatal opening (aperture), and stomatal density. Meanwhile, the application did not affect the anatomy of potato plant leaf cells. Also, foliar application of oil palm empty fruit bunch ash nanoparticles at a concentration of 0.3% is the most effective in increasing the stomatal aperture because it affects the activity of the photosynthetic process.

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