https://scilett-fsg.uitm.edu.my/
Vol. 17(2) JUNE 2023

ISSN: 1675-7785
eISSN: 2682-8626

DOI: 10.24191/sl.v17i2.20724

Pathogenicity of Fungal Pathogens Isolated from Infected Soursop in Bukit Kurau, Perak

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Accepted: 13 April 2023; Published: 21 June 2023

ABSTRACT

Plant rot disease is considered as one of the major constraints in soursop fruit production. Various fungal causal agents caused diverse rot diseases of soursop trees in Peninsular Malaysia but the extensive study in the determination of the species of the pathogen is yet widely demonstrated. Thus, this study was aimed to identify the fungal pathogen associated with the infected soursop trees and its pathogenicity level. Eight different fungal species were isolated from the infected soursop trees on a private farm in Bukit Kurau, Perak. The fungal species identified as *Pseudopestalotiopsis theae*, *Penicillium simplicissimum, Purpureocillium lilacinum*, *Penicillium javanicum*, *Fusarium oxysporum*, *Aspergillus sydowii, Diaporthe phaseolorum* and *Trichoderma asperellum*. Among them, *F. oxysporum* demonstrated the highest pathogenicity activity towards soursop sapling. The symptoms include wilting, black spots, and yellowish-green leaves. Meanwhile, the rest of the species exhibit mild pathogenicity activity whilst *P. theae* does not affect the soursop sapling. This study presents useful insight on the pathogens of soursop rot disease and its pathogenicity activity.

Keywords: *Plant rot disease, pathogen, soursop, molecular identification*

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Vol. 17(2) JUNE 2023
ISSN: 1675-7785
eISSN: 2682-8626

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INTRODUCTION

For many years, plant rot disease has been recognized as one of the major disease problems in fruit production for various types of crops. The study regarding this problem is the centre of interest for many researchers in agriculture. Diverse kinds of rot disease are reported every year caused by various types of causal agents. The attempts to determine the plant pathogens associated with and its pathogenicity effect are ongoing.

Like other plant crops, soursop trees are also susceptible to pests and diseases. The rot diseases can cause great damage to the tree, and in most cases, kill it. There is various part of soursop trees that get easily infected which include the flower, leaves, stem, fruit, and root. A fungal-based pathogen is a famous causal agent causing many cases of soursop rot diseases around the world. A study by Álvarez and colleagues [1] found that anthracnose was caused by a fungi species of *Colletotrichum gloeosporioides*. It manages to generate a loss of up to 90% in grown soursop fruit. The disease was also reported to be active during rainy season, producing black fruit rot. Stem black rot caused by *Botryodiplodia theobrome* is also considered as one of the most vital diseases where the small purple lesion blackens and enlarges [2]. Apart from fungal infection, pests are also responsible for certain soursop rot diseases. In Brazil, low fruit production had been reported due to unfavourable climatic conditions, poor pollination and mostly caused by the attack of destructive pests and diseases [3]. Several pests have been recognized that frequently attack the soursop plantation back then, including some species of Membracidae, Coccidae, Diaspidiae, Alphididae, and especially the fruit borer, *Cerconota anonella* Sepp, the stem borer, *Cratosomus* spp., the seed borer, *Bephratelloides maculicollis* Bondar, and the leaf miner, *Prinomerus anonicola* Bondar.

With regards to fruit production in Peninsular Malaysia, rot diseases are reported to have caused huge economical losses, especially for small farm holders. Lack of environmental awareness and illiterate caused them to take the short way to achieve the mass production of soursop fruit. This led to the application of chemicals pesticides and fungicides widely on the plantation and while it does significantly effective, the residue of those chemicals can negatively affect our land [4, 5]. Hence, an alternative way with environmental-friendly agents is required. However, to implement that method, the investigation of causal agents for the rot disease should be carried out prior to the implementation of those method. Unfortunately, existing studies regarding the pathogen associated with the soursop rot disease are lacking in Malaysia. Thus, the identification of pathogens that contribute to the soursop rot disease in Perak with its pathogenicity level in this study will act as a preliminary effort to help the disease management of soursop trees in Peninsular Malaysia. This experiment consists of three consecutive steps: (i) Morphology observation, (ii) Molecular identification, and (iii) Pathogenicity test.

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EXPERIMENTAL

Isolation

The soursop sample collection was conducted in November 2019 in the farm at Batu Kurau, Perak (5.16560 North, 100.91760 South). The farm was chosen as the representative for small commercial farming that has contributed to the production of various soursop-based products in the local market. These areas are classified as a tropical rainforest climate with a significant amount of rainfall during the year even for the driest month with an accumulative temperature of 26°C, ranging between 2300-2700 mm of annual rainfall [6]. The soursop plants with external symptoms such as discolouration of the stems and bark, leaf browning and wilting were observed, as these properties were indicators of plant rot infection. The soil and leaves from the infected soursop trees were collected and transported with sterile sealed bags and brought to the Cell Biology Laboratory, Faculty of Applied Sciences, Universiti Teknologi MARA where the cultivation of fungi was carried out using potato dextrose agar (PDA) medium.

An optimized and modified serial dilution plate method from Warcup (1960) [7] was used for fungi isolation in soil samples. At least 1 gram of the soil was dissolved in 10 ml of sterile distilled water to create a soil suspension. For the first dilution, an aliquot of 1 ml from the suspension then was transferred to another tube with 9 ml of sterile water (10^{-1} dilution). Next, the second dilution ($10^{-2)}$ dilution was made by transferring 1 ml suspension from the 10^{-1} dilution to another tube that also contained 9 ml sterile water. Therefore, third, fourth and fifth dilutions were made accordingly where the dilution of choice was plated on PDA plates. The plates were then incubated at 30°C for 5-7 days or till the single spore appeared. The single spores were picked aseptically and transferred into new PDA plates to get pure culture. Meanwhile, for soursop leaves, the isolation method was followed and modified according to Quintana-Obregón protocol [8]. The 70% alcohol wipes were used to get rid of debris from the leaf before small pieces of the infected part were picked from the infected area using sterile forceps and transferred to several PDA plates. The agar plates were then incubated at 30°C for 5-7 days. The single spores present after the incubation were also picked aseptically and transferred into new PDA plates to get pure culture.

Morphology Observation

Each of the isolates was macroscopically identified by looking at their colony characterisation on the PDA plates. The characteristics include the colour of the isolate's elevation, texture, type of spore production and the presence of hyphae. The isolated fungi were also microscopically identified via agar block smear. Approximately, 2mm x 2mm mycelium agar block of a 7-day-old fungal isolate was cut using a sterile blade, placed on the glass slide, and covered with a coverslip before transferred into a sterile empty plate. The plates were then half-filled with sterile distilled water to provide humidity to the culture. The set-up apparatus was placed at room temperature for 2-3 days until a small portion of the fungal spore grows surrounding the agar block. The glass slide with the mycelium block was then observed under a light microscope with the power of 400x magnification.

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DNA Extraction

DNA was extracted using Vivantis© GF-1 Plant Extraction Kit. The fungal colony was transformed into a powder form using liquid nitrogen. The colony powder was then proceeded for DNA extraction where the technique was optimised based on the manufacturer's protocol: 0.5-2g colony powder was mixed with 300µl buffer PL and 20µl Proteinase K and vortexed eventually. The mixture was incubated at 65°C for 120 minutes. After that, the mixture was centrifuged for at least 5 minutes at 10,000 rpm speed to get the supernatant, which was then transferred to a new 1.5 ml tube. Next, 640µl of buffer PB was added to the supernatant and the tubes were inverted a few times to get an even mixture. The mixture was then again incubated at 65°C for 10 minutes.

After the incubation, 200µl of absolute ethanol was added and mixed immediately by inverting it a few times to prevent clumping of extracted DNA to form. A maximum of 650µl of the mixture was then transferred to a filter column tube provided. The tube was centrifuged at 10,000 rpm for 1 minute. This step is repeated for the remaining solution. The filter column was then washed using a 650 µl diluted wash buffer. The tube was centrifuged again for 1 minute at 10,000 rpm. The centrifuge step was duplicated to get rid of all the wash buffers from the filter column. Lastly, the upper part of the filter column was transferred to a new 1.5ml tube and preheated 60µl elution buffer was pipetted to the centre of the filter column, to make sure that the amount of the filter was covered evenly before the tube incubated at room temperature for 60 minutes. The tube was then centrifuged, and the extracted DNA was stored at -20°C for long-term storage. The DNA extracted size was determined using the gel electrophoresis technique which was then observed visually under a UV transilluminator.

PCR Amplification and Sequencing

The DNA extracted was amplified using the ITS region universal primer pair, ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') as the forward primer and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') as the reverse primer. The reaction mixture is a mixture of 1X buffer, 2.0 mM $MgCl_2$, 0.2 nM dNTPs, 0.1 µM Forward primer, 0.1 µM Reverse primer, 1.25 U *Taq* polymerase and 2 µl DNA template. The final volume of each reaction mixture was made up to 50 µl with sterile distilled water. The PCR profiles consist of initial denaturation at 94°C for 1 minute followed by 30 cycles of DNA denaturation at 94°C for 30 seconds, DNA annealing at 49°C for 45 seconds, DNA extension at 72°C for 3 minutes and finally, another DNA extension step at 72°C for 7 minutes. The final products were stored at -20°C for future use. The expected size of PCR products from this work is 400-800 base pairs [9]. The amplified gene then was sequenced by Apical Scientific Sdn. Bhd., Selangor, Malaysia. The sequenced DNA provided by the company was then aligned with the NCBI gene bank by using BLAST software (http://blast.ncbi.nlm.nih.gov).

Pathogenicity Test

This experiment was carried out using 5-month-old soursop saplings. The saplings were prepared in a condition of saplings were prior infected with the identified fungi with a concentration of 10^5-10^7 CFU/ml. The observation regarding the severity of the infection was recorded after 7 weeks. Standard soursop tree handlings were applied for all conditions. The saplings were under individual rain shelter conditions. Pest

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covers were applied by using an anti-pest net to avoid other disease-causal agents. The saplings were then water-sprayed every 2 days and the soil needed to be irrigated regularly. Moreover, a clean tree framework was maintained, and partially shaded conditions were placed for all the saplings in which more than 80% humidity was required. These conditions were modified and referred from local small farmers in soursop plantations.

All saplings were transferred to a new pot and were left to adapt for a week before treatment. This experiment ended with the calculation of the percentage of severity infection of the trees which includes two ways of measurement: Disease Incidence (DI) and Disease Severity (DS) respectively [10]. DI measurement is the percentage of diseased leaves per sapling at the beginning of the treatment. The data were calculated based on how many infected leaves can be pulled out after the symptoms appeared by using the Equation 1:

$$\text{Disease Incidence } (\%) = \frac{A}{B} \times 100 \qquad \text{Equation 1}$$

A, the total number of leaves with symptoms that can be pulled off from the plant.
B, the total number of observed leaves of the plant.

Meanwhile, DS measurement is the disease severity determined after the initial symptoms are shown by calculating the area of infection on leaves. The data area for symptoms of the rotting disease was measured by using the grid line [11] by using the Equation 2 as follows:

$$\text{Disease Severity } (\%) = \frac{C}{D} \times 100 \qquad \text{Equation 2}$$

C, the total area of a leaf affected.
D, the total area of the whole leaves.

RESULTS AND DISCUSSION

Fungal Isolation

In sample collection, wilting of leaves and the presence of black spots on the bark near the root were observed and collected from the localities. Figure 1 illustrates the splits of the roots showing black colouration indicates the characteristic of rot disease. However, no unpleasant smell was present. The rotting of roots was also humid and pasty. Meanwhile, the leaves on the tree turned brown with white and black spots of fungi.

A total of thirty-three isolates were obtained from the sample culturing. However, multiples of the fungi isolates were excluded due to their similar morphological characteristics which indicate similar species of fungi obtained, resulting in 10 fungi isolates being chosen from a collection of 21 samples of soil and leaves of the infected soursop trees.

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Figure 1: Soursop leaf and root with symptoms of rot disease collected from the farm in Bukit Kurau, Perak. The symptoms usually appeared as wilting, black spots on the end bark as well as the appearance of the brown lesion and white spots on the leaves.

Fungal Identification

The appearance of 10 identified fungi visualised through microscopic and macroscopic observations were shown in Figure 2 and Figure 3. The descriptions included covered their specific morphology and taxonomy elaboration respectively.

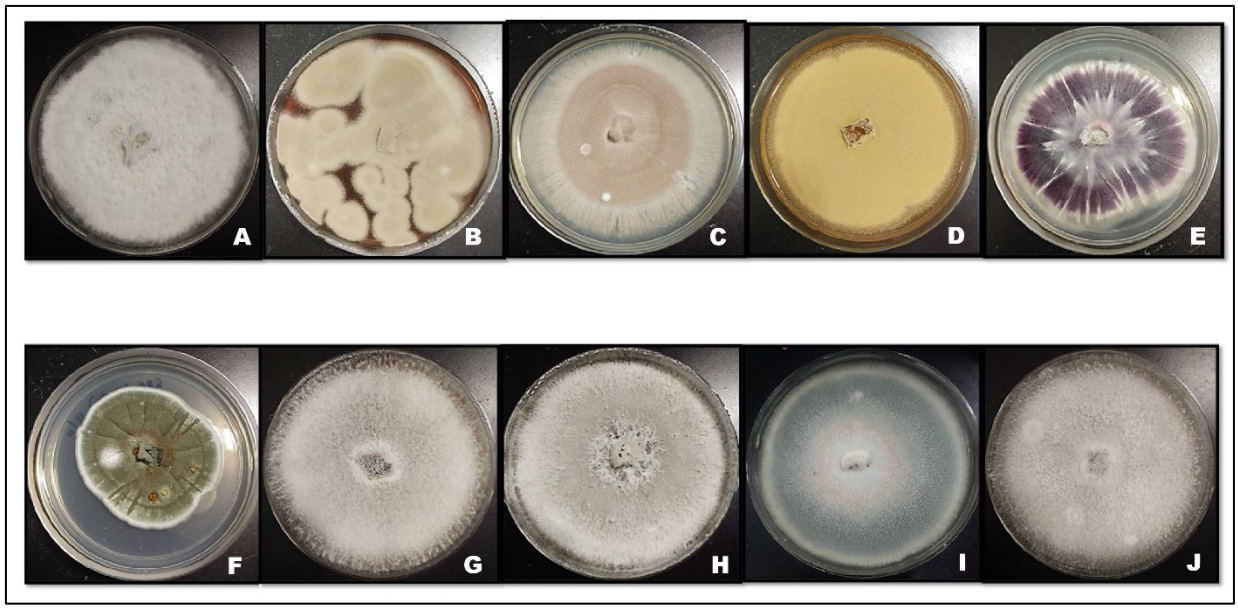

Figure 2: The images show morphological characteristics of fungal isolates on the PDA medium. They possessed distinct appearances in terms of colour, elevation, and texture. A, *P. theae;* B, *P. simplicissimum*; C, *P. lilacinum*; D, *P. javanicum;* E, *F. oxysporum;* F, *A. sydowii;* G, *F. oxysporum*; H, *D. phaseolorum*; I, *T. asperellum;* J, *F. oxysporum.*

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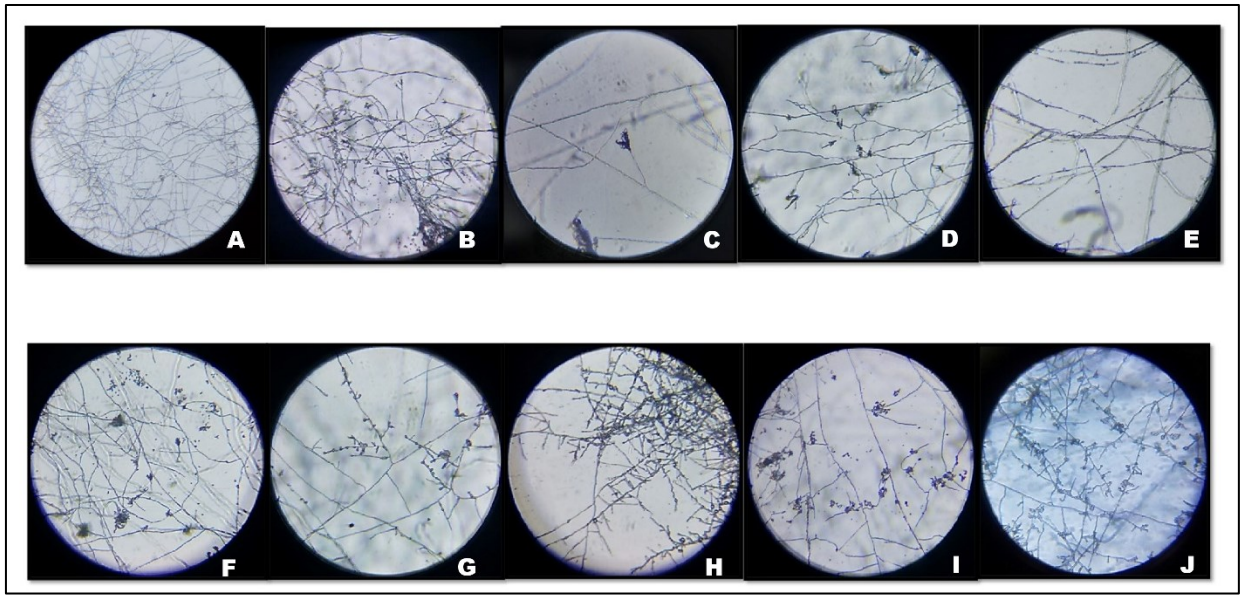

Figure 3: The images show fungal isolates identified under a microscope with 400x magnification. A, *P. theae;* B, *P. simplicissimum;* C, *P. lilacinum*; D, *P. javanicum*; E, *F. oxysporum*; F, *A. sydowii;* G, *F. oxysporum*; H, *D. phaseolorum;* I, *T. asperellum;* J, *F. oxysporum*.

Scientific name for Figure 3(A) is *Pseudopestalotiopsis theae.* The taxonomy as follows: Division, Ascomycota; class, sordariomycetes; order, Xylariales; family, sporocadaceae and genus, *Pestalotiopsis*. The fungal colony grew at a very slow rate at both room temperature and 37°C. The colony grow full plate under 14 days period. The mycelium appeared milky white with a hint of light yellow around the centre. The reverse was light yellow on the edge as well as the centre. The structure was shrub-like, soft surface and appeared velvety. The colony was buff but tough when reattached from the agar medium. The septate hyphae have short branches, smooth and abundant. The conidia were fusiform, three to a five-celled group, and constriction at the septa. Usually reported 18.5-31.5 × 5-7.5 µm in size [12].

Scientific name for Figure 3(B) is *Penicillium simplicissimum* with the taxonomy order as follows: Division, Ascomycota; class, eurotiomycetes; order, Eurotiales; family, trichomacaeae; genus, *Penicillium.* The fungal colony grew at a slow rate at both room temperature and 37°C, the colony was a full plate after 10 days. The mycelium appeared light brown and a lighter colour at the frame edge. The reverse was also light brown both at the centre as well as the edge. The structure is velvety and thin. The sporulation appeared very short. The colony has a soft texture but is flat when reattached from the agar. The fungal compacted with septate hyphae and appeared abundant. The metula and phialides of the conidiophore were clearly visualised and usually exist in groups and long, ranging from 4-6, 8-10 × 2.2- 2.8 µm in size. The phialides also appeared in groups and were reported to be 5-8, 7-9 × 1.8-2.2 µm in size [13]. The conidia were scattered around, ellipsoidal and in chain shape.

For figure 3(C), the scientific name is *Purpureocillium lilacinum*. Taxonomy order are as follows: Division, Ascomycota; class, sordariomycetes; order, Hypocreales; family, ophiocordycipitaceae; genus, *Purpureocillium*. The fungal colony grew at the average rate in both room temperature and 37°C. The colony grow full plate within under 7 to 8 days period. The mycelium appeared rosy vinaceous for most of

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Vol. 17(2) JUNE 2023
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eISSN: 2682-8626

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the part and appeared pale vinaceous around the frame edge. The reverse was dark vinaceous on the edge as well as the centre. The structure is cotton-like, soft surface and velvety. The sporulation is dense. The colony was buff but flat and encrusted when reattached from the agar medium. Conidiophores were erect, arising mainly from sub-merged hyphae, consisting of verticillate branches with whorls of two to four phialides variation of size, ranging 2~3 (~4) µm in width. Conidia, which were observed in short divergent chains, were ellipsoidal to fusiform, apiculate, hyaline, and smooth-walled, and usually 2~3 (~3.5) × 1.9~2.7 µm in size [14].

Scientific name for Figure 3(D) is *Penicillium javanicum*. The taxonomy is as follows: Division, Ascomycota; class, eurotiomycetes; order, Eurotiales; family, trichomacaeae; genus, *Penicillium*. The fungal colony grew at a fast rate in both room temperature and 37°C. The colony grow full plate under 5 days period. The mycelium appeared light yellowish brown for all the parts. The reverse was a little darker than the upper visual at the edge as well as the centre. The structure has a soft surface and is velvety with short mycelium. The sporulation is dense. The colony was thin, flat, and encrusted when reattached from the agar medium. The morphology was similar to *Purpureocillium* species where the conidiophores were produced by rough and wavy hyphae, consisting of verticillate branches with 4 to more phialides. Some of the hyphae visualized with blunt ends indicate the short, rounded protrusions hyphae resembled aborted clamp connection. Meanwhile, the conidia appeared in short divergent chains, apiculate and ellipsoidal shapes.

For Figure 3(E), the scientific name is *Fusarium oxysporum* with the taxonomy order as follows: Division, Ascomycota; class, sordariomycetes; order, Hypocreales; family, nectriaceae and genus, *Fusarium.* The fungal colony grew at a slow rate at room temperature but at the average rate at 37°C. The colony grow full plate within under 7-8 days period. The mycelium appeared purple but cover with white mycelia on the top, especially in the middle and forming a star shape. White mycelia appeared cotton-like and dry meanwhile the purple surface was thin, glassy, and slippery. The reverse was dark purple on the edge as well as the centre with a slightly different range of colour density. The mycelium was consisting of loose knot hyphae branches and mostly appeared with a blunt end of conidiophores. The conidia were unsuccessfully visualized where it was usually oval, curved, or kidney-shaped, without or with one septate and formed over short mono-phialides and conforming pseudo-heads. The matured conidia can vary in size ranging from 6.14 to 9.75 µm [15].

Scientific name for Figure 3(F) is *Aspergillus sydowii*. The taxonomy order as follows: Division, Ascomycota; class, eurotiomycetes; order, Eurotiales; family, trichomacaeae; genus, *Aspergillus*. The fungal colony grew at a very slow rate at both room temperature and 37°C. The colony can grow to 5-6 cm in diameter within 10 days period. The mycelium sporulates in dark greyish blue-green, and funicular hyphal aggregates were often seen centrally with no exudate and no soluble pigment. The structure was velvety, rough surface, thick and appeared dry. The reverse was unpigmented to brownish pink on the edge as well as the centre. The colony was buff but flat and encrusted when reattached from the agar medium. The conidiophore produced loose knot branches and had a long distance from the tip of the phialides. Conidial heads biseriate, metulae covering most of the vesicle, and fragmentary heads resembling penicillate fructifications are abundant. Conidia were in groups with the shape of globose, usually ranging in size from 2.5–3.0 (–5) µm [16].

Scientific name for Figure 3(G) is *Fusarium oxysporum* with the taxonomy order as follows: Division, Ascomycota; class, sordariomycetes; order, Hypocreales; family, nectriaceae; genus, *Fusarium.*

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Vol. 17(2) JUNE 2023
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The fungal colony grew at a fast rate at both room temperature and 37°C. The colony can grow a full plate under 7 days period. The colony appeared pink violet both at the centre and at the edge however the upper part of the mycelium mostly appeared white. The structure is cotton-like, visually rough, and thick. The sporulation was abundant. The reverse was red violet on the edge as well as the centre with a slightly different range of colour density. The colony was buff and soft but encrusted when reattached from the agar medium. The mycelium appeared with loose knot branches of hyphae, signifying the less volume of the hyphae produced. Some of the chlamydospore produced was formed terminally and some were intercalary. They were unicellular and bicellular, denticulate on the surface.

Scientific name for Figure 3(H) is *Diaporthe phaseolorum*. The taxonomy order is division, Ascomycota; class, sordariomycetes; order, diaporthales; family, diaporthaceae; genus, *Diaporthe*. The fungal colony grew at the average rate at both room temperature and 37°C. The colony can grow a full plate in under 7 days period. The colony appeared light grey with a hint of brownish at the edge and white mycelium at the centre. The structure is shrub-like with fewer mycelia and is visually rough, and thin. The sporulation was not abundant. The reverse was dark grey on the edge as well as the centre with a slightly different range of colour density. The colony appeared flat and encrusted when reattached from the agar medium. The mycelium appeared with tight knot branches of hyphae, signifying the volume of the hyphae produced. Most of the chlamydospores produced similarly with *Fusarium oxysporum* formed terminally and some were intercalary. They also were unicellular and bicellular, where they appeared hyaline and glassy on the surface.

For Figure 3(I), the scientific name is *Trichoderma asperellum* with taxonomy order as follows: Division, Ascomycota; class, sordariomycetes; order, Hypocreales; family, hypocreceae; genus, *Trichoderma*. The fungal colony grew at the average rate at both room temperature and 37°C. The colony can grow a full plate under 7 days period. The mycelium appeared in two tones, dark green mycelium at the edge and white shrub-like mycelium at the centre with the existence of red, yellow-orange mycelia intermediate in between. The surface was cotton-like and velvety. The reverse was pale green. The colony appeared buff and soft but encrusted when reattached from the agar medium. The whole conidiophore has a short distance from the phialides, and the conidia appeared to be in groups and globose in shape. However, usually, the conidiophores were branched with bottle-shaped phialides, located with whorls 2–3 or singly, and 6.4 × 1.4µm meanwhile the conidia usually were ellipsoidal, 3.1 × 2.6µm in size [17].

Scientific name for Figure 3(J) is *Fusarium oxysporum*. The taxonomy order as follows: Division, Ascomycota; class, sordariomycetes; order, Hypocreales; family, nectriaceae; genus, *Fusarium*. The fungal colony grew at a fast rate at both room temperature and 37°C. The colony can grow a full plate under 7 days period. The colony appeared pink violet both at the centre and at the edge however the upper part of the mycelium mostly appeared white. The structure is cotton-like, visually rough, and thick. The sporulation was abundant. The reverse was red violet with a slightly different range of colour density. The colony was buff and soft but encrusted when reattached from the agar medium. The mycelium appeared with loose knot branches of hyphae, signifying the less volume of the hyphae produced. The chlamydospore produced was formed terminally; some were intercalary at the ends of the abundance of short hyphae branches. They also appeared unicellular or bicellular, denticulate on the surface.

Further identification of the fungi isolates was done through ITS region amplification. The applications of ITS region are well known as the easiest marker to be amplified, and sequence and this region are highly variable in the Fungi Kingdom allowing them to be used in broad range of fungal

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Vol. 17(2) JUNE 2023
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identification [18,19]. Table 1 shows the difference in fungal species matched for each of the isolates. From the BLAST analysis, eight different fungal species were identified as mentioned earlier in the morphological section.

Table 1: The species matched for the fungal isolates identified with their registered GenBank accession number

Isolates	Species Matched	GenBank Accession Number
LP8	*Pseudopestalotiopsis theae*	OP797630
SP1	*Penicillium simplicissimum*	OP797656
SP2	*Purpureocillium lilacinum*	OP797655
SP7	*Penicillium javanicum*	OP797656
SP8	*Fusarium oxysporum*	OP797657
SP9	*Aspergillus sydowii*	OP797658
SP13	*Fusarium oxysporum*	OP797659
SP16	*Diaporthe phaseolorum*	OP797660
SP17	*Trichoderma asperellum*	OP797661
SP19	*Fusarium oxysporum*	OP797662

Fungal Pathogenicity

Table 2 shows that *P. lilacinum* (SP2), *P. javanicum* (SP7), *F. oxysporum* (SP8), *A. sydowii* (SP9), *D. phaseolorum* (SP16), and *T. asperellum* (SP17) managed to record percentage of disease incidence (DI) that indicates the presence of symptoms of rot disease during the beginning of infection. The highest value recorded was from the plant infected by SP16 with an incidence value of 17.65% whilst, the lowest value was recorded by *P. theae* (LP8) with zero value. On the other hand, the percentage of disease severity (DS) was recorded by most of the fungal isolates including LP8. They showed a high infection rate with a high percentage of disease severity. Generally, the symptoms appeared to worsen as the severity percentage increased. One of the highest DS readings was recorded by *F. oxysporum* (SP8) with 49.94%. The infection symptoms include black spots, huge brownish lesions on the leaves, and wilt that lead the leaves to fall off from the trees (Figure 4). The bark also appeared to be dryer and discoloured. Twelve of the infected leaves fall off from the sapling infected by *F. oxysporum* in week 7th indicating the infection was worsen over time as black spots were spotted everywhere and wilting was shown on most of the leaves.

Despite the symptoms, the sapling did not give any sign of dying as new shoots continue sprouting and the roots appeared healthy. *F. oxysporum* was expected to exhibit high pathogenicity activity towards soursop saplings as it was reported to cause many kinds of diseases on a variety of crops around the world. In the past, *F. oxysporum* causes Fusarium wilt, one of the most destructive diseases of banana trees which is mostly reported in Africa and China [20, 21, 22]. Recently, the fungus was reported as a causal agent that led to early-stage disease in cotton causing root rot, seedlings wilt, and death [23, 24]. Following SP8, *A. sydowii* (SP9) and *D. phaseolorum* (SP8) also recorded a high value of DS with 30.35% and 15.59%

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eISSN: 2682-8626

DOI: 10.24191/sl.v17i2.20724

respectively. Similar symptoms appeared such as wilt, black spots, and lesion on the leaves. The root also appeared to be healthy. Meanwhile, some of the isolates recorded zero percentage of disease severity while having the existence of symptoms such as *P. theae* (LP8) and *P. javanicum* (SP7). However, the infection area was too small to be counted as severity indicating the species exhibit mild pathogenicity activity towards the soursop sapling. In the meantime, the saplings that appeared with no symptoms of disease, where new shoots continuously sprouted and the roots also appeared to be healthy, the fungi could be classified only as fungal endophytes that associated with the soursop tree without causing any disturbance on their growth.

Figure 4: Symptoms of the infected soursop saplings by fungi species identified after 7 weeks infection period. The image shows the wilting, discoloured, and brown lesions of the leaves

Table 2: The measurement of the Disease Incidence (DI) taken on week 1/week 2 and the Disease Severity (DS) taken on week 7 of soursop saplings infected by fungal isolates with the severity condition recorded.

Sample	**Disease Incidence (%) (Week 1)**	**Disease Severity (%) (Week 7)**	**Severity Condition**
LP8 *Pseudopestalotiopsis theae*	0	0	A small area on one of the leaves showed infection symptoms but not severe and no leaf fell off, new shoots grow. Healthy root with thicker size. (***Font checked***)
SP2 *Purpureocillium lilacinum*	9.09	4.30	Some area of the leaves gets infected and one leaf fell off. Many branches of the root start to grow. (***Font checked***)
SP7 *Penicillium javanicum*	8	0	Two leaves fell off at the beginning of the experiment. Some parts got small dots of infection but not severe. The root appeared healthy and new shoots start to grow.

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ISSN: 1675-7785
eISSN: 2682-8626

DOI: 10.24191/sl.v17i2.20724

SP8 *Fusarium oxysporum*	4.17	49.94	Some of the leaves fell off at the beginning of the experiment. Twelve leaves fell off during the experiment due to infection however, new shoots still grow, and the tree has a healthy root with a few branches.
SP2 *Purpureocillium lilacinum*	9.09	4.30	Some area of the leaves gets infected and one leaf fell off. Many branches of the root start to grow.
SP7 *Penicillium javanicum*	8	0	Two leaves fell off at the beginning of the experiment. Some parts got small dots of infection but not severe. The root appeared healthy and new shoots start to grow.
SP9 *Aspergillus sydowii*	9.52	30.35	Many leaves get infected, wilt, and fell off. However, new shoots still grow, and the root appeared to be healthy
SP16 *Diaporthe phaseolorum*	17.65	15.59	Many leaves fell off, but new shoots still grow, and the root still looks healthy but not many branches produced.
SP17 *Trichoderma asperellum*	5	7.97	One leaf fell off. New shoots still grow, and roots appeared healthy with many branches.

Further observations were done to determine the effect of infection on the growth rate of soursop saplings. The height of infected saplings was measured from the ground to the top surface of the topmost leaves of the tree. The measurements were taken every two weeks and the growth rate for each of the saplings was calculated and presented in the bar graph (Figure 5). From the graph, *P. theae* (LP8) was at 1 cm/week, *P. lilacinum* (SP2) at 1.02 cm/week, *P. javanicum* (SP7) at 1.19 cm/week, *F. oxysporum* (SP8) at 1.04 cm/week, *A. sydowii* (SP9) at 1.29 cm/week, *D. phaseolorum* (SP16) at 0.96 cm/week, and *T. asperellum* (SP17) at 1.17 cm/week. The values recorded by these fungi were not considered high compared with the control (0.93cm/week). Recent studies reported that *A. sydowii* was involved in causing diseases mostly in marine plants [25, 5].

A. sydowii produces kinds of secondary metabolites that exhibited antifungal, antibacterial, and cytotoxic activities for the control of marine organisms' diseases [26, 27, 28, 29]. Recently, the fungi demonstrated a positive effect on *Artemisia annua,* a plant-produced compound that has the ability as an anti-malarial called *artemisinin* [25]. Despite their positive previous contribution, this study could be the first report of *A. sydowii* as a possible plant pathogen in crops. For *D. phaseolorum,* various study has shown the pathogenicity of this fungal species towards many types of plants around the world. This fungi species belongs to the phylum Ascomycota - a well-known fungal parasite of plants. *D. phaseolorum* involved in causing brown rot of Red Fuji apple in Hangzhou city, China. The disease condition significantly caused serious economic losses [30]. In another study, China once reported *D. phaseolorum* might contribute to causing soybean stem blight [31]. Another observation regarding this pathogenic fungus

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Vol. 17(2) JUNE 2023
ISSN: 1675-7785
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DOI: 10.24191/sl.v17i2.20724

also led to an occurrence of leaf spots on *Bletilla striata*, a plant used commonly in traditional medicine in China [32].

Meanwhile, *F. oxysporum* (SP8) as discussed earlier was an infamous plant pathogen in the agriculture field, suggesting the fungus undoubtedly would cause either a negative effect on the soursop plant growth or no occurrence may ensure since the disease severity towards the soursop plant was the highest value recorded. Thus, the result showed agreed with the assumption made. However, despite the high percentage of disease severity recorded by *F. oxysporum* (SP8), *A. sydowii* (SP9), and *D. phaseolorum* (SP16), the sapling exposed to these fungi unexpectedly demonstrated a high growth rate. The results suggest that the fungi may trigger the soursop sapling defence system causing the plant to increase its growth rate in response to the infection. However, no replication was done during the test and this limitation of the experiment unfortunately could bring down the level of accuracy and significance of the results. Thus, further study should be done to bring more explicit findings.

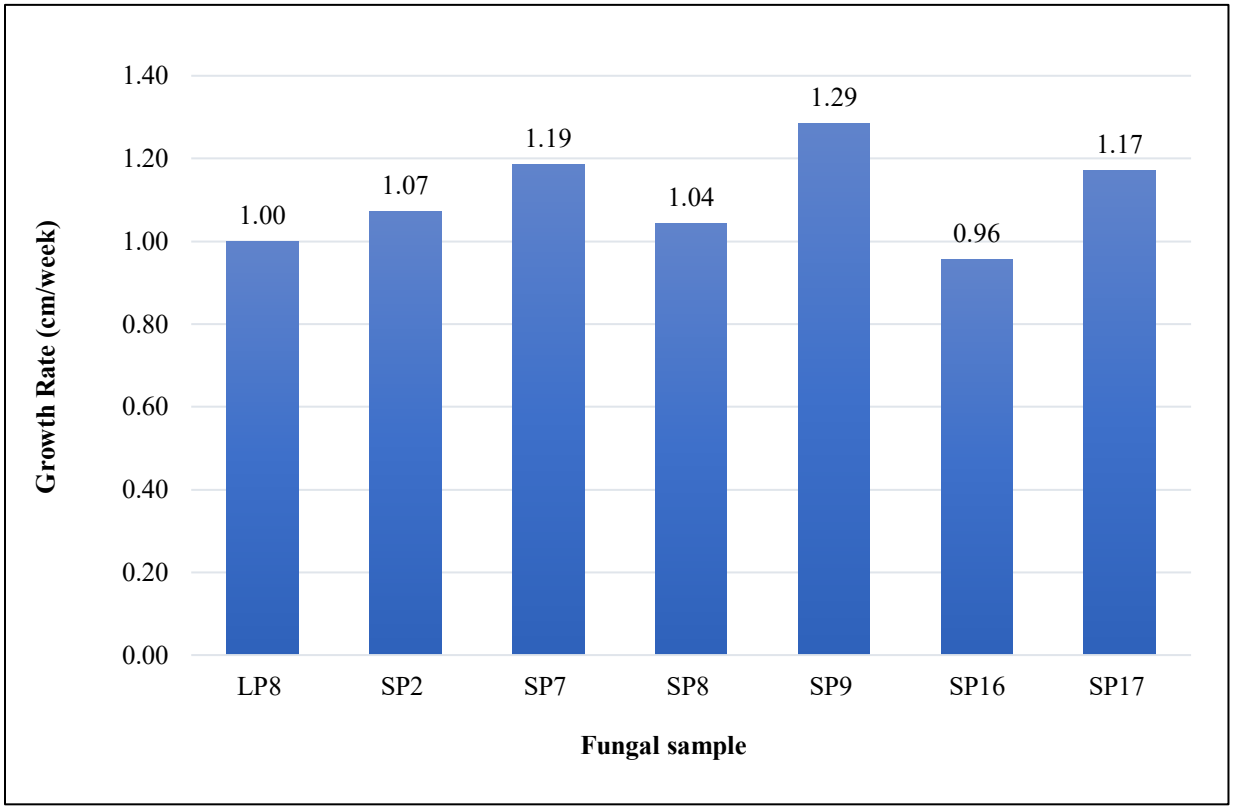

Figure 5: Bar graph for the growth rate of the infected soursop saplings in 7 weeks (LP8, SP2, SP7, SP8, SP9, SP16, and SP17). (No replication – was mentioned as the limitation of the study in the last paragraph next page and in the conclusion)

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Vol. 17(2) JUNE 2023

ISSN: 1675-7785
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DOI: 10.24191/sl.v17i2.20724

CONCLUSION

The conclusion connects the findings to a larger context, such as the wider conversation about an issue and the journal theme. It suggests the implications of your findings or the importance of the topic**.** Asking questions or suggesting ideas for further research and revisiting your main idea or research question with new insight.

ACKNOWLEDGMENTS

The authors would like to acknowledge the support of Universiti Teknologi Mara (UiTM), Cawangan Negeri Sembilan, Kampus Kuala Pilah and Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, Selangor, Malaysia for providing the facilities and financial support on this research.

AUTHOR'S CONTRIBUTION

Each author contribution must be stated clearly reflecting each contribution to the body of the work and manuscript. For example:
Jayaraj carried out the research, wrote and revised the article. Rahmah conceptualised the central research idea and provided the theoretical framework. Rahmah and Chong Ju Lian designed the research, supervised research progress; Rahmah anchored the review, revisions and approved the article submission.

CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts and declare absence of conflicting interests with the funders.

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ISSN: 1675-7785
eISSN: 2682-8626

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