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Role of Microbial Community in Suppressing Development of *Ganoderma* in Oil Palm Seedlings

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Authors' contributions

This work was carried out in collaboration between all authors. Author ARK designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors RO, NSA and MHM managed the analyses of the study. Author FSR managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Ganoderma boninense is a fungal pathogen causing serious basal stem root (BSR) disease in oil palm. The development of this pathogen can be influenced by soil microbial community. A greenhouse study was conducted to determine the effect of indigenous soil microorganisms on growth of *G. boninense* inoculated in oil palm seedlings on different soils. Soil samples were collected from three different locations; *G. boninense* infected soil (S1), non-infected soil (S2) and forest soil (S3). The results showed that sterilized soil without the presence of indigenous microbial population did not suppress the development of *G. boninense* in oil palm seedlings. The *G.*

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boninense inoculated plants grown on sterilized soils had a significantly lower shoot (34.87 g/plant) and root dry weights (9.12 g/plant) as compared to the non-*Ganoderma* inoculated plants. The highest contents of N (724 mg/plant), P (60 mg/plant), and K (605 mg/plant) were found in seedlings grown in non-infected soil. The lignin content and photosynthesis decreased in *G. boninense* infected plants while cellulose content varied. The *Ganoderma* inoculated seedlings showed higher disease severity index and lower shoot yield. Hence, soil devoid of indigenous microorganism negatively affected oil palm seedlings growth due to increased development of *G. boninense* in the seedlings roots.

Keywords: Ganoderma boninense; inoculation; oil palm; indigenous soil microorganisms; soil sterilization.

1. INTRODUCTION

Malaysian oil palm suffer from basal stem rot (BSR) disease caused by *G. boninense* [1]. The BSR is a root disease which can be seen as root deterioration with lower stem growth, can be spread by basidiospores and direct root contact through yet unidentified means [2]. *Ganoderma* species are characterized as white rot fungi that have the ability of breakdown parts of plant cell wall [3]. According to Pilotti [4], the fungi prevent transportation of nutrients and water to the upper part of the oil palm, therefore, triggering palm leaf wilting, yellowness, canopy mottling, unopened leafs, and lower stem appearance of basidiocarps. Singh [5] reported that infected oil palms have low productivity and eventually collapse.

The most serious loss in productivity as a result of BSR have been reported in Malaysia, Indonesia and lesser prevalence documented in Thailand, Africa, and Papua New Guinea [6]. Moreover, Lim et al. [7] reported an average of 50% yield losses of 13 years old oil palms in Malaysian coastal areas. BSR causes tree loss in palm stands and consequently lose in yield [8,9]. Being a major or severe disease of oil palms and with no known remedy at the moment, it is, therefore, a great economic concern to the Malaysian oil palm industry. The fungi naturally infect seedlings in one to two years after planting and increases in four to five years [10]. Young palms usually die from infection within 6–24 months after the first appearance of the symptoms, while mature palms last for 2–3 years or longer [11]. Oil palm has an economic lifespan of 25-30 years. Basal stem rot can destroy more than 80% of palm stands by the time they are half-way through normal economic life [12].

Oil palm seedlings grow in rich and temporarily moist alluvial soils [11], with the roots deeply embedded in the soil. The primary route of infection for BSR has been identified as roots in accordance with general consensus within the industry [13]. Utomo [14] stated that *Ganoderma* attacked the root system during planting cycles. Tisné et al. [15] revealed that this happened during seedlings growing stage. Likely infection of oil palm occurs after the roots come into contact with inoculums from the residues left on the ground [16], or roots nearest to the infected palms.

Soil has suppressive characteristics with the ability to keep disease incidence or severity at a low level [17]. Actinobacteria and pseudomonads are associated with disease suppression and incidences in soils [18]. A bulk of microbial biomass was previously anticipated to form a competitive environment lethal for the pathogenic indigenous microorganism in the soil. Leon et al. [19] reported a significant negative relationship between disease severity and microbial biomass in soil using microwave irradiation as sterilization technique [20]. Soil sterilization lowers the microbial populations of both the soil-borne pathogenic and non-pathogenic microbes which in turn affect plant growth and vitality [21]. Higher microbial diversity is closely connected to pathogenic disease suppression in plants [22]. It is necessary to determine the role of those indigenous microbes which would likely to suppress the presence of BSR disease in oil palm seedlings.

A profound practical solution is needed to be addressed to this problem. So far no effective method or approach has been provided by previous studies to combat the severance and damages caused by *G. boninense* to oil palm seedlings. Currently, there is little work done on the role of soil microbial community on the suppression of *G. boninense* in oil palm. The hypothesis of this study was that the presence of

indigenous microorganisms in soils from oil palm plantations might have an effect on the development of *G. boninense* in oil palm seedlings. Therefore, this study aimed to determine the effect of indigenous soil microorganisms on the growth of *G. boninense* inoculated oil palm seedlings on different soils.

2. MATERIALS AND METHODS

2.1 Soil Sample Collection and *Ganoderma* Preparation

The soil samples were collected from *G. boninense* soil (S1), non-infected soil (S2), and forest (S3) from oil palm plantation sites of United Malacca Berhad in Macap, Malacca, Malaysia. No physical differences existed among soils collected from these sites. Each sample was taken from 0-30 cm soil depth. All soil samples were divided into two groups; sterilized and non-sterilized. The soils were sterilized using autoclave at 121ºC for 1.40 kg/cm^2 pressure for 30 min, and this procedure was repeated three times for each soil.

2.2 Microbial Analysis

Populations of bacteria, fungi, and actinomycetes were determined for all samples. A series of 10-fold dilutions were prepared up to 10^{-7} and 0.1 ml aliquots were spread onto the selective media and incubated at 28±2°C in an incubator for three days [23].

2.3 Preparation of Rubber Wood Block and *Ganoderma* Inoculum

A total of 30 rubber wood blocks (6.0 × 6.0 × 12.0 cm) were prepared according to the method of Naidu et al. [24]. Each rubber wood was placed into a heat-resistant polypropylene bag of 10.0 × 32.0 cm and autoclaved at 1.40 kg/cm^2 pressure for 121ºC for 25 min. The blocks were then soaked in Malt Extract Broth (MEB) overnight in basins. The following day, the blocks were again placed in heat-resistant polypropylene bags and 100 ml of molten Malt Extract Broth (MEB) was added into each bag. The bags were then tied with raffia string and autoclaved for the second time under the mentioned conditions. Inoculum preparation was made by placing ten plugs sized 6 mm from eight days old *Ganoderma* mycelium grown on potato dextrose agar (PDA) that was obtained using a core-borer onto each surface of the autoclaved rubber wood blocks. Then the bags were tied quickly and carefully to avoid contamination and incubated in the dark at 27 ± 1ºC for 10 to 12 weeks. Blocks fully colonized with *Ganoderma* and uncontaminated were used for inoculation of the oil palm seedlings.

2.4 Planting of Oil Palm Seedlings

The planting of oil palm seedlings was conducted in a greenhouse at Universiti Putra Malaysia, Malaysia. Three months old seedlings were placed in polythene bags containing 5 kg soil. The soils used were from three locations; i) *Ganoderma* infected soil, ii) non-*Ganoderma* infected soil and iii) forest soil. Half of the soils were subject to sterilization and half of the seedlings were inoculated with *Ganoderma*. The experiment was conducted in a factorial randomised complete block design (RCBD) with five replicates. Oil palm seedlings were grown for three months.

2.5 Disease Incidence

Disease development was examined based on quantitative valuation measured as disease incidence (DI) percentage at four weeks intervals. The DI denoted the number of seedlings visually evaluated as disease type (leaves necrosis and chlorosis, with or without sporophore production) as described by Wong et al. [25]:

DI = (Number of seedlings infected/ Total number of seedlings assessed) × 100.

The monomolecular model (Monit) was used to obtain the slopes of the curves of DI data transformation [25]. At the end of the study (three months), the seedlings were split longitudinally to examine stem and root decay and to evaluate the visual severity of the internal symptoms established on the proportion of bole and root tissues damaged by *G. boninense*. The assessment was based on the following modified parameters [26].

2.6 Disease Severity Index (DSI)

The progress of the disease in the seedlings was assessed by disease severity index (DSI). The symptoms were indexed by formula as described by Abdullah [27]:

0 = healthy seedlings; 1 = appearance of three necrotic leaves; 2 = appearance of more than

three necrotic leaves; 3 = appearance of the fruiting body at the bowl; 4 = dying/dead seedling.

The DSI was calculated at the end of study (120 days) based on the following formula:

Disease severity index

$$(DSI) = \sum(A \times B) \times \frac{100}{\sum B} \times 4$$

Where: A= disease class (0, 1, 2, 3 or 4)

B= number of seedlings showing disease class per treatment.

2.7 Photosynthetic Leaf Determination

Leaves were measured for photosynthesis at three months after planting when older leaves had acclimatized to the light condition. A portable photosynthesis meter was used to measure the leaf gas exchange rate. All measurements were carried out between 0800 to 1100 h in the morning to avoid depression cause by mid-day photosynthesis [28]. The relationship between the carbon assimilation rate and photon flux density was measured for apparent non-senescing and fully expanded leaves [29].

2.8 Seedling Harvest

Plants were harvested after three months of growth. Fresh and dry weights of shoot and roots were determined. Root samples were also scanned by the aid of a scanner (Epson Perfetion V700 Photo**)** to determine root development.

2.9 Cellulose and Lignin Determination

After an acid-detergent fiber pre-extraction, cellulose was removed with 72% sulphuric acid using the gravimetric method and the percentage of lignin was calculated by weight differences. Lignin removal was determined by weight loss upon ashing and hydrolysis with 72% sulphuric acid [30].

2.10 Statistical Analysis

Data were analyzed using analysis of variance (ANOVA), and the means were presented as Mean ± SEM. The difference between the means was compared using Tukey. SAS software (version 9.3) program was used to analyze the data. The significance level was set at $P \leq 0.05$ and the *Ganoderma* inoculation and soil sterilization for the root development were set at $P \leq 0.001$.

3. RESULTS

3.1 Population of Microorganisms

The populations of microorganism in the different soil samples collected prior to sterilization are presented in Table 1. In general, the higher ($P \leq 0.05$) bacterial population were found in the forest soil compared to *Ganoderma* infected and non-*Ganoderma* infected soils. However, the low fungal population was observed in the forest and non-infected compared to *Ganoderma* infected soils. Actinomycetes population was quite similar in all soils.

However, after seedlings were harvested the bacterial and fungal populations varied significantly (Table 2). Non-infected plants displayed significantly higher bacterial and fungal populations than the infected plants. The highest significant bacterial and fungal populations were observed in S3T2G- whereas the lowest bacterial and fungal populations were noted in S1T2G+ and S2T1G+, respectively.

3.2 Root Development

The *Ganoderma* inoculation and soil sterilization significantly ($P \leq 0.001$) affected the growth and spread of *Ganoderma* disease in oil palm seedlings (Table 3). Highest root length, root surface and root tips were found in plants grown in the forest soil that was neither sterilized nor inoculated with *Ganoderma*. Meanwhile, the highest root volume was found in plants from the non-*Ganoderma* infected soil, non-sterilized and without *Ganoderma* inoculation. The result indicated that better root growth was found in soil free of *Ganoderma* infection either from soil or by inoculation.

3.3 Plant Biomass

The shoot highest dry weight was observed in plants grown in forest soil which was not sterilized and not inoculated with *Ganoderma* (S3T2G-). The highest root and shoot

Table 1. The original populations of bacteria, fungi, and actinomycetes in three soil type

Soil type	Bacteria (Log_{10} cfu g^{-1} soil)	Fungi (Log_{10} cfu g^{-1} soil)	Actinomycetes (Log_{10} cfu g^{-1} soil)
Ganoderma infected soil (S1)	5.99	3.74	4.09
Non-*Ganoderma* infected soil (S2)	4.81	2.93	4.03
Forest soil (S3)	6.70	2.39	3.97

Table 2. Populations of bacteria and fungi in three soils as affected by sterilization and *Ganoderma* inoculation at 4 months of oil palm growth

	Treatments		Bacteria (Log_{10} cfu g^{-1} soil)	Fungi (Log_{10} cfu g^{-1} soil)
S1	T1	G+	nd	1.57d
	T1	G-	nd	nd
	T2	G+	3.72b	1.62c
	T2	G-	4.13a	1.73c
S2	T1	G+	nd	1.36e
	T1	G-	nd	nd
	T2	G+	3.82a	3.72a
	T2	G-	3.94a	3.91a
S3	T1	G+	nd	2.3b
	T1	G-	nd	nd
	T2	G+	4.13a	3.24a
	T2	G-	4.65a	3.92a

S1= Ganoderma infected soil, S2= Non-Ganoderma infected soil, S3= forest soil, T1= sterilized soil, T2= non-sterilized soil, G+=inoculated with Ganoderma, G- = Non-inoculated with Ganoderma nd=not detected. Means in a column with the same letters are not significantly different at P ≤ 0.05

Table 3. Effect of *Ganoderma* inoculation and soil sterilization on development of oil palm seedlings roots after 3 months of growth

Treatments			Root length (cm)	Surface area (cm^2)	Root volume (cm^3)	Root tips
S1	T1	G+	443.49 ± 1.23^{l}	307.43 ± 0.53^{j}	2.73 ± 0.05^{gf}	4393.4 ± 1964.79^{c}
		G-	2454.77 ± 0.88^{e}	669.06 ± 0.10^{g}	1233 ± 0.22^{gf}	15442.8 ± 6906.23^{abc}
	T2	G+	1384.76 ± 3.63^{i}	514.23 ± 0.35^{h}	11.52 ± 0.15^{g}	15063.6 ± 6736.65^{abc}
		G-	2054.06 ± 2.92^{g}	835.54 ± 1.64^{e}	26.00 ± 0.36^{b}	17113.2 ± 7653.26^{ab}
S2	T1	G+	1353.65 ± 1.90^{j}	$277.872.35^{k}$	8.25 ± 0.26^{h}	6533.4 ± 2921.83^{bc}
		G-	2617.17 ± 1.30^{d}	913.52 ± 1.63^{d}	23.38 ± 0.14^{c}	16583 ± 7416.14^{abc}
	T2	G+	1043.96 ± 1.17^{k}	305.54 ± 0.38^{j}	12.35 ± 0.18^{fg}	8385 ± 3749.89^{abc}
		G-	2856.38 ± 0.78^{b}	969.61 ± 1.86^{c}	30.24 ± 0.86^{a}	19957.6 ± 8925.31^{a}
S3	T1	G+	1480.72 ± 2.31^{h}	376.76 ± 0.97^{i}	8.13 ± 0.36^{h}	10090.4 ± 4512.56^{abc}
		G-	$2818..75\pm0.33^{c}$	1059.08 ± 3.31^{b}	13.17 ± 0.11^{f}	13759.2 ± 6153.30^{abc}
	T2	G+	2355.57 ± 1.39^{f}	744.39 ± 1.49^{f}	17.28 ± 0.14^{d}	13508 ± 6040.96^{abc}
		G-	3225.70 ± 1.80^{a}	1265.99 ± 1.78^{a}	15.60 ± 0.28^{e}	19825.2 ± 8866.10^{a}

S1= Ganoderma infected soil, S2= Non-Ganoderma infected soil, S3= forest soil, T1= sterilized soil, T2= non-sterilized soil, G+=inoculated with Ganoderma, G- = Non-inoculated with Ganoderma, L=length, SA=Surface area, V=volume, N=number tips, Means in column with the same letters are not significantly different at P ≤ 0.05

weights were observed in *Ganoderma* infected non-sterilized and non-inoculated soil followed by the forest non-infected, non-sterilized and non-inoculated soil (Fig. 1). The increase in both root and shoot yields in the infected groups of non-inoculated seedlings might be the result of

tissue or cell differentiation and physiological deviations from normal function. There was no significant interactions in term of root and shoot yield per plant for inoculated seedlings (Table 3).

3.4 Disease Incidence

The disease incidences in seedlings of the forest, infected and non-infected soils after three months of *Ganoderma* inoculation were found positive (Figs. 2 a, b & c). In all groups, the incidences were increased, though those of sterilized soils demonstrated a higher ($P \leq 0.05$) increased compared to non-sterilized soil.

The disease severity increased with the increase of *G. boninense* inoculation period of oil palm seedlings. Initially, it was less but gradually increased with time. The highest disease severity was noted at the week 12. The significantly highest disease severity in oil palm seedlings was found in *Ganoderma* infected soil for both sterilized and non-sterilized soils (Fig. 3).

3.5 Nutrient Uptake

The tissue N, P, and K levels of the treated seedlings for the soils were significantly affected by *Ganoderma* development after three months (Table 4). Inoculated and non-inoculated seedlings of harvested seedlings varied in tissue N levels. The maximum N (724 mg/plant), P (60 mg/plant) and K (605 mg/plant) were significantly found in the inoculated oil palm seedlings of non-sterilized and non-infected soil (Table 4). Hence, it appears that there was stability in N, P, and K between infected sterilized and inoculated soils in response to *Ganoderma* infection.

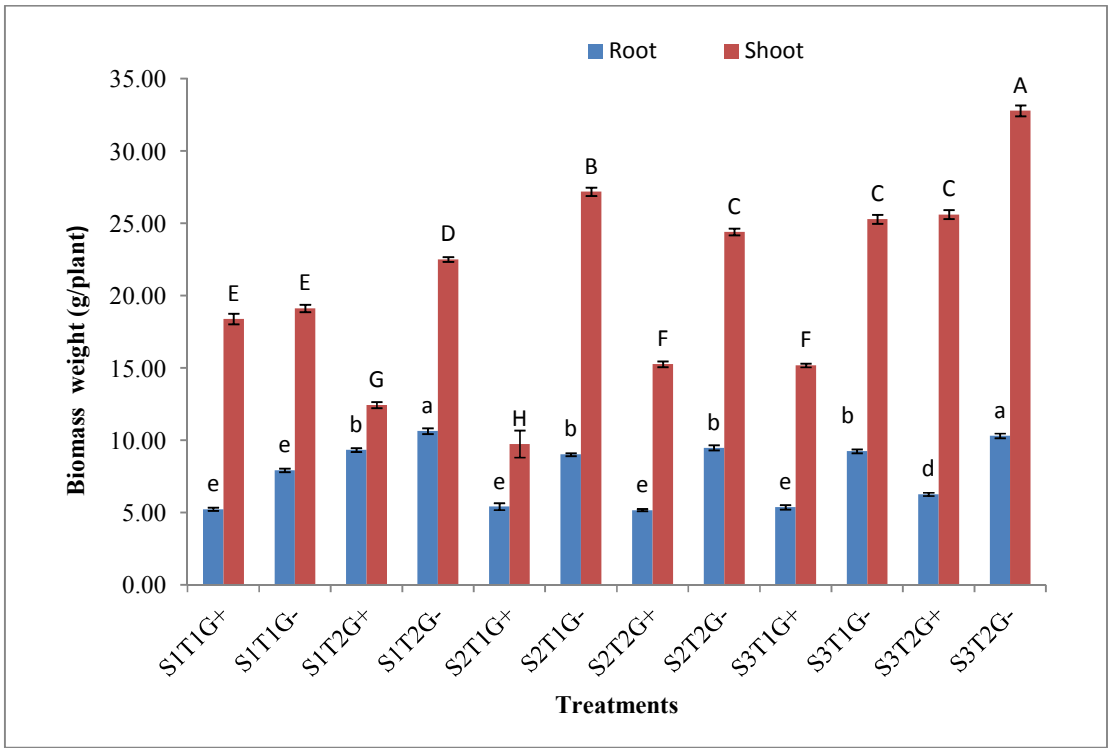
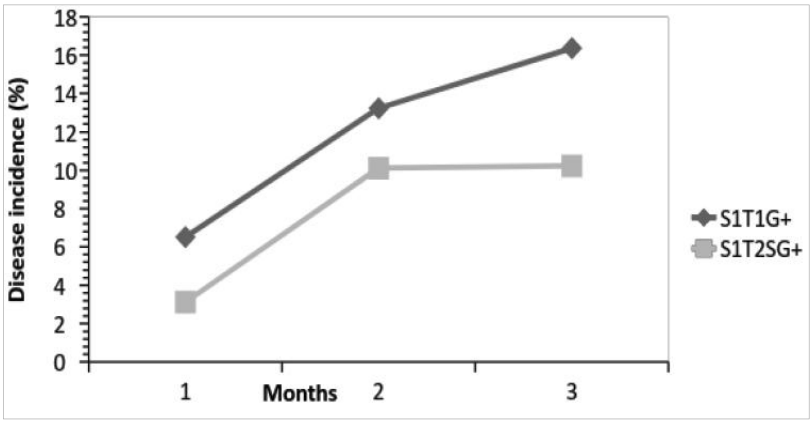


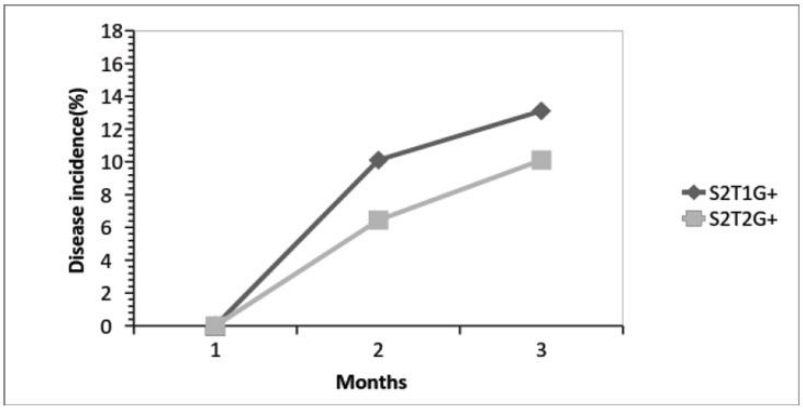
Fig. 1. Effect of *Ganoderma* and soil sterilization on Root and shoot dry weights of oil palm grown on three soils

S1= Ganoderma infected soil, S2= Non-Ganoderma infected soil, S3= Forest soil, T1=Sterilized, T2= Non-sterilized, G+ = with Ganoderma inoculation, G- = without Ganoderma inoculation. Capital letters are for shoot biomass and the small letters are for root biomass. Means with same letters are not significantly different at P ≤ 0.05

a) *Ganoderma* infected soil



b) Non-infected soil



c) Forest soil

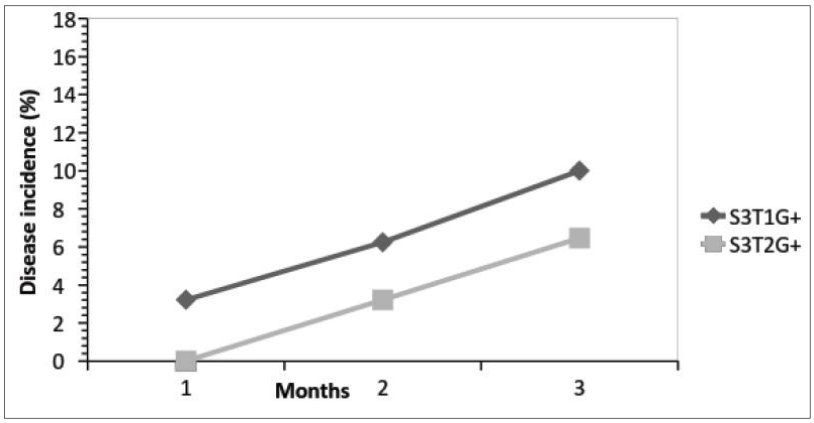


Fig. 2. Effect of *Ganoderma* and soil sterilization on incidences of *G. boninense* infection in oil palm seedlings grown in three soils a) forest, b) infected and c) non-infected soils

S1= Ganoderma infected soil, S2= Non-Ganoderma infected soil, S3= Forest soil, T1=Sterilized, T2= Non-sterilized, G+ = with Ganoderma inoculation, G- = without Ganoderma inoculation. Means with same letters are not significantly different at P ≤ 0.05

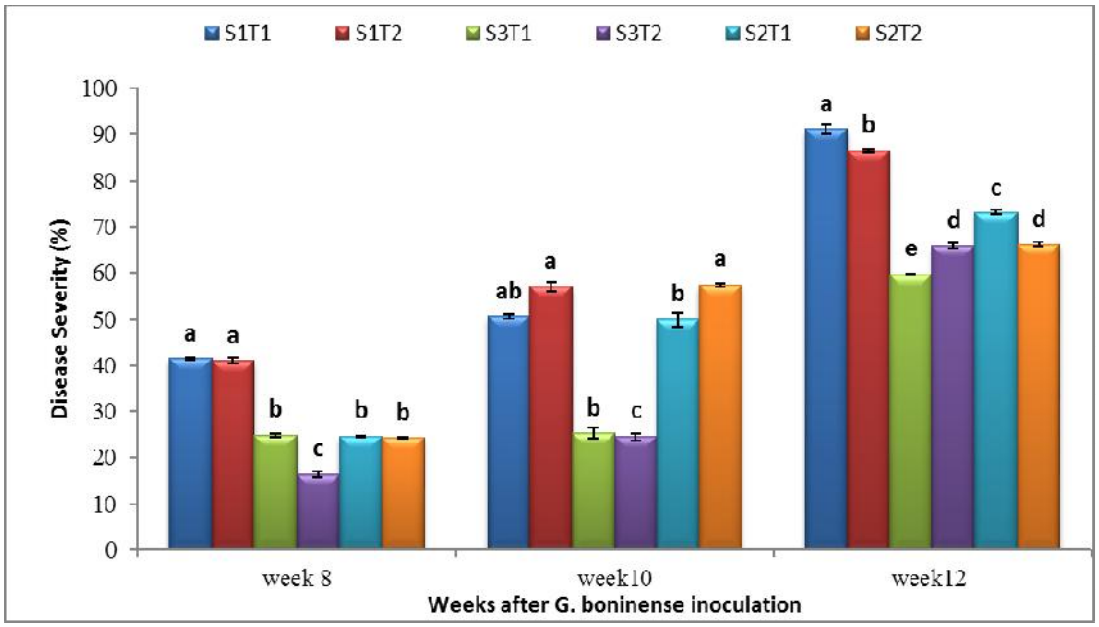


Fig. 3. Disease severity in response to *Ganoderma* growth on forest, infected and non-infected soils from 8-12 weeks

S1= Ganoderma infected soil, S2= Non-Ganoderma infected soil, S3= Forest soil, T1=Sterilized, T2= Non-sterilized, G+ = with Ganoderma inoculation, G- = without Ganoderma inoculation. Means with the same letters are not significantly different at P ≤ 0.05

Table 4. Effect of *G. boninense* inoculation and soil sterilization on N, P, and K up take (mg/plant)

Treatments			N uptake (mg/plant)	P uptake (mg/plant)	K uptake (mg/plant)
S1	T1	G+	463 ± 0.007^{de}	35 ±0.0005^{c}	337 ±0.009^{ed}
		G-	449 ±0.006^{e}	29 ±0.0006^{d}	286 ±0.007^{ef}
	T2	G+	337 ±0.005^{f}	15 ±0.0003^{e}	236 ±0.004^{fg}
		G-	499 ±0.022^{cde}	39 ±0.0002^{bc}	407 ±0.007^{bc}
S2	T1	G+	181 ±0.014^{g}	13 ±0.0012^{f}	146 ±0.016^{h}
		G-	649 ±0.009^{b}	45 ±0.0005^{b}	349 ±0.016^{cd}
	T2	G+	322 ±0.003^{f}	24 ±0.0004^{ed}	286 ±0.024^{ef}
		G-	528 ±0.005^{cd}	36 ±0.0004^{bc}	453 ±0.008^{b}
S3	T1	G+	326 ±0.006^{f}	23 ±0.0006^{e}	197 ±0.004^{gh}
		G-	610 ±0.013^{b}	44 ±0.0011^{bc}	265 ±0.005^{f}
	T2	G+	538 ±0.027^{c}	42 ±0.0008^{bc}	350 ±0.003^{cd}
		G-	724 ±0.023^{a}	60 ±0.0014^{a}	605 ±0.011^{a}

S1= Ganoderma infected soil, S2= Non-Ganoderma infected soil, S3= Forest soil, T1=Sterilized, T2= Non-sterilized, G+ = with Ganoderma inoculation, G- = without Ganoderma inoculation. Means in a column with the same letters are not significantly different at P ≤ 0.001

3.6 Plant Physiological Activities

The plant photosynthesis was equally affected by *Ganoderma* infection. The photosynthesis varied considerably among all treated groups. The highest photosynthesis (10.56 μ mol photon m^{-2} s^{-1}) was found in *Ganoderma* infected soil of non-sterilized and *Ganoderma* inoculated then

non-infected (7.12 μ mol photon m^{-2} s^{-1}) unsterilized and non-inoculated soil (Table 5 and Fig. 4). The cellulose percentage varied among the treatments. The highest cellulose (35.28 %) was found in the forest sterilized non-inoculated soil (Table 5). However, there were no significant differences found among the rest of other treatments. Moreover, the lignin contained the young seedlings showed an increase from *Ganoderma* infection soil to the non-infected soil (Table 5). The maximum lignin (27.28%) was significantly ($P < 0.05$) found in the non-infected sterilized soil with non-sterilized and non-inoculated treatments, whereas it varied among the other treatments (Table 5).

4. DISCUSSION

The soil sterilization exposed the seedlings to *Ganoderma* infection by decreasing the soil suppressiveness when the plants of the forest, infected, and non-infected soils were compared. The seedlings of sterilized and non-sterilized soils inoculated with *G. boninense*, exhibited clear negative effects of soil biotic response on the seedlings growth, as previously reported [31]. Non-inoculated (G-) plants grown on sterilized soils generally had positive effect on the plant growth than inoculated (G+) soils. The sterilization destroyed the microbial diversity in the soil (forest, non-*Ganoderma*, and *Ganoderma* infected soils) which in turn reduced the soil capacity of disease suppressiveness and increased the pathogenic activities as indicated by the progress of *Ganoderma* disease in the seedlings. The populations of bacteria, fungi, and actinomycetes varied among the three soils and were affected by the *Ganoderma* infection and soil sterilization.

Disease suppressive soil contained higher indigenous microbial diversity when its beneficial microorganism was destroyed, and the pathogenic microbes increased via recolonization [17]. The competition between soil microflora and fungi can decrease their activity in the soil [32]. The presence of soil microorganisms influences the functions and development of plant [33]. Feedback of soil biota contributes to plant rarity and invasiveness in the communities [34]. As a result of eradication of the indigenous soil microbes by sterilization elevate the soil pathogens as a result of the killing of the beneficial microbes that may have directly participated in the suppression of growth and development of *G. boninense* in the seedlings. The present results agreed with previous studies that these microbial activities are directly correlated with suppression of *G. boninense* development in the soil. Alabouvette et al. [35] reported that disease suppression and expression in natural soil, such as the forest soil in the present study, occurred with different percentage of incidence and severity. The sterilization increased the disease in all treated soils. A *G. boninense* suppressive soil can thus be described as a soil in which disease incidence and severity remain low, in spite of the presence or inoculation of *G. boninense*. Soil sterilization decreases the indigenous microbial community, and this affected the development of *Ganoderma*

Table 5. Effect *G. boninense* inoculation and soil sterilization on root cellulose and lignin content

Treatments			Cellulose (%)	Lignin (%)
S1	**T1**	G+	23.18e	16.1e
		G-	25.3d	18.5de
	T2	G+	30.76c	20.15cde
		G-	33.56 ab	27.00ab
S2	**T1**	G+	26.31d	20.43dc
		G-	31.16bc	27.60ab
	T2	G+	31.50 bc	16.98de
		G-	32.26 bc	25.28ab
S3	**T1**	G+	31.11 bc	17.70de
		G-	30.88bc	20.66 cd
	T2	G+	31.38bc	23.66bc
		G-	35.28 a	27.88 a

S1= Ganoderma infected soil, S2= Non-Ganoderma infected soil, S3= Forest soil, T1=Sterilized, T2= Non-sterilized, G+ = with Ganoderma inoculation, G- without Ganoderma inoculation. Means in a column with the same letters are not significantly different at P ≤ 0.05

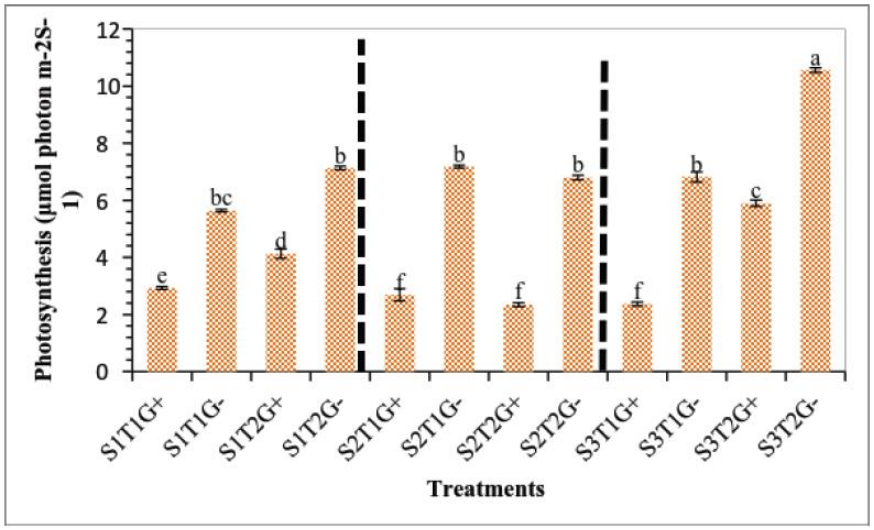


Fig. 4. Effect of *G. boninense* inoculation and soil sterilization on plant photosynthesis
S1=BSR-infected soil, S2=uninfected soil, S3= Forest soil, T1=Sterilized, T2= Non-sterilized, G+ = with Ganoderma inoculation, G- without Ganoderma inoculation. Means with the same superscript in the same week do not differ significantly at (P = .05).

in plant roots. The primary route of infections of palms by *G. boninense* has been reported to occur through direct contact of palm roots with microbial colonized debris within the soil [17]. All components such as root length, volume, surface area, root tip number, and dried root weight of *Ganoderma* infected seedlings were reduced in inoculated groups as compared to non-inoculated groups as a result of sterilization treatment. This showed that some essential biological elements in the soil which could have contributed to the growth and development of the plant were destroyed by sterilization. Olff et al. [36] revealed that sterilization improved the relative root biomass more than the shoot biomass. The growth reduction in the sterilized group (relative to non-sterilized) of *Ganoderma* infected soil was much greater than non-infected and forest soil in the present study. Forest sterilized non-inoculated seedlings had significantly higher cellulose (35.28 %) as compared to other treatments. Cellulose content (20-30%) is commonly expressed as dry weight in the primary cell wall, while in the secondary cell wall ranges from 40 to 50% [37]. No significant variation was observed among the *Ganoderma* infected plants in relation to cellulose content except for the non-infected sterilized soil, which demonstrated a slightly lower cellulose compared to other treated plants. This may be attributed to metabolism and physiological disparity. Moreover, the lignin content of the seedlings increased from *Ganoderma* infected plants to the non-infected group. This revealed that lignin synthesis was significantly affected by soil sterilization and absence of soil microbial diversity. Generally wood rot disease causes root, butt, and stem rots in plants [38]. These rots grow within the wood cells and degrade the cell wall components. White rot fungi (*Ganoderma* species) degrade lignin and other wood components [39].

Soil sterilization may have a negative effect on nitrogen fixing or nitrifying bacteria and other beneficial which result in the growth reduction of the seedlings [36]. The lower tissue N in the sterilized soil as compared to non-sterilized soils may suggest that these bacteria were killed by the sterilization treatment. Usually, plants obtain N by absorbing either nitrate or ammonium ion via the roots [40]. However, treated plants showed net negative responses to N uptake, indicating that all beneficial microbes were eliminated by the sterilization. Olff et al. [36] previously showed that increase of N levels as determined by soil sterilization was due to effects of sterilization on soil chemical properties. In addition, higher tissue N content in seedlings of non-sterilized compared with sterilized soils after inoculation showed that sterilization influenced the growth of *Ganoderma* and reduced the general growth performance of the oil palm seedlings. The soil sterilization significantly affected shoot P concentration of inoculated plants in all soil types. *Ganoderma* inoculated

plants had lower tissue P as compared to non-inoculated plants. While shoot P concentration in sterilized soil was significantly higher than non-sterilized soil. This increase may be attributed to presence of soil organic matter which is a vital reservoir for P immobilization (20 to 80%) in the soil [41], and just 0.1% of total P in soluble form is freely available for plant uptake [42]. This could be the reason why there was a negative effect on *Ganoderma* response to nutrient uptake due to sterilization treatment. Furthermore, plant tissue K concentration in the current study showed a reduced trend in all soil groups with high increased in the non-*Ganoderma* infected non-sterilized soil. These suggest that sterilization affected the growth and development of *Ganoderma* in the tissue of the infected seedlings. Similar results were observed from the earlier studies [43]. We observed that disease severity in all groups significantly increased but not uniformly within the weeks of inoculations. Seedlings grown in *Ganoderma* infected of non-sterilized soils exhibited higher disease severity as compared to other soils. This finding agreed with the results reported by El-Gali [44] that all sterilized plants had higher disease severity after treated with hot water. This can be attributed to low disease severity in the infected seedlings which showed the progress of the *Ganoderma* infection in oil palm. The seedlings start to show disease severity after two months of inoculation. This further explained that the affected seedlings might have produced dry rot of internal tissues at the base and the roots of the seedling stems. This severely limited the supply of nutrients and water to the upper part of the seedlings which later produced yellowing and wilted leaves. Disease suppression in seedlings of *Ganoderma* infected but sterilized soil was mainly due to the induction of the seedling's defense mechanism such as lignified cell walls, to create a barrier for pathogen and the production of antifungal metabolites to slow down the progress of infection which increases growth and plant strength [45]. There was no significant difference between the sterilized and non-sterilized plants in term of the rate of photosynthesis. However, *Ganoderma* inoculated plants exhibited a decrease photosynthesis as compared to non-inoculated plants. This implied that sterilization affected the photosynthesis of plants inoculated with *Ganoderma*. Plants synthesize up to 42% photosynthesis that reaches the roots into the rhizosphere [46], diseased plants showed lower photosynthesis. The absence of microbial population in the root region after the sterilization may be due to lack of nutrient exchange between the plants and the differences in indigenous microbial communities surrounding the root, which may have an impact on the photosynthesis.

5. CONCLUSION

The indigenous microbial community was significantly destroyed by sterilization resulted in growth reduction of oil palm seedlings. The seedlings inoculated with *Ganoderma* showed higher disease severity and lower shoot yield as compared to seedlings of forest soil. The *Ganoderma* infected seedlings demonstrated lower N, P, K, cellulose, lignin, and photosynthesis as compared to seedlings of forest and non-infected soils. Hence, soil sterilization has a negative effect on the indigenous microbial community involved in suppressing the development of *Ganoderma* in oil palm seedlings.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bridge PD. Molecular and morphological characterization of *ganoderma* in oil-palm plantings. *Ganoderma* Diseases of Perennial Crops. 2000;159. DOI:10.1079/9780851993881.0159.
2. Rees RW, Flood J, Hasan Y, Wills MA, Cooper RM. *Ganoderma boninense* basidiospores in oil palm plantations: Evaluation of their possible role in stem rots of *Elaeis guineensis*. Plant Pathology. 2012;61:567-78.
   DOI:10.1111/j.1365-3059.2011.02533.x
3. Paterson RR. *Ganoderma* disease of oil palm - A white rot perspective necessary for integrated control. Crop Protection. 2007;26:1369-76.
   DOI:10.1016/j.cropro.2006.11.009.
4. Pilotti CA. Stem rots of oil palm caused by *Ganoderma boninense*: Pathogen biology

and epidemiology. Mycopathology. 2005;159:129-37.

DOI:10.1007/s11046-004-4435-3

5. Singh G. *Ganoderma*-the scourge of oil palm in the coastal area. In Proceedings of *Ganoderma* workshop, Bangi, Selangor, Malaysia, 11 September 1990. 1991 (pp. 7-35). Palm Oil Research Institute of Malaysia.

   DOI:10.1007/978-3-319-54969-9_2.5.

6. Idris A, Kushairi A, Ismail S, Ariffin D. Selection for partial resistance in oil palm progenies to *Ganoderma* basal stem rot. Journal of Oil Palm Research. 2004;16:12-8.

7. Lim TK, Chung GF, Ko WH. Basal stem rot of oil palm caused by *Ganoderma boninense*. Plant Pathology Bulletin. 1992;1:147-52.

8. Ariffin D, Idris AS, Singh G. Status of *Ganoderma* in oil palm. *Ganoderma* Disease of Perennial Crops. 2000;49-68. DOI: 10.1079/9780851993881.0049.

9. Mazliham MS, Pierre L, Idris AS. Towards automatic recognition and grading of *Ganoderma* infection pattern using fuzzy systems. Tropical Engineering and Computer Technology. 2007;19.

   DOI=10.1.1.193.4093&rep=rep1.

10. Ariffin DG, Singh Lim TK. *Ganoderma* in Malaysia-current status and research strategy. In1989 PORIM International Palm Oil Development Conference. Agriculture. 1989. September 5-9, Kuala Lumpur, Malaysia 1989 (No. L-0076). PORIM.

    DOI: 10.1007/978-3-319-54969-9_2.

11. Corley RH, Tinker PB. The oil palm. John Wiley & Sons. 2008. DOI: org/10.1017/S001447970422244X.

12. Ho CL, Tan YC. Molecular defense response of oil palm to *Ganoderma* infection. Phytochemistry. 2015;114:168-77.

    DOI: 10.1016/j.phytochem.2014.10.016.

13. Hushiarian R, Yusof NA, Dutse SW. Detection and control of *Ganoderma* boninense: Strategies and perspectives. Springer Plus. 2013;2(1):555.

    DOI:10.1186/2193-1801-2-555

14. Utomo C, Werner S, Niepold F, Deising HB. Identification of *Ganoderma*, the causal agent of basal stem rot disease in oil palm using a molecular method. Mycopathology. 2005;159:159-70.

    DOI:https://doi.org/10.1007/s11046-004-4439-z.

15. Tisné S, Pomiès V, Riou V, Syahputra I, Cochard B, Denis M. Identification of *Ganoderma* disease resistance loci using natural field infection of an oil palm multiparental population. G3: Genes, Genomes, Genetics. 2017; 7(6):1683-92. DOI: 10.1534/g3.117.041764.

16. Flood J, Keenan L, Wayne S, Hasan Y. Studies on oil palm trunks as sources of infection in the field. Mycopathology. 2005;159:101-7.

    DOI: 10.1007/s11046-004-4430-8.

17. Bezemer TM, De Deyn GB, Bossinga TM, Van Dam NM, Harvey JA, Van der Putten WH. Soil community composition drives aboveground plant–herbivore–parasitoid interactions. Ecology Letters. 2005;8:652-61.

    DOI:10.1111/j.1461-248.2005.00762.x

18. Janvier C, Villeneuve F, Alabouvette C, Edel-Hermann V, Mateille T, Steinberg C. Soil health through soil disease suppression: which strategy from descriptors to indicators?. Soil biology and Biochemistry. 2007;39:1-23.

    DOI: 10.1016/j.soilbio.2006.07.001.

19. Leon MC, Stone A, Dick RP. Organic soil amendments: Impacts on snap bean common root rot (*Aphanomyes euteiches*) and soil quality. Applied Soil Ecology. 2006;31:199-210.

    DOI :10.1016/j.apsoil.2005.05.008.

20. Islam KR, Weil RR. Microwave irradiation of soil for routine measurement of microbial biomass carbon. Biological Fertilization of Soils. 1998;27:408-16.

    DOI: 10.1007/s003740050451.

21. Drenovsky RE, Duncan RA, Scow KM. Soil sterilization and organic carbon, but not microbial inoculants, change microbial communities in replanted peach orchards. Research Article. 2005;59:3.

    Available:http://CaliforniaAgriculture.ucop.edu

22. Rees RW, Flood J, Hasan Y, Wills MA, Cooper RM. *Ganoderma boninense* basidiospores in oil palm plantations: evaluation of their possible role in stem rots of *Elaeis guineensis*. Plant Pathology. 2012;61:567-78.

23. Benson HJ. Microbiological applications 8th Edition. New York: McGraw Hill. Bacterial Population Counts. 2002;87.
DOI: 10.12691/jaem-2-4-1.

24. Naidu Y, Siddiqui Y, Rafii MY, Saud HM, Idris AS. Biodegradation of basal stem rot-affected oil palm stumps by white-rot Hymenomycetes: Potential disease management by prevention of inoculum spread. The 9th Australasian Soilborne Diseases Symposium, Lincoln University, Canterbury, New Zealand. 2016.

25. Wong L, Bong CF, Idris AS. *Ganoderma* species associated with basal stem rot disease of oil palm. American Journal of Applied Sciences. 2012;9(6):879-85.
DOI: 10.3844/ajassp.2012.879.885.

26. Breton F, Hasan Y, Lubis Z, De Franqueville H. Characterization of parameters for the development of an early screening test for basal stem rot tolerance in oil palm progenies. Journal of Oil Palm Research. 2006;24-36.

27. Abdullah F, Ilias GN, Nelson M, Izzati NA, Yusuf UK. Disease assessment and the efficacy of *Trichoderma* as a biocontrol agent of basal stem rot of oil palms. Science Putra (Malaysia). 2003;11:31-33.

28. Kenzo T, Ichie T, OGAWA T, Kashimura S, Hattori D, Irino KO, Kendawang JJ, Sakurai K, Ninomiya I. Leaf physiological and morphological responses of seven dipterocarp seedlings to degraded forest environments in Sarawak, Malaysia: A case study of forest rehabilitation practice. Tropics. 2007;17:1-6.
DOI.org/10.3759/tropics.17.1.

29. Kenzo T, Yoneda R, Matsumoto Y, Azani AM, Majid MN. Growth and photosynthetic response of four Malaysian indigenous tree species under different light conditions. Journal of Tropical Forest Science. 2011;271-81.

30. Van Soest PV, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science. 1991;74:83-97.
DOI: 10.3168/jds.S0022-0302(91)78551-2.

31. Kardol P, Cornips NJ, van Kempen MM, Bakx-Schotman JM, van der Putten WH. Microbe-mediated plant–soil feedback causes historical contingency effects in plant community assembly. Ecological monographs. 2007;77:147-62.
DOI: 10.1890/06-0502.

32. Ruiz-Lozano JM, Azcón R. Specificity and functional compatibility of VA mycorrhizal endophytes in association with Bradyrhizobium strains in Cicer arietinum. Symbiosis (USA); 1993.
DOI:10.1111/j.1365- 294X.2009.04359.x

33. Linderman RG. Vesicular-arbuscular mycorrhizae and soil microbial interactions. Mycorrhizae in Sustainable Agriculture. 1992;45-70.

34. Klironomos JN. Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature. 2002;417:67-70.
DOI:10.1038/417067a.

35. Alabouvette C, Hoeper H, Lemanceau P, Steinberg C. Soil suppressiveness to diseases induced by soilborne plant pathogens. Soil biochemistry. 1996;9:371-413.
DOI: 10.12691/ajmr-5-1-2.

36. Olff H, Hoorens B, De Goede RG, Van der Putten WH, Gleichman JM. Small-scale shifting mosaics of two dominant grassland species: The possible role of soil-borne pathogens. Oecologia. 2000;125:45-54.
DOI: 10.1007/PL00008890.

37. Mellerowicz EJ, Baucher M, Sundberg B, Boerjan W. Unravelling cell wall formation in the woody dicot stem. In Plant Cell Walls 2001 (pp. 239-274). Springer Netherlands.
DOI:10.1023/A:1010699919325.174.

38. Arya A, Perelló AE, editors. Management of fungal plant pathogens. Cabi; 2010.
DOI: 10.1079/9781845936037.0000.

39. Goh KM. Induction of defence response in lignin biosynthesis of Elaeis guineensis during an interaction with *Ganoderma* boninense (Doctoral dissertation, University of Nottingham Malaysia Campus); 2016.

40. Lovelock CE, Feller IC, Ball MC, Engelbrecht BM, Ewe ML. Differences in plant function in phosphorus- and nitrogen-limited mangrove ecosystems. New Phytologistry. 2006;172:514-22.
DOI: 10.1111/j.1469-8137.2006.01851.x.

41. Richardson AE. Soil microorganisms and phosphorus availability. Soil Biota. 1994;50:35-9.
DOI: 10.12691/ajn-4-2-1.

42. Zou X, Binkley D, Doxtader KG. A new method for estimating gross phosphorus mineralization and immobilization rates in soils. Plant and Soil. 1992;147:243-50.
DOI: 10.1007/BF00029076.

43. Alemán F, Nieves-Cordones M, Martínez V, Rubio F. Root K+ acquisition in plants: The Arabidopsis thaliana model. Plant Cell Physiology. 2011;52:1603-12.
DOI: 10.1093/pcp/pcr096.

44. El-Gali ZI. Comparison of natural soil sterilization methods and their effects on soil inhabitant fungi. Nature Science. 2014;12:72-8.

45. Hammerschmidt R, Kuc J, editors. Induced resistance to disease in plants. Springer Science and Business Media. 2013.
DOI:http://dx.doi.org/10.1007/978-94-015-8420-3.

46. Balakrishnan K, Rajendran C, Kulandaivelu G. Differential responses of iron, magnesium, and zinc deficiency on pigment composition, nutrient content, and photosynthetic activity in tropical fruit crops. Photosynthesis. 2000;38:477-9.
DOI:10.1023/A:1010958512210.

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