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Inhibition of Dark Septate Endophyte Fungus Metabolites Against Oil Palm Basal Stem Rot Disease Caused by Ganoderma boninense.

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Abstract

The extensification of oil palm plantations in Indonesia has been accompanied by an increase in the occurrence of Basal Stem Rot (BSR) disease caused by *Ganoderma boninense*. Biological control is one of the alternative efforts to suppress *G. boninense* infection by the application of biological control agents such as Dark Septate Endophyte (DSE) fungus. This research aimed to assess the potential of three DSE fungi metabolites to control *G. boninense* in oil palm trees and to detect the active ingredients contained therein. The abilities of filtrates from three DSE fungi, i.e. *Cladophialophora nyingchiensis* S5.1, *Exophiala pisciphila* S1.4, and *Diaporthe pandanicola* TM.1.1 were evaluated in vitro for their suppression to *G. boninense* at concentrations of 0%, 15%, 30%, 45%, 60%, and 75% (v/v). Filtrates were also evaluated in vivo using oil palm seedlings with treatments consisting of positive and negative controls, once and/or twice applications of DSE filtrate with concentrations of 27% and 75%. At the end of the experiment, the seedlings were disassembled to evaluate the severity of internal symptoms. *C. nyingchiensis* S5.1 incubated in Potato Dextrose Broth (PDB) medium for two weeks produced most effective filtrate (code: S5.1) with IC55 value was the lowest, i.e. up to 43.78%. The filtrate of S5.1 at concentration of 75% caused the highest colony inhibition of *G. boninense* up to 88.63%. Twice applications with 75% of S5.1 filtrate significantly increase plant height and stem diameter. Filtrate isolate S5.1 contains metabolite compounds *Linolein* and *5- Oxoproline* which can act as antifungals that play a role in inhibiting the growth of *Ganoderma boninense.*

Introduction

Indonesia is the largest producer of palm oil (*Elaeis guineensis* Jacq.) in the world with total production reaching 45.5 million metric tons/year or almost 69.7% of total oil palm production in the world (USDA 2022). Susanto *et al*. (2005) stated that basal stem rot (BSR) can affect oil palm production because it causes the death of up to 50% of the entire oil palm population per hectare. Losses arising from every 1% *G.boninense* attack on Indonesian palm oil plantations are equivalent to 256 million USD per year (Morel et al. 2016). The disease caused by the fungus *G. boninense* is reported to have a high incidence at all growth stages of oil palm plants (Santoso 2020), with a high attack rate that can reach 60% (Bharudin et al. 2022). This disease cannot be optimally controlled because it does not show symptoms in the early period of infection and is non-specific. Specific symptoms will appear after the advanced stages of the disease and for a long time after the initial infection (Suryantini & Wulandari 2018).

Some of the controls that have been carried out to suppress *G. boninense* fungal infection include technical cultural control, planting holes in holes, making isolation trenches, and using several resistant varieties (Naher et al. 2013). Control using technical and mechanical culture is less efficient and often fails because of the characteristics of *G. boninense* as a soil-borne pathogen that has a high saprophytic ability and a very wide host range (Ress 2009). *G. boninense* is also often controlled using chemical fungicides, but this is still not successful. Apart from inconsistent results, its use also causes residues, resistance to pathogenic fungi, environmental pollution, and failure to overcome the spread of stem rot disease (Sinaga et al. 2003; Susanto 2002). Many control failures are caused by the nature of *G.*

boninense, which has several types of tools to survive in extreme conditions, such as resistant mycelium, basidiospores, and chlamydiospores, which have a wide host range. Biological control has the greatest chance of success based on the biology of *G. boninense* (Susanto, 2013). The direct mechanism involves the production of antibiosis compounds and cell wall-lysing enzymes, while the indirect mechanism is carried out by inducing plant resistance responses, triggering the formation of secondary metabolite compounds, and triggering plant growth and physiology (Gao et al. 2010).

Dark Septate endophyte (DSE) fungus is a biological agent that has the potential to control BPB in palm oil plants. DSE are a group of endophytic fungi that have melanized hyphae, form dark colonies on various agar media, and can colonize plant roots without causing disease symptoms and increase plant growth (Jumpponen 2001; Khan *et al*. 2007; Surono & Narasiwa 2017). DSE colonization has been reported to occur in approximately 600 plant species covering 320 genera and 114 families and is widespread in tropical, arctic, and mountainous areas (Jumpponen & Trappe 1998). Added by Knapp *et al*. (2015), DSE formed dark-colored colonies on a growth medium. DSE has the characteristics of dark-colored hyphae owing to the presence of melanin compounds (Bonfim *et al*. 2016).

Several studies have reported that the DSE fungus has a good ability to inhibit the development of soil-borne pathogens such as *Fusarium* sp. and *Rigidoporus microporus* (Surono & Narisawa 2017; Dalimunthe et al. 2019; Zaffan *et al.* 2019). DSE has also been reported to inhibit the growth of *G. boninense* in vitro, with an inhibition level above 50%. DSE, which acts as a biocontrol agent, can be influenced by several factors, including the production of secondary metabolites (Rahayu *et al.* 2021). Endophytic fungi have been reported to produce metabolite compounds that can inhibit and control the growth of pathogenic fungi (Suryanarayanan et al. 2009). The content of secondary metabolite compounds in endophytic fungi can be determined by liquid chromatography-mass spectrometry (LC-MS) (Siless et al. 2018). This study aimed to assess the potential of DSE filtrate metabolites from the National Research and Innovation Agency (BRIN) collection, namely *Cladophialophora nyingchiensis* isolate with isolate code S5.1, from oil palm plants from Subang (West Java), *Exophiala pisciphila* isolate with isolate code S1.4, from oil palm plants from Subang (West Java), and *Diaporthe pandanicola* isolate with isolate code TM.1, from rubber plants from Deli Serdang (North Sumatera), to control *G. boninense* on oil palm plants and evaluate their mechanisms.

Materials and methods

Preparation of Ganoderma boninense inoculum

The *G. boninense* inoculum was propagated on a sterile rubber wood substrate cubes 6 cm × 6 cm × 6 cm. *G. boninense* inoculum was prepared according to the method described by Idris et al. (2004). A piece of Potato Dextrose Agar (PDA) media growing *G. boninense* made with cork borer with diameter 1 cm Potato Dextrose Agar (PDA) medium was inoculated into heat-resistant plastic containing some pieces of sterilized rubber wood cubes, and incubated at 28°C for one to two months to produce mature inoculum.

DSE filtrate preparation

Production of metabolite compounds contained in the DSE filtrate was carried out according to the method described by Achmad (1997). Each isolate of DSE fungus which had been re-cultured on CMMYA media was cut into four pieces using cork borer (diameter 0.5 cm) and placed each piece into an erlenmeyer flask containing 100 mL of Potato Dextrose Broth (PDB) medium. The inoculated PDB medium were incubated on incubation-shaker at a speed of 100 rpm for one, two, three and four weeks, respectively. According to the incubation time, the inoculated PDB medium was centrifuged at 6000 rpm for 20 min to harvest the supernatants. The supernatants were filtered sterilized with a 0.2 µm syringe to obtain a DSE filtrate.

Testing the effectiveness of DSE filtrate in vitro

This test was performed to determine the inhibitory concentration (IC) value by poisoning the media using the method described by Meyer et al. (1982). Filtrate of DSE TM.1, S1.4, and S5.1, from each incubation treatment were mixed with PDA according to the treatment concentration, namely 15, 30, 45, 60, and 75%, and without the addition of filtrate as a negative control. The mixture of PDA and filtrate was poured into a petri dish. Once solid, a piece of *G. boninense* culture with a diameter of 0.5 cm was inoculated on the media. Each treatment of concentration was repeated five times and incubated at room temperature for seven days. Observations were made every day for seven consecutive days by measuring the diameter of the *G. boninense* fungus colony. The relative inhibition (RI) of the filtrate was calculated using the formula as follow:

Relative Inhibition (RI) = $\frac{D1-D2}{D1} x 100\%$

Information:

D1 = diameter of pathogenic fungus hyphae as negative control (cm)

D2 = diameter of pathogenic fungus hyphae as treatment (cm)

Data of RI was then analyzed using POLO PC program (Finney 1971) to obtain inhibition concentration of 50% (IC_{50}).

Identification of compounds content in the filtrate using LCQTOF-MS/MS

The selected filtrate was chosen for metabolites analysis using qualitative tandem liquid chromatography quadrupole time of flight mass spectrometry (LCQTOF-MS/MS) (Xevo G2-XS Waters, USA). The process was started by filtering the filtrate through a 0.2 µM membrane and directly injected to the LCQTOF-MS/MS for 10 µl with flowrate 0.6 ml/min. The ionization mode used in this LCQTOF-MS/MS was Electrospray ionization (ESI) with negative and positive modes at 6 eV, while the MS profile was $TofMS^E$ with acquisition range 50-1200 Da. The separation system used was C18 column with

column and autosampler temperature was 40 and 15 ^{0}C, respectively. Meanwhile, the mobile phase was 0.1% formic acid in aquades and 0.1% formic acid in acetonitrile for 20 minutes. The metabolites identification was carried out by comparing the fragmentation MS spectra of sample with database through UNIFI software. The criteria of identification process were mass error ≤ 5 ppm, isotope match MZ RMS ≤ 6 ppm and 10%, intensity of analyte ≥ 300, and fragment match ≥ 1.

Testing the effectiveness of DSE filtrate in vivo

Preparation of planting media

The planting medium used in the test was a mixture of topsoil and sand in a 1:1 ratio, which was first sterilized. A total of 5 kg of planting medium was placed in a polybag measuring 40 × 50 cm.

Inoculation of G. boninense and seedling planting

G. boninense inoculation was performed simultaneously with planting of oil palm seedlings. The method used was to create a planting hole in a polybag, immerse it in *G. boninense*, stick it tightly to the plant roots to a depth of 10 cm, and then cover it with the soil. The oil palm seeds used for testing in the greenhouse were three-month-old healthy seedlings of the DXP Sriwijaya variety.

Application of S5.1 endophytic fungus filtrate

The application of the S5.1 endophytic fungus filtrate was carried out using one experiment and two waterings. Watering is carried out when planting oil palm seedlings and inoculating pathogens. The second watering is carried out one week after the first watering.

Experimental design

Experiments in the greenhouse were carried out using a Randomized Complete Block Design (RCBD). The experiment consisted of six treatments and five replications (Table 1). Each treatment in each replication consisted of three plant units so the total number of plant seeds tested in one experiment was 90 seeds. Experimental treatments include:

Table 1
Treatment S5.1 filtrate testing on oil palm seedlings

Treatment codes	Treatments
F27A	Watering once with 27% of filtrate
F27B	Watering twice with 27% of filtrate
F75A	Watering once with 75% of filtrate
F75B	Watering twice with 75% of filtrate
PC	Positive control (plant + *G.boninense*)
NC	Negative control (plant)

Table 1 Treatment S5.1 filtrate testing on oil palm seedlings

Observations were made on plant growth, namely: plant height, number of leaves, and stem diameter, carried out every two weeks for 4 MAI (months after inoculation). Observations of disease incidence and severity were carried out every two weeks for 4 MAI (months after inoculation) by observing external symptoms on oil palm seedlings with external disease category values based on Rakib et al. (2015). At the end of the experiment, destructive disease incidence and severity were observed by observing internal symptoms in oil palm seedlings with internal disease category values based on Peng et al. (2022).

Table 2 Scoring of oil palm seedlings infected with *G. boninense* based on the external symptoms of oil palm seedlings (Rakib et al. 2015)

Table 2
Scoring of oil palm seedlings infected with *G. boninense* based on the external symptoms of oil palm seedlings (Rakib et al. 2015)

Score	Symptoms of *G. boninense* attack on oil palm seedlings
0	Healthy plants, green leaves, and no fungal masses forming on any part of the plant.
1	There are 1–3 leaves that experience chlorosis and no fungal mass is formed on all parts of the plant.
2	There are fungal masses accompanied or not accompanied by symptoms of chlorosis.
3	There are more than 3 leaves that are experiencing symptoms of chlorosis, there are leaves that are necrotic, whether accompanied or not accompanied by the formation of fungal masses on parts of the plant.
4	There are at least 4 leaves experiencing chlorosis or necrosis, whether accompanied or not accompanied by the formation of fungal masses.
5	Plants die, whether accompanied or not accompanied by the formation of fungal masses.

Table 3 Scoring of oil palm seedlings infected with *G. boninense* based on internal symptoms in the tubers and roots of oil palm seedlings (Peng et al. 2022)

Table 3
Scoring of oil palm seedlings infected with *G. boninense* based on internal symptoms in the tubers and roots of oil palm seedlings (Peng et al. 2022)

Score	Symptoms of *G. boninense* attack on oil palm seedlings
0	There are no necrotic symptoms on the roots and base of the stem
1	There is necrosis on the roots but not yet at the base of the stem
2	There is necrosis in the roots, necrosis is starting to occur in the parts stem base < 5%
3	There is necrosis at the roots, necrosis at the base of the stem 5%-25%
4	There is necrosis at the roots, necrosis at the base of the stem > 25%, *G. boninense* fruit bodies appear at the base of the stem, necrotic to death.

After knowing the value of the disease category, the incidence and severity of the disease were determined using the following formula (Cooke 2006):

$$\mathbf{DI}(\%) = \frac{n}{N} X 100\%$$

Note

DI = disease incidence; n = number of infected plants, N = number of plants observed.

$$\mathrm{DS} \quad (\%) = \frac{\sum(ni \; x \; vi)}{N \; X \; Z} \; X \; 100\%$$

Note

DS = disease severity (%); ni = number of infected plants at score i; vi = ith score value; N = number of plants observed; Z = highest score in the scoring reference.

Statitistical analyses

Data from in vitro testing in the laboratory and in vivo testing in the greenhouse were tabulated using Microsoft Excel 2019 and analyzed using the POLO PC program and analysis of variance (ANOVA). Significantly different treatments were tested using Tukey and Dunnett tests at the 5% level using SAS 9.4 software.

Results

Effectiveness of DSE filtrate in vitro

Effect of DSE filtrate in inhibiting G. boninense .

The results showed that the best incubation period for the filtrate based on the IC_{55} value from the calculation of the Relative Resistance Level (THR) on the seventh day was to incubate the filtrate for 2 weeks. This was indicated by the lowest concentration value with the addition of S5.1 isolate filtrate 43.78% which was able to suppress *G. boninense* (Fig. 1). The results of this test selected the two best isolate filtrates with the highest inhibition which will be further tested, namely the TM.1 and S5.1 isolate filtrates. Media poisoning revealed the ability of *G. boninense* colonies to grow on PDA media with DSE filtrate concentrations ranging from 15–75% (Fig. 2). The growth diameter of each *G. boninense* colony with the addition of S5.1 and TM.1 filtrate at 2 MSI showed different growth rates. The relative level of inhibition with the addition of both filtrates showed an increase in inhibition every week with increasing concentrations in the media (Fig. 3). The highest inhibition (88.63%) was observed when the S5.1 filtrate was added at a concentration of 75%.

Figure 1 IC_{55} value of DSE isolate filtrate at various concentrations with a four-week incubation period; *Diaporthe* (TM.1), *Exophiala* (S1.4), and *Cladophialophora* (S5.1) Lihat koreksi

Figure 2 Growth of *G. boninense* colonies at various concentrations of S5.1 and TM.1 filtrate; S5.1 Filtrate; (A) Control, (B) 15% (C) 30%, (D) 45%, (E) 60% (F) 75% and TM.1 filtrate; (G) Control, (H) 15%, (I) 30%, (J) 45% (K) 60% and (L) 75% Lihat koreksi

Figure 3 Relative levels of inhibition of TM.1 and S5.1 filtrate against *G. boninense* media poisoning Lihat koreksi

Effect of DSE filtrate on disease severity and incidence in vivo

Effect of S5.1 filtrate treatment based on external and internal symptoms

The effect of S5.1 filtrate treatment incubated for two weeks on the severity and incidence of disease based on external symptoms is presented in Tables 2 and 3, and external symptoms began to appear in the fourth week after inoculation. The results of calculating the severity and incidence of disease externally showed that the positive control was significantly different from all S5.1 filtrate watering treatments. The symptoms that were visible during observation were that the leaves turned yellow and, over time, dried out. The seed shell was covered with *G. boninense* hyphae. According to Susanto (2013), *G. boninense* is known to be a hidden disease (cryptic disease) because it often does not cause visible symptoms in infected plants.

The severity and incidence of internal disease were observed at the end of the observation period. The positive control treatment was significantly different from the 75% S5.1 filtrate watering treatment (Table 4). Symptoms that can be seen are that the roots look brown to rot and there are signs of the

appearance of *G. boninense* fruiting bodies at the base of the stem. When the palm weevil is cut open, it can be seen that the infected weevil is yellowish to brown.

Table 4
Effect of S5.1 filtrate treatment on disease severity based on external symptoms

Treatment DSE filtrate**	External disease severity (%) at n-weeks after inoculation (WAI)			
	2 WAI	4 WAI	6 WAI	8 WAI
F27A	0,00 ± 0,00[a]	0,00 ± 0,00[a]	0,00 ± 0,00[b]	0,00 ± 0,00[b]
F27B	0,00 ± 0,00[b]	0,00 ± 0,00[a]	0,00 ± 0,00[b]	0,00 ± 0,00[b]
F75A	0,00 ± 0,00[a]	0,00 ± 0,00[a]	0,00 ± 0,00[b]	0,00 ± 0,00[b]
F75B	0,00 ± 0,00[a]	0,00 ± 0,00[a]	0,00 ± 0,00[b]	0,00 ± 0,00[b]
KP	0,00 ± 0,00[a]	5,20 ± 0,72[a]	22,6 ± 1,03[a]	30,80 ± 0,86[a]
KN	0,00 ± 0,00[a]	0,00 ± 0,00[a]	0,00 ± 0,00[b]	0,00 ± 0,00[b]

*Note: Numbers in the same column followed by different letters indicate significant differences in the Dunnet test α = 5% **F27A = watering once with 27% filtrate; F27B = watering twice with 27% filtrate; F75A = watering once with 75% filtrate; F75B = watering twice with 75% filtrate; PC = positive control with *G. boninense*; NC = negative control without *G. boninense*.

Table 4 Effect of S5.1 filtrate treatment on disease severity based on external symptoms

*Note: Numbers in the same column followed by different letters indicate significant differences in the Dunnet test α = 5% **F27A = watering once with 27% filtrate; F27B = watering twice with 27% filtrate; F75A = watering once with 75% filtrate; F75B = watering twice with 75% filtrate; PC = positive control with *G. boninense*; NC = negative control without *G. boninense*.

Table 5 Effect of S5.1 filtrate treatment on disease incidence based on external symptoms

Table 5
Effect of S5.1 filtrate treatment on disease incidence based on external symptoms

Treatment DSE filtrate**	Internal disease incidence (%) at n-weeks after inoculation (WAI)			
	2 WAI	4 WAI	6 WAI	8 WAI
F27A	0,00 ± 0,00[a]	0,00 ± 0,00[a]	0,00 ± 0,00[b]	0,00 ± 0,00[b]
F27B	0,00 ± 0,00[b]	0,00 ± 0,00[a]	0,00 ± 0,00[b]	0,00 ± 0,00[b]
F75A	0,00 ± 0,00[a]	0,00 ± 0,00[a]	0,00 ± 0,00[b]	0,00 ± 0,00[b]
F75B	0,00 ± 0,00[a]	0,00 ± 0,00[a]	0,00 ± 0,00[b]	0,00 ± 0,00[b]
KP	0,00 ± 0,00[a]	20,00 ± 1,91[a]	73,40 ± 0,45[a]	100,00 ± 0,00[a]
KN	0,00 ± 0,00[a]	0,00 ± 0,00[a]	0,00 ± 0,00[b]	0,00 ± 0,00[b]

*Note: Numbers in the same column followed by different letters indicate significant differences in the Dunnet test α = 5% **F27A = watering once with 27% filtrate; F27B = watering twice with 27% filtrate; F75A = watering once with 75% filtrate; F75B = watering twice with 75% filtrate; PC = positive control with *G. boninense*; NC = negative control without *G. boninense*.

*Note: Numbers in the same column followed by different letters indicate significant differences in the Dunnet test α = 5% **F27A = watering once with 27% filtrate; F27B = watering twice with 27% filtrate; F75A = watering once with 75% filtrate; F75B = watering twice with 75% filtrate; PC = positive control with *G. boninense*; NC = negative control without *G. boninense*.

Table 6 Severity and incidence of internal diseases

Table 6
Severity and incidence of internal diseases

Treatment DSE filtrate**	Internal disease incidence (%)	Internal disease severity (%)
F27A	13,20 ± 1,21[b]	2,80 ± 0,83[b]
F27B	0,00 ± 0,00[b]	0,00 ± 0,00[b]
F75A	0,00 ± 0,00[b]	0,00 ± 0,00[b]
F75B	0,00 ± 0,00[b]	0,00 ± 0,00[b]
KP	100 ± 0,00[a]	33,20 ± 0,60[a]
KN	0,00 ± 0,00[b]	0,00 ± 0,00[b]

*Note: Numbers in the same column followed by different letters indicate significant differences in the Dunnet test α = 5% **F27A = watering once with 27% filtrate; F27B = watering twice with 27% filtrate; F75A = watering once with 75% filtrate; F75B = watering twice with 75% filtrate; PC = positive control with *G. boninense*; NC = negative control without *G. boninense*.

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Effect of DSE filtrate treatment on agronomic characteristics of oil palm seedlings

The treatment of S5.1 filtrate which was incubated for two weeks on plant height, number of leaves, and tuber diameter are presented in Figs. 4, 5, and 6, and the mean plant height and tuber diameter showed significant differences from the control in the treatment of watering with 75% S5.1 filtrate twice (F75B). This is following the results of the S5.1 filtrate test using LCQTOF-MS/MS, which showed that there are growth regulator (IAA) components in the S5.1 filtrate metabolite compounds (Table 4). The S5.1 Filtrate S5.1 did not show significant differences with the control regarding the number of leaves, but the average number of treated leaves was higher than the control so the filtrate S5.1 is not phytotoxic to oil palm seedlings. This is also supported by data on the intensity and severity of the disease, based on internal and external symptoms.

Figure 4 Height of oil palm seedlings in the greenhouse after treatment

Figure 5 Number of leaves of oil palm seedlings in the greenhouse after treatment

Figure 6 Stem diameter of oil palm seedlings in the greenhouse after treatment

Results of analysis of metabolite compounds in the DSE filtrate using LCQTOF-MS/MS

The results of these study reported that the chromatograms of all selected filtrates have almost similar. The peak appeared at the the end of separation process, especially between 15-18.8 minutes in Figs. 7. This data was accordance with the characteristic of separation column used (C18) which relatively non-polar, thus, the compound within the peak was possible to be non-polar also. Moreover, there were several peak at < 0.5 and between 9–10 minutes on all selected filtrate. Each peak was then analyzed to obtain putative metabolites within the filtrate. All of the putative compounds within the selected filtrate was reported at Table 7. It can be seen that in all filtrate, the ion positif mode produced more metabolites dan negative mode. Other than that, the the putative metabolites in positive mode was different than negative mode. The putative metabolites in S51 on negative mode was only *5-Oxoproline*, while in positive mode was *heptadecylamine, yadanzioside A* and *P*, *decumbesterone A*, *protodiosgenin, linolein, androstenediol* and *11- O-p-coumarylnepeticin*

Table 7 Compound identification in selected filtrate : (a) Compound identification in selected filtrate on ion positif mode; (b) Compound identification in selected filtrate on ion negative mode

Table a. Compound identification in selected filtrate on ion positif mode

Observed Retention Time (min)	Component name	Formula	Precursor ion	Mass error (ppm)	Adducts	Intensity
TM-1						
0.44	Betaine	C5H11NO2	118.08599	-2.4	+H	5413
16.51	Heptadecylamine	C17H37N	256.28422	2.2	+H	1154
16.63	Furanodiene	C15H20O	217.26003	1	+H	1690
16.66	Diphenylamine	C12H11N	170.09642	-0.1	+H	3205
16.88	Protodiosgenin	C33H54O9	595.38123	-4.4	+H	9268
17.15	(3,3-Dimethyldecyl)-benzene	C18H30	247.2406	-3.7	+H	1782
17.25	11-O-p-Coumarylnepeticin	C39H56O4	589.42584	1.2	+H	10676
18.5	Linolein	C57H98O6	879.7401	-4	+H	157043
S14						
0.55	Maltol	C6H6O3	127.98351	-4.7	+H	365
0.75	Guanine	C5H5N5O	152.05621	-3.1	+H	369
16.5	Heptadecylamine	C17H37N	257.27439	0.2	+H	2119
16.88	Protodiosgenin	C33H54O9	595.4263	-4.3	+H	10996
17.13	Androstenediol	C19H30O2	291.28916	0.5	+H	1437
17.25	11-O-p-Coumarylnepeticin	C39H56O4	589.40053	1.8	+H	9474
18.51	Linolein	C57H98O6	879.73934	-4.4	+H	219192
S51						
16.5	Heptadecylamine	C17H37N	257.27438	0.5	+H	1728
16.68	Yadanzioside P	C34H46O16	685.70647	-4.4	+H	1510
16.88	Decumbesterone A	C29H46O7	507.32936	-3.8	+H	11452
16.93	Yakuchinone B	C20H22O3	311.32895	-1.8	+H	3434
16.98	Protodiosgenin	C33H54O9	595.38161	-4.9	+H	10373
17.1	Linolein	C57H98O6	879.73969	-4.4	+H	70798
17.13	Androstenediol	C19H30O2	291.23075	-2.5	+H	1560

17.24	11-O-p-Coumarylnepeticin	C39H56O4	589.42574	1.6	+H	10276

Table b Compound identification in selected filtrate on ion negative mode

Observed Retention Time (min)	Component name	Formula	Precursor ion	Mass error (ppm)	Adducts	Intensity
TM						
0.46	Raffinose	C18H32O16	503.15783	-1.9	-H	4277
16.82	(E)-9-Octadecenoic acid	C18H34O2	281.24756	-2.1	-H	325
16.84	Pseudosantonin	C15H20O4	263.12816	1.1	-H	2129
16.88	Sugiol	C20H28O2	299.20034	0.1	-H	459
16.98	Dehydroabietic Acid	C20H28O2	299.18496	-2.6	-H	418
17.3	3β-Hydroxy-12,17-diene-28-nordeane-23-al	C29H44O2	423.32575	-1.2	-H	5086
S14						
0.43	Glutamic acid	C5H9NO4	146.00894	-2.4	-H	2336
9.93	3-Acetamino-4-hydroxy benzoic acid	C9H9NO4	194.04536	-2.7	-H	1759
16.72	Dihydroxy stearic acid	C18H36O4	315.25199	-5.1	-H	1606
16.76	Oxyphyllol B	C15H22O2	233.24205	-5.3	-H	926
16.68	Dibutyl sebacate	C18H34O4	313.22992	-5.5	-H	1315
16.83	Ethyl palmitoleate	C18H34O2	281.18778	-5.3	-H	346
16.89	Sugiol	C20H28O2	299.24192	-6.1	-H	364
17.31	3β-Hydroxy-12,17-diene-28-nordeane-23-al	C29H44O2	423.32581	-2.5	-H	5384
S51						
0.47	5-Oxoproline	C5H7NO3	128.03477	-4.3	-H	1946

Figure 7 LCQTOF-MS/MS Spectra of filtrate: (a) TM; (b) S14; (c) S51

Table 7 Compound identification in selected filtrate : (a) Compound identification in selected filtrate on ion positif mode; (b) Compound identification in selected filtrate on ion negative mode

Discussion

Root stem rot (BPB) caused by *Ganoderma boninense* is one of the biggest threats to the quality and production of oil palm in Southeast Asia (Rees et al. 2009). *G. boninense* can reduce production yields by 50–80% (Corley RHV and Tinker PB 2016). The current control strategies for stem rot disease consist of physical, chemical, and biological controls. This control is intended to reduce the incidence of stem rot disease after replanting and increase the productive life of infected oil palms (Siddiqui et al. 2021). Several controls have been implemented but none have been effective and efficient. Biological control approaches to control diseases caused by fungal pathogens are currently being studied and have become an alternative option. Biological control involves the use of natural enemies to reduce the damage caused by harmful organisms or the regulation of disease populations by their natural enemies. One control strategy that can be taken to overcome the presence of pathogens is the use of biological agents (Djaenuddin & Muis 2015).

The Dark Septate Endophyte (DSE) fungus was used to control *G. boninense*. DSE fungi can be found in various places, from tropical to polar regions, and in the Alps. However, DSE generally live abundantly in coniferous forests. DSE is a heterogeneous group of fungi whose function and ecology overlap with soil fungi, saprophytic fungi, rhizoplane fungi, pathogenic fungi (obligate or facultative) and mycorrhizal fungi (Jumpponen & Trappe 1998). The fungus used in the test was the DSE fungus from the BRIN collection which has the highest level of inhibition in suppressing *G. boninense* in vitro. *Dark septate endophyte*(DSE) is useful for plants because it can produce secondary metabolites that can prevent the host plant from being attacked by pathogenic fungi. DSE fungi are capable of producing bioactive compounds such as antibacterial and antifungal compounds (Hasanah 2015). DSE fungi synergize with their host plants through a mutualistic symbiotic relationship. DSE fungi can be used to treat plant diseases. Several studies state that the DSE fungus can produce metabolite compounds to suppress the pathogenic fungus *G. boninense*

The production of fungus metabolites can be influenced by several factors, one of which is the incubation period of the filtrate (Irawati et al. 2019). According to Syarifah et al. (2021), fungi during growth and metabolite production require time to hydrolyze available nutritional sources.

The longer the incubation period, the more mycelium growth can continue to increase until a certain time limit so that metabolite production increases. In testing, the best incubation period for DSE filtrate was obtained by incubating the filtrate for two weeks. The three DSE filtrates used in this study could inhibit *G. boninense* in vitro, as shown by their relative inhibition levels. The S5.1 isolate filtrate showed the best results in inhibiting the growth of *G. boninense*. The effectiveness of the S5.1 filtrate was tested on a greenhouse scale, showing its effect on relative disease incidence and severity. Testing for four months showed that plants applied with S5.1 filtrate showed no external or internal symptoms, indicating the

influence of DSE on disease progression. This is in accordance with previous research that has reported the role of DSE in increasing tolerance to biotic stress (Mateu *et al*. 2019) and as a fungicide (Surono & Narisawa 2018). The effect of S5.1 filtrate application on agronomic growth in plants, including plant height, tuber diameter, and number of leaves, was not significantly different between treatments, but when compared with the positive control treatment, there was a significant difference in plant height and tuber diameter of oil palm seedlings. Metabolite profiling on selected filtrate was carried out using LCQTOF-MS/MS with a C18 as separation column and ionization mode were positive and negative. This technique was usually used to analyze the metabolite profiles in natural resources because the process of analysis is easy, sensitive and the identification of metabolites can be run by comparing to the database, thus it is simple (Syabana et al 2021). The identification process was carried out by determining the parent and product ions from the mass spectra of the sample and comparing them with the database contained in the UNIFI software. Moreover, The putative matabolites was also verified by comparing the fragmentation profile of the sample with previous literature related to the metabolites. *Diphenylamine* compound in the TM-1 filtrate was identified by the presence of the precursor ion at m/z 170.09642 and product ion at 153.05741, 107.08553, and 65.03456 (Schymanski *et al* 2017). Meanwhile, the fragmentation pattern of *furanodiene* in TM-1 was in agreement with Rafı et al (2022) that reported the parent and product ion at m/z 217.26003, 119.08554 and 123.11656. Other compound, *pseudoantonin* (class of sesquiterpene) was observed by the presence of parent ion at m/z 263.12816, and fragmentation ion at 225.00663, 219.13825, and 194.08209 (Trifan et al 2022). Filtrate isolate S5.1 is the best filtrate containing metabolite compounds *Linolein* and *5-Oxoproline* which can act as antifungals that play a role in inhibiting the growth of *Ganoderma boninense.*

This is in accordance with the statement that DSE fungi can help host plants in various ways, including adaptation to less favorable habitats, protection against environmental stress both biotic and abiotic, and increasing growth and nutrient absorption (Maciá-Vicente et al. 2009). Several types of DSE have been reported to have benefits in improving plant performance (He et al. 2019) and plant growth (Manalu et al. 2020), increasing tolerance to biotic and abiotic stresses (Mateu *et al*. 2019), increasing plant nutrient absorption (Yakti *et al.* 2019), and helps dissolve P elements in the soil (Baressi *et al.* 2022). Furthermore, DSE has been reported to act as a herbicide (Kumar et al. 2009), insecticide (Bahar et al. 2011), fungicide (Surono & Narisawa 2018), bactericide (Chu et al. 2019), producing secondary metabolites (Bai et al. 2019) and growth hormone (Hamayun et al. 2010), as well as as a phytoremediation (Santos *et al.* 2021), reclamation (Akhtar et al. 2022) and mitigation agent on non-optimal land (He et al. 2019).

In summary, filtrates from three DSE fungi, i.e. *Cladophialophora nyingchiensis* S5.1, *Exophialxa pisciphila* S1.4, and *Diaporthe pandanicola* TM.1 have the potential to inhibit the development of *G.boninense*. The filtrate of *C. nyingchiensis* S5.1 at concentration of 75% caused the highest colony inhibition *G. boninense* up to 88.63%. Twice application with 75% of S5.1 filtrate significantly increase plant height and stem diameter, and reduce the incidence and severity of *G.boninense.*

Declarations

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Authors' contribution

All authors contributed to the concept and design of the research flow. Material preparation, data collection and analysis were mainly carried out by Fransiska Natalia Purba, Abdjad Asih Nawangsih, Efi Toding Tondok and Surono. The secondary metabolite analysis process was conducted mainly by Mohamad Ana Syabana and Mirta Ardi Lestari. The first draft of the manuscript was written by Fransiska Natalia Purba. All authors commented and provided improvements on earlier versions of the manuscript. All authors read and approved the final manuscript.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Ethical approval Not applicable.

Conflict of Interest The authors declare that they have no conflicts of interest.

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Figures

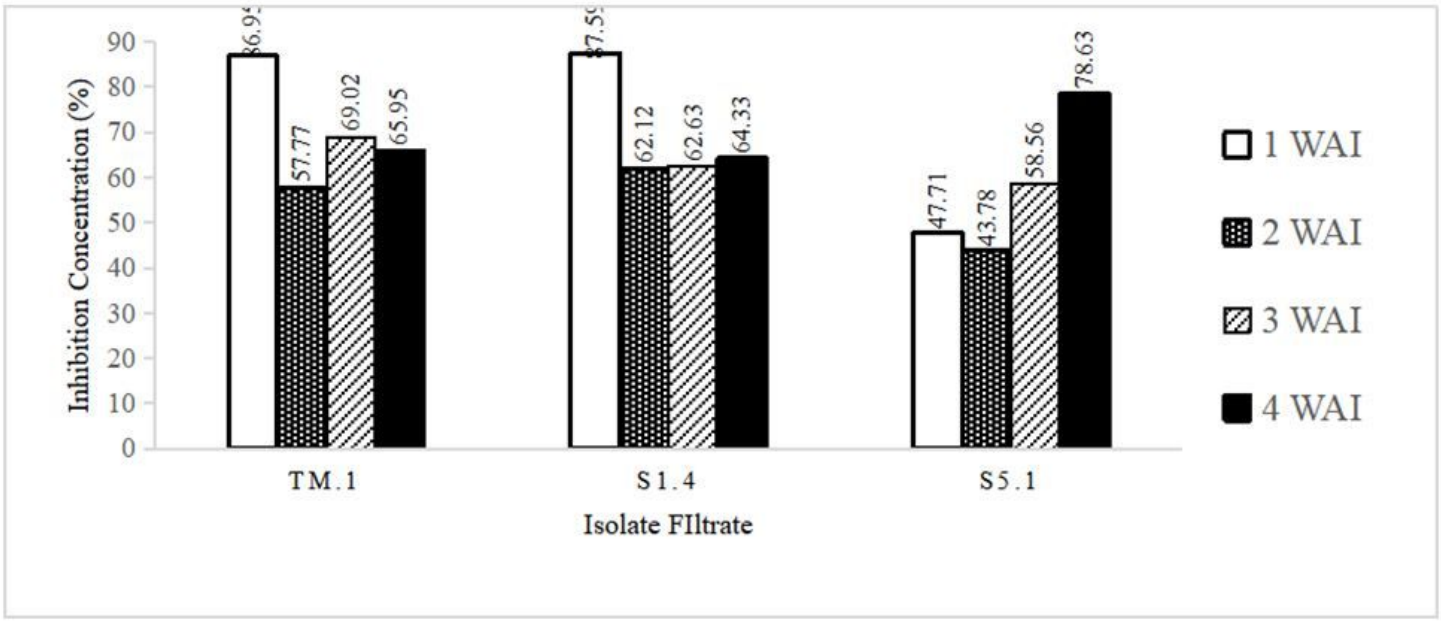


Figure 1

IC_{55} value of DSE isolate filtrate at various concentrations in four different incubation periods; *Diaporthe* (TM.1), *Exophiala* (S1.4), and *Cladophialophora* (S5.1)

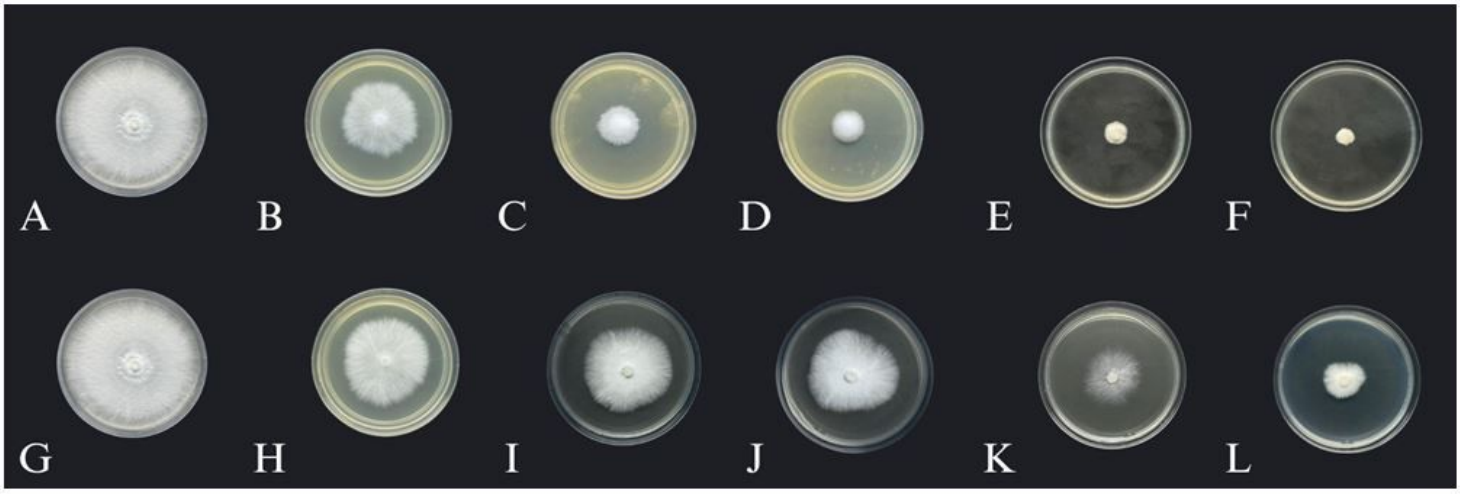


Figure 2

Growth of *G. boninense* colonies at various concentrations of S5.1 and TM.1 filtrate; S5.1 Filtrate; (A) Control, (B) 15% (C) 30%, (D) 45%, (E) 60% (F) 75% and TM.1 filtrate; (G) Control, (H) 15%, (I) 30%, (J) 45% (K) 60% and (L) 75%

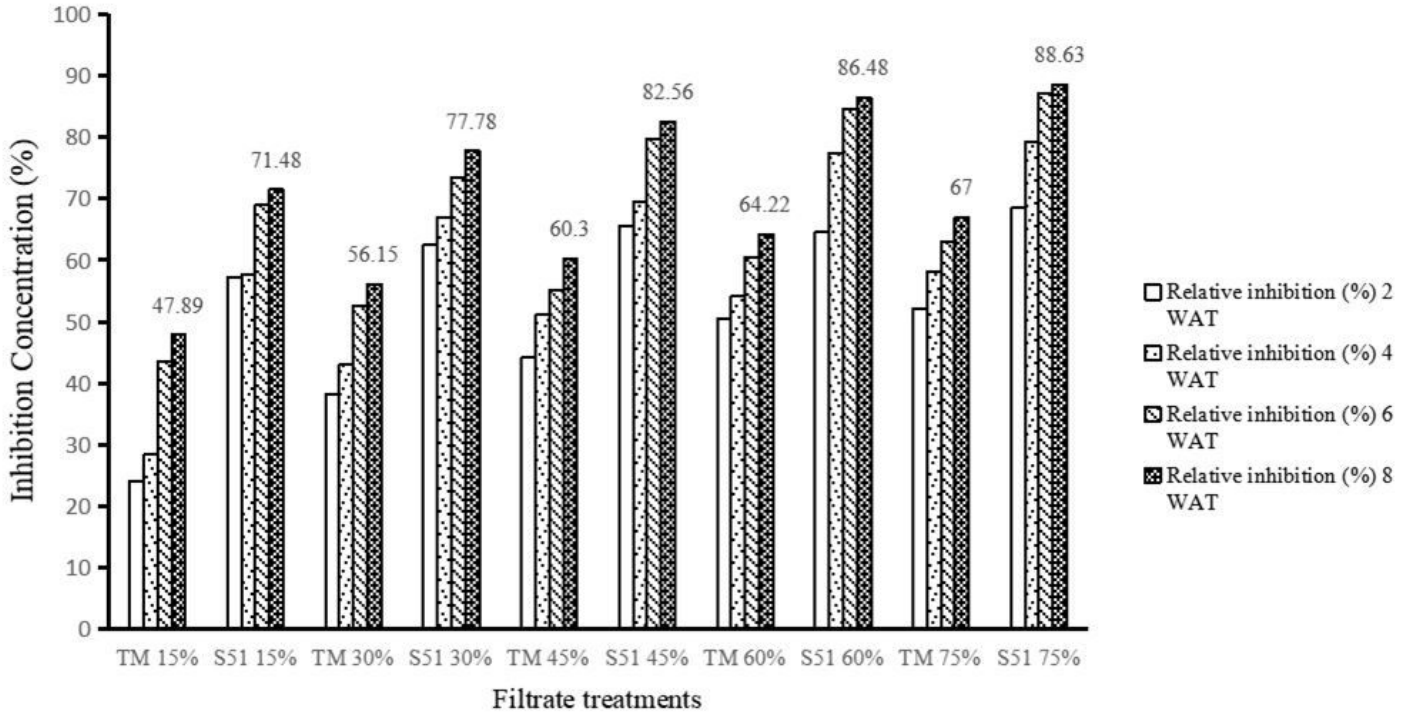


Figure 3

Relative inhibition of TM.1 and S5.1 filtrate against *G. boninense* by media poisoning

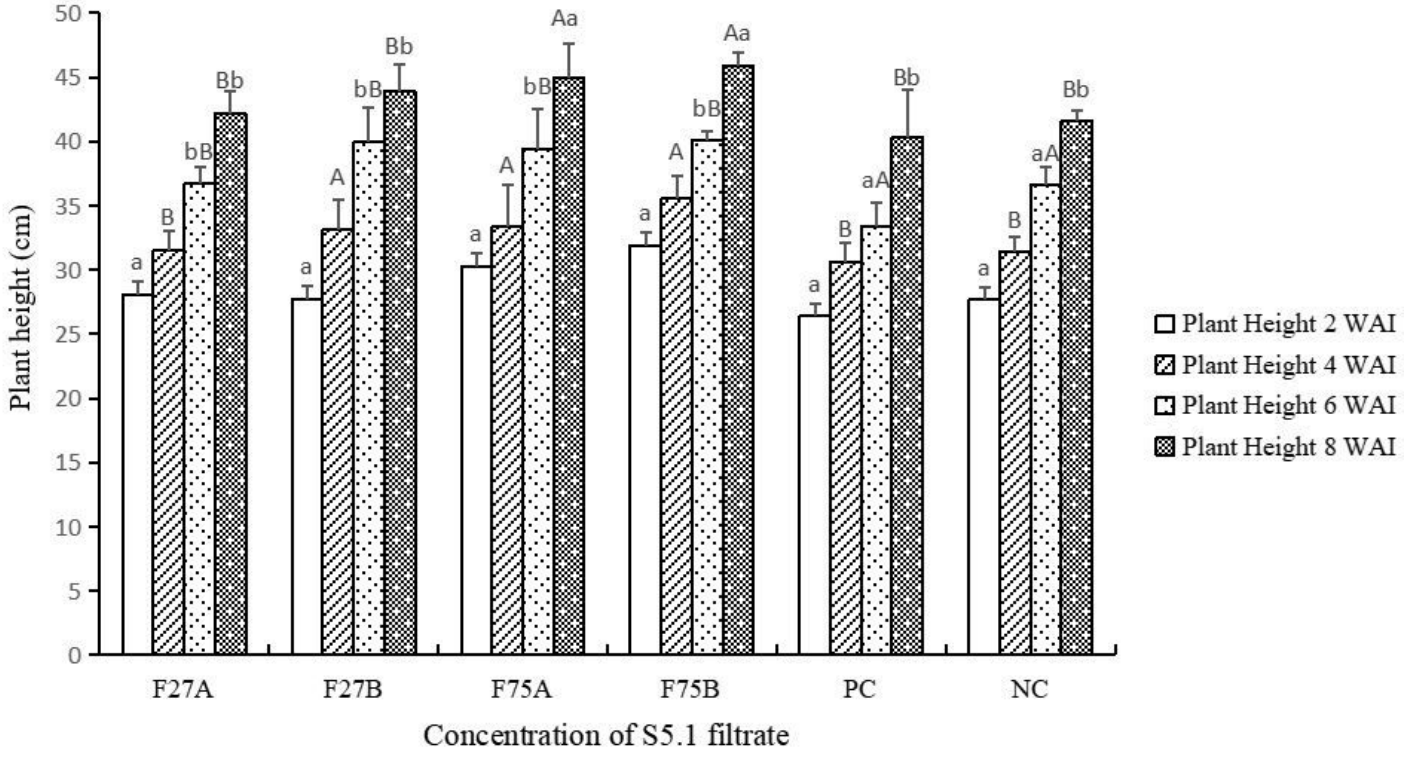


Figure 4

Height of oil palm seedlings in the greenhouse after treatment; F27A = watering once with 27% filtrate; F27B = watering twice with 27% filtrate; F75A = watering once with 75% filtrate; F75B = watering twice with 75% filtrate; PC = positive control with *G. boninense*; NC = negative control without *G. boninense*.

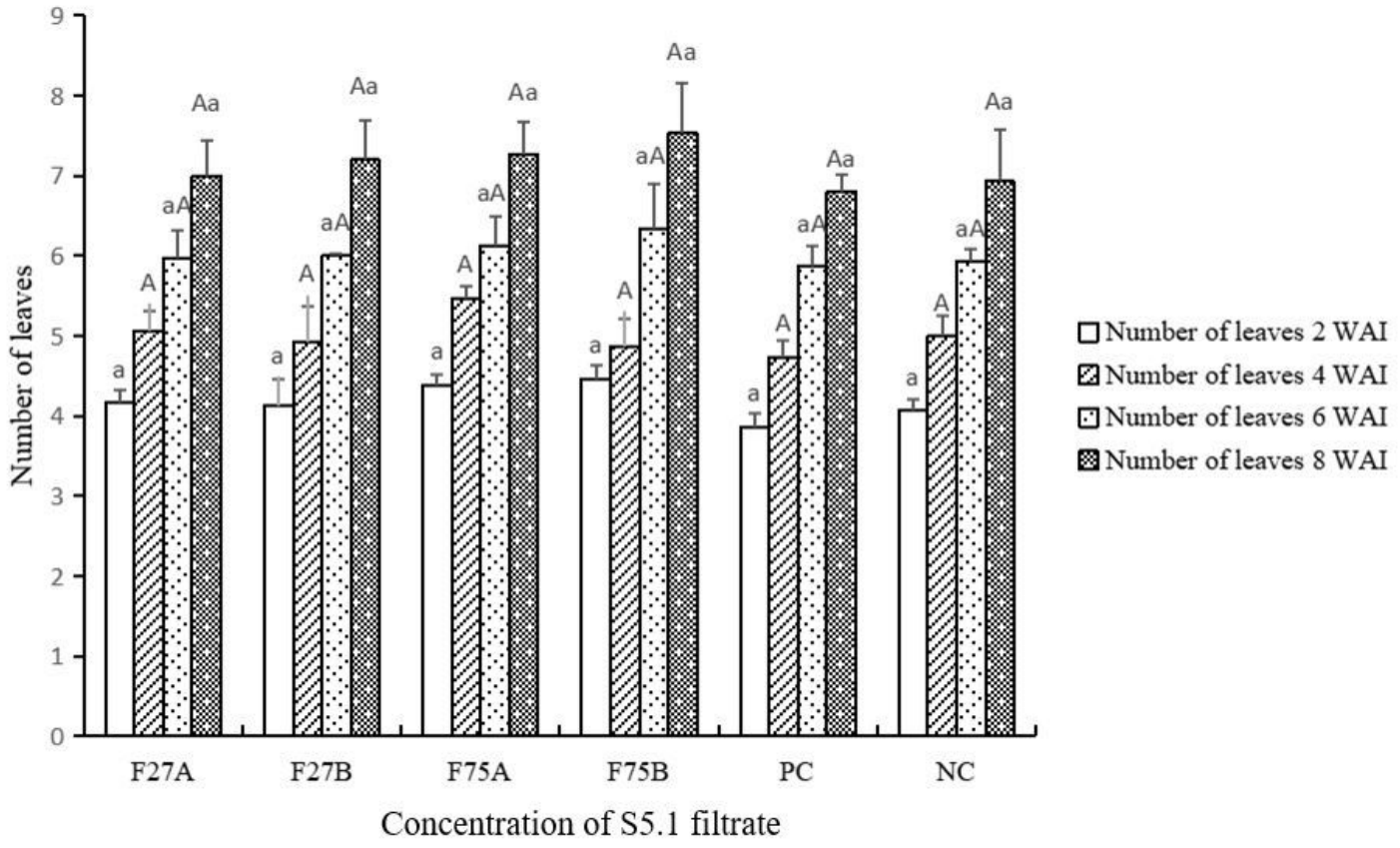


Figure 5

Number of leaves of oil palm seedlings in the greenhouse after treatment; F27A = watering once with 27% filtrate; F27B = watering twice with 27% filtrate; F75A = watering once with 75% filtrate; F75B = watering twice with 75% filtrate; PC = positive control with *G. boninense*; NC = negative control without *G. boninense*.

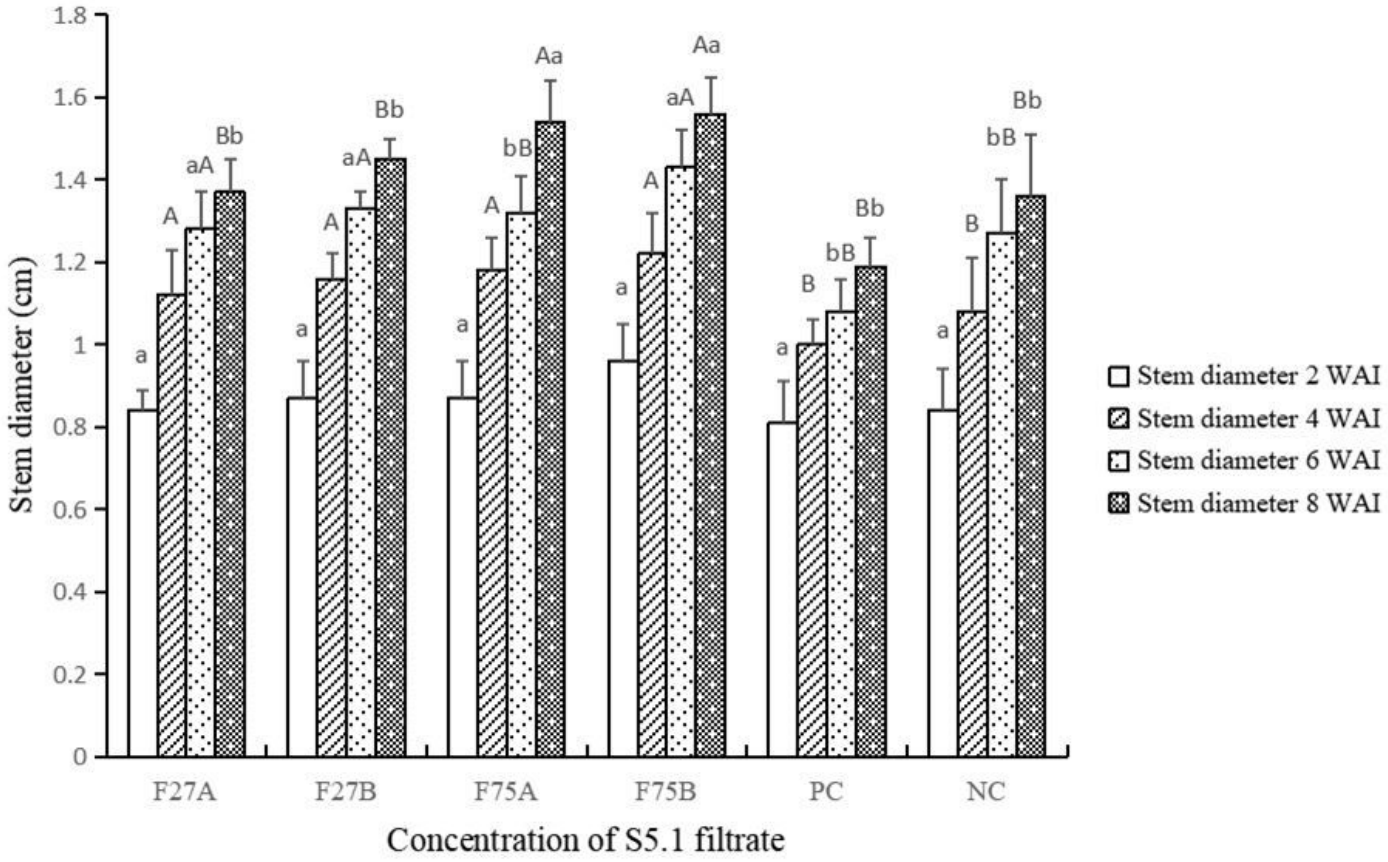


Figure 6

Stemdiameter of oil palm seedlings in the greenhouse after treatment; F27A = watering once with 27% filtrate; F27B = watering twice with 27% filtrate; F75A = watering once with 75% filtrate; F75B = watering twice with 75% filtrate; PC = positive control with *G. boninense*; NC = negative control without *G. boninense*.

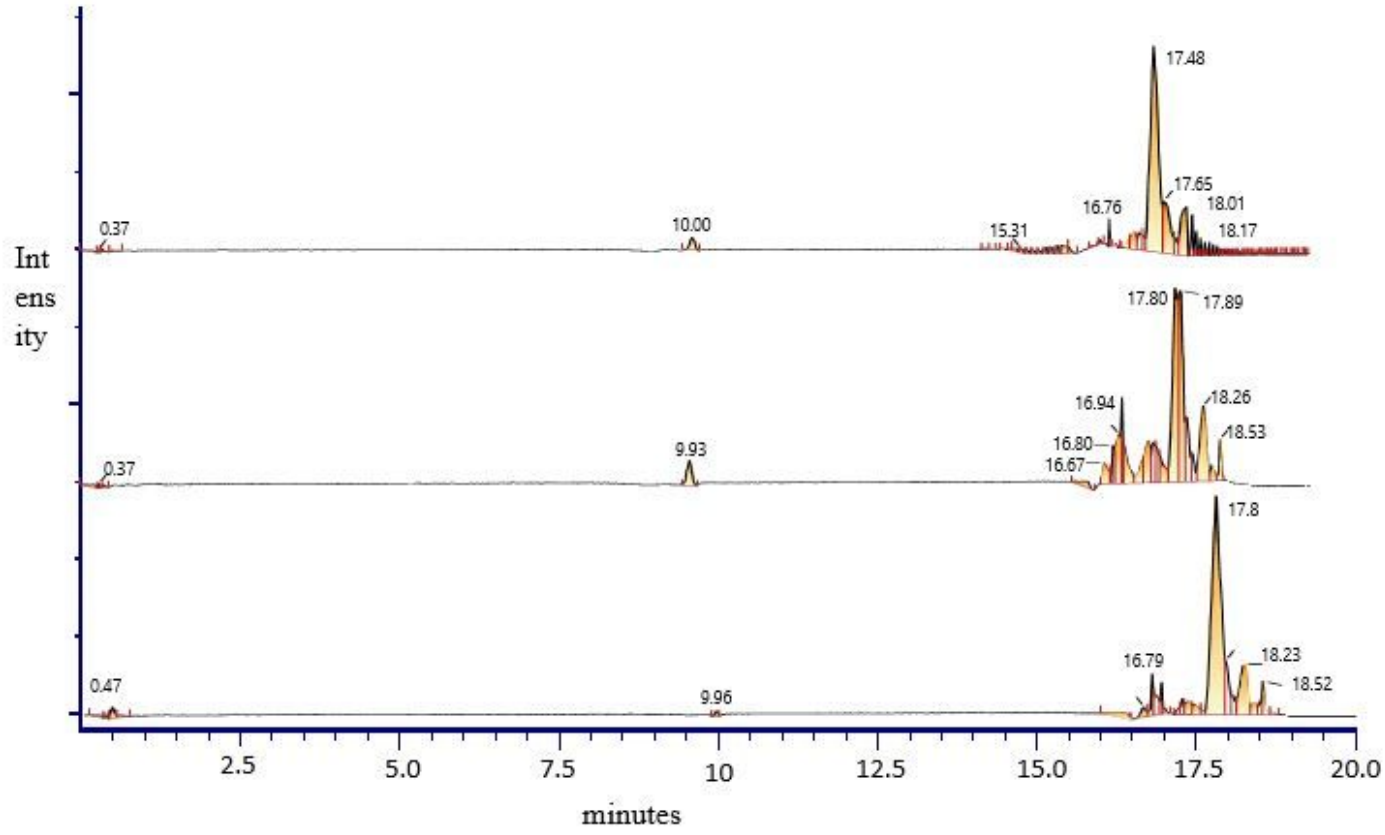


Figure 7

LCQTOF-MS/MS Spectra of filtrate: (a) TM; (b) S14; (c) S51