Prosiding Seminar Nasional Biologi Tropika 2018, Gajah Mada University, Yogyakarta 28 July 2018
"Pemanfaatan biodiversitas tropika untuk mewujudkan *bio-based economy*"
http://doaj.org/toc/2540-9581
ISBN: 902896-61-0

EMPTY FRUIT BUNCHES AS A POTENTIAL SOURCE FOR BIOSILICA FERTILIZER FOR OIL PALM

Laksmita Prima Santi, Donny Nugroho Kalbuadi, and Didiek Hadjar Goenadi

Indonesian Research Institute for Biotechnology and Bioindustry
PT Riset Perkebunan Nusantara
Bogor, Indonesia
Correspondence: laksmita.santi@gmail.com

Abstract

The development of oil palm plantations in Indonesia has been going on very extensively, covered about 14.03 million hectares in 2017. This means it generated a huge amount of biomass per year in the form of both solid and liquid wastes. The processing of fresh fruit bunches (FFB) in palm oil mill (POM) produces wastes that primarily empty fruit bunches (EFB) amounting up to 25% (w/w) of FFB. It has been indicative that EFB contains a considerable amount of silica (Si) so then the Indonesian Research Institute for Biotechnology and Bioindustry (IRIBB) has investigated the potential use of EFB as source for bio-available Si, in the form of H_4SiO_4 (mono silicic acid, BioSilAc). Experiment was carried out at Sungai Mirah Minting Estate, PT Bumitama Gunajaya Agro-Central Kalimantan. EFB materials were obtained from POM and chopped into 2.5-5.0 cm in length size. A four-week bio-decomposition process was employed by using a bio-decomposer containing *Trichoderma pseudokoningii*, *T. polysporum*, and *Phanerochaete chrysosporium*. Chemical analyses of composted EFB were conducted before and 28-days after decomposer application. The presence of Si in the compost was observed by scanning electron microscopy (SEM). Effect of Si-containing EFB compost on the immature and mature of oil palm was evaluated. Seven treatments, i.e. combination of EFB compost and BioSilAc application with reduced dosages of NPK fertilizers, were arranged in a random block design with three replicates. The results show that large quantities of silica bodies attached to the surface of EFB fibers and amounting to 0.44% soluble Si. The FFB data indicated that application of 75% NPK + 500 kg composted EFB + 2 L BioSilAc/ha/year on a five-year old plant resulted in higher yield than that obtained from 100% standard dosage of NPK. The study also revealed that the application of EFB compost reduced 50% of BioSilAc dosage.

Keywords: bio silica, silica body, empty fruit bunch, bio decomposition, mono silicic acid.

Introduction

Indonesia has been placed as the world's first producer of palm kernel and crude palm oils. Oil palm industry is dependent on the processing of the fruit to obtain results that are traded internationally. In practice, the processing of fresh fruit bunches (FFB) at palm oil mill (POM) in producing crude palm oil (CPO) and palm kernel oil (PKO) also produces waste that is primarily in the form of solid organic waste [i.e. empty fruit bunch (EFB)] with the volume reaches 25% of FFB. Therefore, if Indonesia's production in 2017 reached 38 million tons of CPO or the equivalent of 190 million tons of FFB (CPO yield 20% of FFB), the potential availability of EFB will be more than 47 million tons of EFB per year. In addition, the nonsolid biomass, i.e. palm oil mill effluent (POME) discharged from washing and sterilization of the palm fruits, is available in huge quantity.

Oil palm EFB fiber has been identified as the single most important agriculture biomass in Indonesia. The EFB remaining after the processing usually contains 30–35 % lignocellulose, 1–3 % residue oil, and roughly 60 % of moisture (Gunawan *et al*. 2009). Its lignocellulose or fiber consists of cellulose, hemicellulose, and lignin. Many researches on EFB had been focused on energy, biochemicals, and wood-related products development purposes (Geng, 2013) as well as for compost (Goenadi, 2006). Therefore, this material could become part of the important portfolio to sustain the development and growth of energy, biochemicals, industrial materials, and source of available nutrients for plant. It has been indicative to that EFB contains a considerable amount of silica (Si) so then the Indonesian Research Institute for Biotechnology and Bioindustry (IRIBB) has investigated the potential use of EFB as source for bio-available Si, in the form of H_4SiO_4 (mono silicic acid) (Santi *et al*. 2017). They have developed a bio-available Si product derived from quartz sand enriched with Si-solubilizing microbes (BioSilAc).

Silica (Si) is the second most abundant element in soils and can be found in noticeable concentration in many terrestrial plants (Epstein, 1994; Keeping & Reynolds, 2009). Plant species vary in their ability to take up and accumulate Si as silicon dioxide (SiO_2) in their tissues. Depending on this characteristic, plants are classified as excluders, intermediate types, or accumulators (Mitani & Ma, 2005; Montpetit *et al.* 2012). Most dicots accumulate less than 0.1% Si on a dry weight basis, but many grass species are able to accumulate as much as

10% (Montpetit *et al.* 2012; Ma *et al.* 2002; Vivancos *et al.* 2015). It has been widely reported that Si is able to suppress both physical stress, such as drought, high temperature, UV, loading, and freezing, and chemical stress, including salinity, nutrient imbalance, and metal toxicity (Ma, 2004; Ma & Yamaji 2015). Silica has not been recognized as an essential element, although numerous studies have clearly demonstrated that Si is beneficial for plant growth and development, especially under a wide range of a biotic stress conditions (Sanglard 2016; Shi *et al.* 2013; Yin *et al*. 2014). Si deposition occurs mainly as phytoliths (SiO2_nH2O) (Ye *et al.* 2013). It acts as a physical barrier and thus improves plant resistance to pathogens and insects. Najihah and colleagues observed that the accumulation of Si in epidermal and endodermal cell walls protected oil palm roots from the penetration of the fungus *Ganoderma boninense* (Najihah *et al.* 2015). The objective of our study reported here is to determine the potential use of composted EFB as a source for Si available to oil palm.

Material and Method

1. *Composting Technology*

Experiment was carried out at Sungai Mirah Minting Estate, PT Bumitama Gunajaya Agro-Central Kalimantan. EFB materials were obtained from POM and chopped into 2.5-5.0 cm in length size. A four-week bio-decomposition process was employed by using a bio-decomposer containing *Trichoderma pseudokoningii*, *T. polysporum*, and *Phanerochaete chrysosporium.* In brief, composting steps involved EFB collection, shredding, mixing with bio-decomposer, incubation, and harvesting. Dosages of bio-decomposer was 0.2% (w/w) with four weeks incubation period without turning following the process outlined by Goenadi (2006).

2. *Chemical Analysis*

2.1. Analysis of EFB

A number of chemical characteristics of EFB before and after bio-decomposition were determined at laboratory of IRIBB. EFB samples were air dried and passed through 100 mesh sieves and analyzed for the following: pH, nitrogen (Kjeldahl), phosphorus (spectrophotometer), potassium (Atomic Absorption Spectrophotometer, AAS), total SiO_2

(gravimetry), soluble Si (spectrophotometer), calcium (AAS), magnesium (AAS), cation exchange capacity (CEC) by using SNI 13-3494-1994 standard method, and C-organic (spectrophotometer).

2.2. Soil and leaf analysis

Soil samples were air dried and passed through 2 mm sieve and analyzed for the following: pH, soil texture, C-organic, nitrogen, phosphorus, potassium, calcium carbonate, CEC, Boron, and exchangeable Al and H. Whereas leaf nutrient analysis was N, P, K, Mg, and SiO_2.

3. *BioSilAc Preparation*

A 150 g of 325-mesh Belitung quartz sand sample was boiled in 100 mL HCl at 5 N concentrations, until almost all of solution evaporated to dissolve any contaminant elements present. The treated samples were then washed out with tap water several times to eliminate the contaminants and the rest of HCl solution. Wet samples were transferred on a sheet of paper and dried out at 100^{o}C in an oven until completely dry. A 60 g washed sample was then mixed with 80 g NaOH (s) in a stainless pan and heated on stove at 330^{o}C while stirred manually until melted. The melted mixture was kept stirred until it dried out. After cooling at room temperature, a 60 g pre-treated quartz sand was dissolved in 400 mL distilled water. The liquid obtained was the soluble silica (H_4SiO_4) (Santi *et al*. 2017), whereas the solid formula was prepared by inoculation of acid-base-pretreated 325-mesh quartz sand with Si-solubilizing microbes i.e. *Burkholderia cenocepacia*, *B. vietnamiensis*, *Aspergillus niger*, *Aeromonas punctata* (Santi & Goenadi, 2017). These two Si sources are called as BioSilAc. Silica concentration was determined by spectrophotometer.

4. *Scanning Electron Microscopy Analyses*

This analysis was performed to confirm the present of Si in EFB tissues. All solid material both fresh and composted EFB were examined with a Scanning Electron Microscope (SEM). A slice cut of EFB fiber was taken and prepared for SEM analyses. The electron beam is accelerated through a high voltage 20 kV and pass through a system of apertures and electromagnetic lenses to produce a thin beam of electrons (Zhou *et al.* 2006). In the early stages a material sample leveled with a special tool. After sputter coating the cast with 35 nm of gold-palladium (Au-Pd), electron micrographs were generated using a Jeol JSM-5310LV SEM.

5. *Field experiment*

A field experiment was conducted at Sungai Mirah Minting Estate, Central Kalimantan and arranged in seven treatments, i.e. combination of EFB compost and BioSilAc application with reduced dosages of NPK fertilizers, were arranged in a random block design with three replicates. The BioSilAc used was in liquid and solid forms. Soil chemical characteristics of immature-plant plot are pH 5.0; 83% sand; 14.5% clay; 2.5% silt; 0.12 (N); 1.98% (C-organic); 0.79 cmol+/kg (exchangeable Al); 0.32 cmol+/kg (exchangeable H); 3.67 cmol+/kg CEC; 6.4 ppm (B); 0.006% (P_2O_5); 0.18% (K_2O); and 0.05% (CaO). The data from mature-plant plot are pH 4.8; 61.0% sand; 25.7% clay; 13.3% silt; 0.17 (N); 3.02% (C-organic); 1.4 cmol+/kg (exchangeable Al); 0.74 cmol+/kg (exchangeable H); 6.8 cmol+/kg CEC; 5.5 ppm (B); 0.102% (P_2O_5); 0.17% (K_2O); and 0.34% (CaO). Applied on a two-year (immature) and five-year (mature) old plants the treatments consist as follows: (i) 100% NPK standar dosage (T1); (ii) T1+ 225 kg BioSilAc /ha/year; (iii) 75%(T1) + 225 kg BioSilAc/ha/year; (iv) T1+ 4 L BioSilAc/ha/year; (v) 75% (T1) + 4 L BioSilAc/ha/year; (vi) T1 + 500 kg EFB compost + 2L BioSilAc/ha/year; (vii) 75% (T1) + 500 kg EFB compost + 2L BioSilAc/ha/year. The oil palm was planted in 2013 (mature) and 2015 (immature). The treatment each was applied on a plot consisting of 25 trees with nine of them in the middle as observation trees. Selected parameters observed included leaf nutrient content (N, P, K, and Mg) of leaf no. 9, average weight and number of FFB. Data were analyzed by ANOVA and Duncan's Multiple Range Test (DMRT).

Results and Discussion

1. *Fresh and composted EFB chemical characteristics*

Chemical characterization of the fresh- and composted-EFB was conducted to determine the potential level of dissolved Si and other nutrients that can be utilized by plants.

The characterization results are presented in Table1. The results indicate that the levels of N, P, K, Ca, and Mg from the composted-EFB increased. Similarly, total SiO_2 and dissolved Si contents increased significantly, i.e. 2.5 and 14.6 times, respectively, in comparison to non-decomposed EFB. Furthermore, there is an increase in CEC and pH values, whereas the C/N ratio was decreased drastically from 59.9 to 19.4. Therefore, composting EFB with bio-decomposer for 28 days incubation have improved the quality of organic material, the nutrient~~s~~ content, and the availability of Si for plants.

Table 1. Chemical characteristics of fresh and composted EFB

Parameters	Fresh EFB	EFB compost
pH	6.3	8.0
N (%)	0.7	1.9
P_2O_5 (%)	0.4	0.6
K_2O (%)	2.5	3.8
SiO_2 (%)	11.3	28.7
dissolved Si (%)	0.03	0.44
Ca (%)	0.4	1.3
Mg (%)	0.3	0.58
CEC (cmol+/Kg)	7.3	52.1
C-organic (%)	41.9	36.9
C/N	59.9	19.4

2. *The present of silica body in EFB*

In general, EFB have thicker cell wall, thinner lumen, smaller diameter, and shorter fibers. EFB fibers have similar cell wall thickness and fiber length, while having thicker lumen and larger diameter when compared with hardwood species (Law *et al*. 2007; Jinn *et al*. 2015). The evidence of Si present in fresh and composted EFB was collected by using scanning electron microscope (SEM). The SEM analysis showed that the amount of crystallite Si in the cross-sectional slices of EFB quite abundant both on fresh (Fig 1.) and composted EFB (Fig.2). Silica bodies were bounded between the fibers. The silica bodies observed on fiber strands are round spiky shape. This evidence is in agreement with those reported by Harun *et al*. (2013) and Jinn *et al*. (2015). In composted EFB Si position nearly separated from fiber tissue because the tissue conditions of EFB has become weak. The tissue of EFB compost structure is somewhat fragile that enables the release of silica from these tissues will become easier.

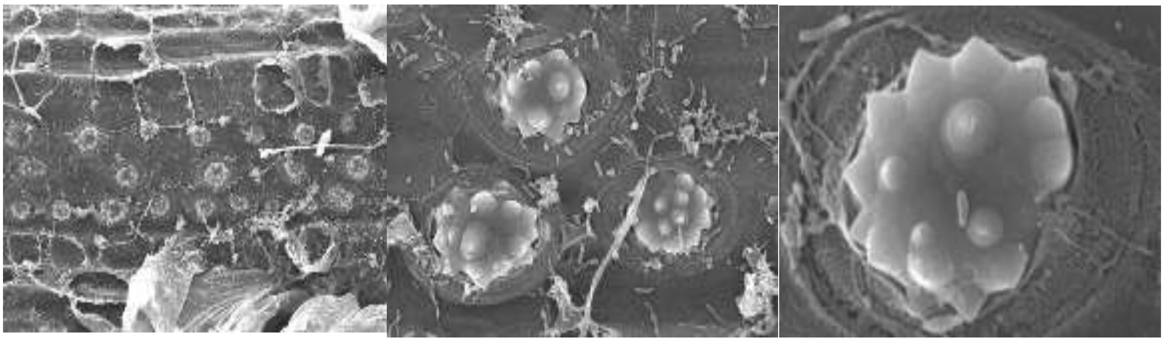

Figure 1. The silica body of fresh EFB found between fiber under microscopic magnification of 500 x (left); 2,000 x (middle); and 7,500 x (right).

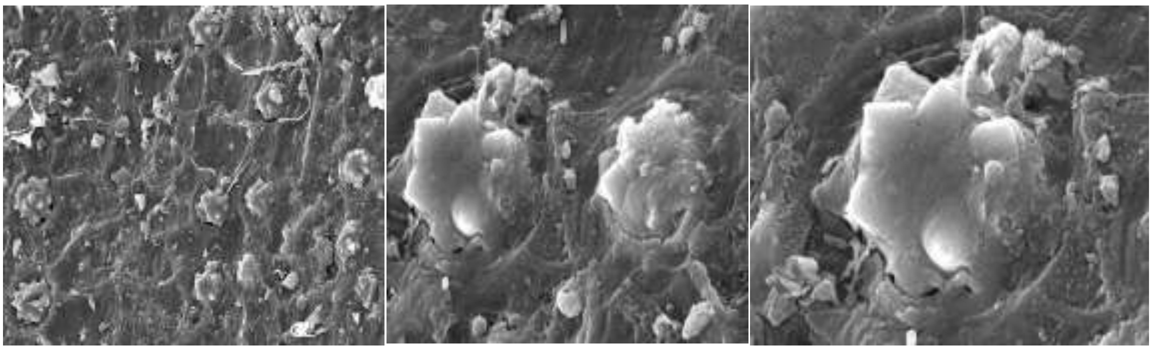

Figure 2. The silica body of EFB compost under microscopic magnification of 700 x (left); 3,500 x (middle); and 5,000 x (right).

3. *Field experiment*

The first application of EFB compost and BioSilAc was conducted in January 2017. The data from this experiment show that the application of EFB compost and BioSilAc after one year on a immature oil palm could maintain P, K, Mg and Si absorptions better than those of other treatments including normal dosage of NPK fertilizer. When applied in combination with 500 kg EFB compost + 2 L BioSilAc/ha/year they reduced rate of NPK up to 75% standard dosage. Leaf P, K, and Mg content of 500 kg EFB compost combined with 75% NPK dosages was considered to be optimum, i.e. 28.6% (P); 11.1% (K); 33.3% (Mg); and 12.2% (SiO_2) compared to the standard NPK dosage treatment. Meanwhile, data analyses indicated that total P content was relatively high (0.27%), whereas N, K, and Mg content of leaf among treatment plots of BioSilAc was at optimum level according to classification made by Fairhurst & Hardter (2003) (Table 2). Furthermore, aplication EFB compost and BioSilAc inreased SiO_2 content on leaf of immature oil palm. Application of 500 kg EFB compost + 2 L BioSilAc combined with 75% dosage of NPK resulted in significantly higher vegetatif growth of frond length, width and dense of petiole than those of other treatments (Table 3).

Table 2. The leaf nutrient contents of immature oil palm one year after treatments.

Treatments	Leaf nutrient contents (%)				
	N	P	K	Mg	SiO_2
NPK 100% standard dosage (T1)	2.7	0.21	0.9	0.15	0.98
(T1) + 225 kg BioSilAc/ha/year	2.7	0.28	1.3	0.18	2.5
75% (T1) + 225 kg BioSilAc/ha/year	2.6	0.26	1.2	0.20	2.9
(T1) + 4 Liter BioSilAc/ha/year	2.7	0.24	1.0	0.18	1.04
75% (T1) + 4 Liter BioSilAc/ha/year	2.7	0.27	1.3	0.21	1.78
(T1) + 500 kg EFB compost + 2 L BioSilAc/ha/year	2.5	0.26	0.8	0.21	1.90
75% (T1) + 500 kg EFB compost + 2 L BioSilAc/ha/year	2.7	0.27	1.0	0.20	1.10

**) Classification of leaf nutrient contents of oil palm according to Fairhurst dan Hardter (2003): N (%) = <2.50 (deficient), 2.6-2.9 (optimum), >3.1 (high); P(%) = <0.15 (deficient), 0.16-0.19 (optimum), >0.25 (high); K (%) = <1.00 (deficient), 1.1-1.3 (optimum), > 1.8 (high); and Mg (%) = <0.20 (deficient), 0.30-0.45 (optimum), >0.70 (high).

Table 3. Growth of immature oil palm one year after treatments.

Treatments	Frond length (cm)	Number of leave (sheet)	Width of petiole (cm)	Dense of petiole (cm)
NPK 100% standard dosage (T1)	340.3 ab*)	218 ab	4.9 ab	3.2 a
(T1) + 225 kg BioSilAc/ha/year	328.0 b	211 b	4.9 ab	3.0 ab
75% (T1) + 225 kg BioSilAc/ha/year	372.8 a	208 b	5.2 a	3.3 a
(T1) + 4 Liter BioSilAc/ha/year	354.6 ab	230 ab	4.2 c	2.5 b
75% (T1) + 4 Liter BioSilAc/ha/year	320.9 b	251 a	4.3 bc	2.4 b
(T1) + 500 kg EFB compost + 2 L BioSilAc/ha/year	342.9 ab	239 ab	4.7 abc	2.9 ab
75% (T1) + 500 kg EFB compost + 2 L BioSilAc/ha/year	383.2 a	223 ab	5.4 a	3.6 a
Coefficient variable (%)	6.6	7.7	8.0	11.4

*) Figures in the same column followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test (P<0.05).

Table 4 presents the effect of treatments on the yield of a five-year-old palm. It is evidenced that the highest yield average in term of FFB was obtained from the application of 75% dosage of NPK combined with 500 kg EFB compost + 2 L BioSilAc/ha/year. The application of these treatments resulted in higher yield (than that obtained from 100% standard dosage of NPK. This study revealed that the use of EFB compost reduced the need of Si up to 50% for both immature and mature oil palms which promote both better vegetative and productive performances of the palm.

Table 4. The productivity of 2013 planting year oil palm, one year after treatments.

Treatments	Number of FFB	Average weight of FFB (kg)	FFB production (ton/ha/year)
NPK 100% standard dosage (T1)	1,823 bc	4.83 a	8.80 ab
(T1) + 225 kg BioSilAc/ha/year	1,750 c	4.70 b	8.23 c
75% (T1) + 225 kg BioSilAc/ha/year	1,800 c	4.70 b	8.47 bc
(T1) + 4 Liter BioSilAc/ha/year	1,889 bc	4.50 c	8.50 a
75% (T1) + 4 Liter BioSilAc/ha/year	1,973 ab	4.63 b	9.13 a
(T1) + 500 kg EFB compost + 2 L BioSilAc/ha/year	1,875 bc	4.70 b	8.80 ab
75% (T1) + 500 kg EFB compost + 2 L BioSilAc/ha/year	2,084 a	4.70 b	9.23 a
Coefficient variable (%)	4.6	1.4	2.9

*) Figures in the same column followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($P<0.05$).

The effect of treatments on selected soil chemical properties after one year of application is shown in Table 5. In general, the selected chemical properties of the soil were not so much different among those treatments. No clear evidences were noticed showing relationships between Si application and P and Al contents. However, it is observed that in general application of Si tended to decrease the Al content of the soil. Britez *et al.* (2002) reported that silicon (Si) can make stable complexes with Al and reduce the harmful Al effects. Si can potentially increase root elongation rate (RER) in Al-toxic solutions, with the magnitude of the effect increasing with the concentration of Si (Koppittke *et al.* 2017). Moreover, these researchers also confirmed that Si is not only deactivated Al in the rhizosphere but also in plant shoot tissue avoiding Al toxicity to the plant.

Table 5. Soil characteristics determined at one year after treatments on mature plant plots.

Treatments	pH H_20	N (%)	P_2O_5 (%)	K_2O (%)	C-Org (%)	Al (%)	exchangeable Al ($cmol^+kg^{-1}$)
NPK 100% standard dosage (T1)	4.9 a	0.13 d	0.05 b	0.01 c	2.5 b	0.11 ab	1.50 bc
(T1) + 225 kg BioSilAc/ha/year	4.4 de	0.16 bc	0.01 c	0.03 a	2.6 b	0.06 d	1.81 b
75% (T1) + 225 kg BioSilAc/ha/year	4.7 bc	0.08 e	0.02 c	0.01 c	1.3 c	0.06 d	1.36 c
(T1) + 4 Liter BioSilAc/ha/year	4.4 de	0.18 ab	0.02 c	0.007 cd	2.9 a	0.10 abc	2.67 a
75% (T1) + 4 Liter BioSilAc/ha/year	4.5 de	0.19 a	0.08 a	0.01 c	3.1 a	0.12 a	1.74 b
(T1) + 500 kg EFB compost + 2 L BioSilAc/ha/year	4.7 b	0.08 e	0.04 c	0.004 d	1.2 c	0.10 abc	1.71 b
75% (T1) + 500 kg EFB compost + 2 L BioSilAc/ha/year	4.6 cd	0.15 cd	0.06 b	0.02 b	2.9 a	0.09 c	1.21 c
Coefficient variable (%)	1.2	7.0	19.2	14.4	4.0	9.6	7.7

*) Figures in the same column followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($P<0.05$).

Conclusions

The application of Si (BioSilAc) improved the vegetative performance of immature and yield of mature oil palm (4.9%) and increased NPK fertilizer use efficiency (25%) one year after treatment. A 28-day bio-composted EFB could provide Si available to the plant and the addition of 500 kg EFB compost/ha/year combined with 75% NPK fertilizer reduced 50% the need for BioSilAc. Further research is needed to evaluate the long-term effect of Si application on yield of oil palm.

Acknowledgements

We would like to extend our sincere gratitude to The Indonesia Estate Crop Fund Management Agency for Palm Oil, for valuable supports in funding this research (Contract No. PRJ - 52 /DPKS/2016) and PT Bumitama Gunajaya Agro, for their kind co-operation in field implementation project and logistical support.

References

Britez, R.M, Watanabe T, Jansen S, Reissmann C.B, & Osaki M. 2002, The relationship between aluminium and silicon accumulation in leaves of *Faramea marginata* (Rubiaceae). *New Phytologist* 156, 437–444.

Epstein, E. 1994, The anomaly of silicon in plant biology. Proc. Natl. Acad. Sci. USA 91, 11–17.

Epstein, E. 2009, Silicon: Its manifold roles in plants. *Ann. Appl. Biol*. 155, 155–160.

Fairhurst, T. & Hardter, R. 2003, Management for large and sustainable yields. Potash and Phosphate Institute of Canada. 382p.

Geng, A. 2013, Conversion of oil palm empty fruit bunch to biofuels. In: Fang Z (ed) Book of liquid, gaseous and solid biofuels—conversion techniques. Tech Open, Croatia.

Goenadi, D.H. 2006, Developing Technology for Biodecomposition of fresh solid wastes of plantation crops under tropical conditions. IPB Press.

Gunawan, F.E., Homma H, Brodjonegoro, S.S, Baseri-Hudin A, & Zainuddin A. 2009, Mechanical properties of oil palm empty fruit bunch fiber. *J Solid Mech Mater Eng*, 3(7), 943–951.

Harun, N.A.F., Baharuddin, A.S, Zainudin, M.H.M, Bahrin, E.K, Naim M.N, & Zakaria, R. 2013, Cellulose production from treated oil palm empty fruit bunch degradation by locally isolated Thermobifidafusca. *Bioresources*, 8(1), 676 – 687.

Jinn, C.M., H’ng P.S, Chin K.L., Chai E.W., Paridah M.T., Lee S.H., Lum W.C., Luqman C., & Mariusz, M. 2015, Agricultural biomass based potential materials. *In* Empty Fruit Bunches in the Race for Energy, Biochemical, and Material Industry. Springer International Publishing Switzerland. K. R. Hakeem (eds.). 375-389 p.

Keeping, M.G. & Reynolds, O.L. 2009, Silicon in agriculture: New insights, new significance and growing application. *Ann. Appl. Biol.* 155, 153–154.

Koppittke, P.M., Gianoncelli A., Kourousias G., Green K. & McKenna, B.A. 2017, Alleviation of Al toxicity by Si is associated with the formation of Al–Si complexes in root tissues of sorghum. *Frontiers in plant science*, 8: 1-9.

Law, K.N., Daud, W.R. & Ghazali A. 2007, Morphological and chemical nature of fiber strands of oil palm empty-fruit-bunch (OPEFB). *Bioresource Technology,* 2(3),351-362.

Ma, J.F. 2004, Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. *Soil Science and Plant Nutrition,* 50(1), 11–18.

Ma J.F. & Yamaji N.A. 2015, Cooperative system of silicon transport in plants. *Trends Plant Sci,* 20, 435–442.

Ma J.F, Tamai K, Ichii M. & Wu G.F. 2002, A Rice Mutant Defective in Si Uptake. *Plant Physiol,* 130, 2111–2117.

Mitani N, & Ma J.F. 2005, Uptake system of silicon in different plant species. *J. Exp. Bot,* 56, 1255–1261.

Montpetit J., Vivancos J., Mitani-Ueno N., Yamaji N., Rémus-Borel W., Belzile F., Ma J.F., & Bélanger R.R. 2012, Cloning, functional characterization and heterologous expression of TaLsi1, a wheat silicon transporter gene. *Plant Mol. Biol,* 79, 35–46.

Najihah, N.I, Hanafi, M.M, Idris, A.S. & Hakim, M.A. 2015, Silicon treatment in oil palms confers resistance to basal stem rot disease caused by Ganoderma boninense. *Crop Prot,* 67, 151–159.

Sanglard, L.M.V.P, Detmann, K.C, Martins, S.C.V, Teixeira, R.A, Pereira, L.F, Sanglard, M.L, Fernie, A.R, Araújo, W.L. & DaMatta, F.M. 2016, The role of silicon in metabolic acclimation of rice plants challenged with arsenic. *Environ. Exp. Bot,* 123, 22–36.

Santi, L.P, & Goenadi, D.H. 2017, Solubilization of silicate from quartz mineral by potential silicate solubilizing bacteria. *Menara Perkebunan,* 85(2), 96-105.

Santi, L.P, Mulyanto D., & Goenadi, D.H. 2017, Double acid-base extraction of silicic acid from quartz sand. *Journal of Minerals and Materials Characterization and Engineering*, 5(6), 362-373.

Shi, Y., Wang Y., Flowers, T.J. & Gong, H. 2013, Silicon decreases chloride transport in rice (*Oryza sativa* L.) in saline conditions. *J. Plant Physiol,* 170, 847–853.

Standar Nasional Indonesia (SNI). 1994, Mineral zeolit, Pengukuran kapasitas pertukaran kation. 13-3494-1994.

Vivancos, J., Labbé C., Menzies, J.G. & Bélanger, R.R. 2015, Silicon-mediated resistance of Arabidopsis against powdery mildew involves mechanisms other than the salicylic acid (SA)-dependent defence pathway. *Mol. Plant Pathol,* 16, 572–582.

Yin, L., Wang S., Liu P., Wang W., Cao D., Deng X. & Zhang S. 2014, Silicon-mediated changes in polyamine and 1-aminocyclopropane-1-carboxylic acid are involved in silicon-induced drought resistance in Sorghum bicolor L. *Plant Physiol. Biochem,* 80, 268–277.

Ye, M., Song Y., Long J.W.R., Baerson, S.R., Pan Z, Zhu-Salzman, K., Xie J., Cai K. & Luo S. 2013, Priming of jasmonate-mediated antiherbivore defense responses in rice by silicon. *Proc. Natl. Acad. Sci,* USA 110, E3631–E3639.

Zhou, W., Apkarian R., Wang, Z.L. & Joy, D. 2006, Fundamentals of Scanning Electron Microscopy (SEM). In: Zhou W. & Wang Z.L, Eds., Scanning Microscopy for Nanotechnology Techniques and Applications, Springer Science Business Media, New York.