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Stem canker of dragon fruit (*Hylocereus polyrhizus*): *Neoscytalidium* sp. is a pathogen of the disease and its control using sodium salt

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Abstract: This study aimed to isolate and characterise a fungal pathogen associated with stem canker on dragon fruit and to evaluate the efficacy of sodium salt as an alternative control against it. The fungal pathogen was isolated and identified by morphological and cultural methods. SMNND11 and ARPN11 isolates, which are morphologically alike *Neoscytalidium* sp., were selected for the present study. The colony's mycelial mass was greyish-white and turned to dark greenish-grey. The shape of the arthroconidia was ellipsoid to ovoid and hyaline to dark brown with septate arthrospores. The hyphae were brown in colour, septate, branched and constricted into spore chains before disarticulation. Based on the blast analysis using the aligned sequences of the internal transcribed spacer, the SMNND11 isolate was highly genetically identical (100%) to *Neoscytalidium dimidiatum*, the ARPN10 isolate was 98.6% identical to *Neoscytalidium* sp. and the neighbour-joining analysis revealed that both isolates were grouped into the same genus, *Neoscytalidium* sp. The *in vitro* study revealed that sodium salt at a concentration of > 3% showed high potential in suppressing the mycelial growth of the SMNND11 isolates. In a field trial, a sodium salt solution at 30 g/L applied twice a week, along with rotating chemical fungicides applied once a week, were able to reduce the disease severity of the stem canker disease on the dragon fruit. This study revealed that *Neoscytalidium* sp., as well as *N. dimidiatum,* is the positive pathogen that infects dragon fruit plants in IP2TP Sumani and Aripan West Sumatra. Thus, the culture and field studies support the potential control technique to alternating chemical fungicide on dragon fruit.

Keywords: pitaya; *Neoscytalidium* sp.; canker disease

Dragon fruit (*Hylocereus* sp.) or pitaya is a type of cactus that has potential as a "cash fruit" in Indonesia. This exotic plant is native to Latin (North and Central) America and was introduced to Indonesia in the 1990s. The agribusiness of this fruit grew rapidly at a farmer level in a short time; besides being easy to cultivate, it also has numerous selling points for the market such as its unique shape and attractive colour (Le Bellec et al. 2006). Red-fleshed dragon fruit (*H. polyrhizus*) is the most popular species,

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more than white-fleshed dragon fruit (*H. undatus*), super red-fleshed dragon fruit (*H. costaricensis*), and white yellowish-fleshed dragon fruit (*Selenicerius megalanthus*). The species is widely cultivated in Indonesia due its high value income. The plantation areas for this species cover almost all the regions in Indonesia, such as the provinces of Riau, Riau Islands, West Sumatra, Daerah Istimewa Yogyakarta, Central Java, East Java, East Kalimantan, Central Kalimantan, and Papua. Recently, the Solok Regency, West Sumatra, plans to develop > 100 ha of the red-fleshed species.

Similar to other cacti, this exotic plant is predicted to have a resistant response to extreme environmental conditions, but, recently, the crops have been destroyed by stem canker disease and flower rot (Ruangwong et al. 2022). Stem canker disease has been reported to be the cause of the collapse of the red-fleshed dragon fruit in various producing countries such as Vietnam, Myanmar, Philippines, Malaysia, Thailand, Taiwan, and China (Chuang et al. 2012; Mohd et al. 2013; Yi et al. 2015; Dy et al. 2022). In Indonesia, the first report was in the Batam area (Jumjunidang et al. 2019). In a short time, outbreaks also occurred in North Sumatra, West Sumatra, Riau Islands, and Riau. Stem canker is caused by the fungus *Neoscytalidium dimidiatum*, which affects young cladodes, young to ripe fruit, and old branches. Symptoms of stem canker generally show white spots on the young tissue (in the early stages). The symptoms develop into coal spots that coalesce, dry up like brown scabies, and form a hollow circle on the old branches (Jumjunidang et al. 2019). Identification by molecular characterisation is an important method for determining of the pathogen and helping in considering effective control measures against the pathogen.

The currently used management approach to prevent devastation to dragon fruit orchards still recommends using synthetic pesticides. The recommended pesticides to be used are copper-based pesticides such as propineb and difenoconazole (Riska et al. 2021). Nevertheless, most consumers are now concerned for their health, and more wary of using chemical pesticides due to the negative impact on the environment such as residual toxicity, the long degradation period, and pollution (Unnikrishnan & Nath 2000). Additionally, controlling stem canker with botanical pesticides or biological agents is only effective on an *in vitro* assay (Taguiam et al. 2020). One alternative is to use naturally-derived chemicals, which are relatively safe.

Salt is an abundantly available natural ingredient that is generally recognised as safe by the United States Food and Drug Administration (Food and Drug Administration 2021). Mostly, it has a broad inhibitory effect against a range of fungal plant pathogens including *Fusarium oxysporum* f. sp. *cyclaminis* (Elmer 2002), *Alternaria alternata*, *F. solani* var. *coeruleum*, *Phytophthora erythroseptica*, *P. infestans*, *Verticillium alboatrum*, *V. dahliae* (Mills et al. 2004), *A. solani, F. solani, F. oxysporum* and *Pythium* sp. (Abdel-Kader et al. 2012). Salt can prevent the germination of microorganisms through toxin induction and water evaporation (Elmer 2002). Thus, this study aimed to identify the stem canker pathogen using morphological and molecular methods and to evaluate the potential of sodium salt as a fungicide in suppressing the development of the fungus *N. dimidiatum* using *in vitro* methods and in field trials.

MATERIAL AND METHODS

Sample collection and isolation

A total of ten symptomatic stem canker cladodes of *H. polyrhizus* were collected from a dragon fruit plantation field in IP2TP Aripan [100°37'19.1"E 0°44'22.4"S; at an altitude of 500 m above sea level (ASL)] and IP2TP Sumani (0°43'17"E 100°35'5"184°S; at an altitude of 362.1 m ASL), from the Indonesian Tropical Fruit Research Institute, Solok, West Sumatera, then kept in a paper bag and taken to a laboratory. The isolation of the fungal pathogens was performed using a tissue transplantation technique with a few modifications. Small pieces (1.5–2 cm) of infected tissue were dipped into 70% alcohol for 5 min as a surface disinfectant, rinsed three times with sterilised distilled water, air-dried, placed on 1/3 potato dextrose agar (PDA) (contains 13 g of PDA, 10 g of agar and 1 000 mL of distilled water), and incubated for 24 h at 28 ± 2 °C with 12 h of natural light. The hyphal tips growing from the isolated tissue were cut and transferred to the PDA, and incubated at 28 ± 2 °C with a natural light cycle. A single spore of the isolates obtained by cutting the one day-germinated hyphae on water agar was cultured on a PDA medium and incubated at 28 ± 2 °C with 12 h of natural light for seven days. The

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fungal isolates were then used for the microscopical morphological identification, pathogenicity testing, and molecular analysis.

Morphology study

The morphology of the colonies is resolved by the ability of the isolates to grow on the PDA, with observations of the colony traits, such as the colour and shape. Three plates for each isolate were incubated at 28 ± 2 °C with 12 h of natural light, and the morphology of the colonies was observed daily until the maximum growth was reached (the colonies reached the edge of the plate). The general morphological characteristics of the fungal isolates were observed using an Olympus ULWC 0.30 Phase Contrast Microscope (Japan) with 40× magnification. For observing the character of each isolate, three Petri plates and two fungal preparations were taken as replicates.

Pathogenicity test of the isolates

This study tested two isolates (SMNND11 and ARPN10) on the dragon fruit (*H. polyrhizus*). One young cladode was cut into 5 cm lengths, and each piece of cladode was put on a Petri plate. The cladodes' surface was punctured by a needle (1 cm in diameter) to make the lesion, and a 0.5 cm plug of the isolate was put on the lesion site. The plug was watered with 10 µL of sterile water, and the Petri plates were sealed. The experiment was carried out with two isolates and three replications (two Petri plates of each isolate).

DNA isolation, PCR amplification and sequence analysis

The total DNA genome of two isolates, accession SMNND11 and ARPN10, were isolated at five days old in the PDA medium using a Quick-DNA Fungal/Bacterial Microprep Kit (Zymo Research, California USA) according to the manufacturer's instructions. The quantity and quality of the obtained DNA were measured on an absorbance ratio of A 260/280 nm using a BioSpectrometer Basic, Eppendorf (Eppendorf AG, Germany).

The DNA genomes were amplified with the universal primer internal transcribed spacer (ITS1) (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-CCT CCG CTT ATT GAT ATG C-3') using a thermocycler (Eppendorf® Mastercycler® Nexus Thermal Cyclers, Germany). The thermal cycler conditions were denaturation at 94 °C for 1 min, 28 cycles of denaturation at 95 °C for 15 s, primer annealing at 50 °C for 15 s and extension at 72 °C for 5 s, and a final extension at 72 °C for 10 minutes.

The amplification fragment was separated from 1.2% agarose gel using electrophoresis at 100 V for 30 minutes. The obtained fragment was compared with a 1-Kb DNA ladder and the target fragment was at 500 kb. The DNA product was sequenced using the sequence 1st base technique performed by PT. Genetika Science Jakarta. The obtained sequences were compared to the related sequences using the BLAST search in the GenBank database. The assembled sequence alignment and pairwise comparisons of the nucleotide and amino acid identity were calculated using the Bioedit software and Emboss Needle, and the phylogenetic tree was constructed using MEGA 11 software (Tamura et al. 2021).

***In vitro* antifungal assay**

Assessment of mycelial growth. The isolate used in the *in vitro* assay was SMNND11, (identified as *N. dimidiatum*). The experiment was conducted in Petri plates (90 mm) containing PDA (10 mL per plate) with six concentrations of sodium salt, four replications and four plates for each treatment. Six different treatments were applied, the first of which was conducted in PDA, the second was in PDA with an additional 1% (w/v) sodium salt; the third was in PDA with an additional 2% (w/v) sodium salt, the fourth was conducted in PDA with an additional 3% (w/v) sodium salt, the fifth was carried out in PDA with 4% (w/v) sodium salt, and the sixth was performed in PDA with 5% (w/v) sodium salt. In every plate, 10 ppm chloramphenicol was added to avoid bacterial contamination. For seven days, 5 mm rounded-plugs from the SMNND11 cultures (seven days old) were isolated and incubated at 25–28 °C. We recorded the diameter colony growth of the fungi every day for six days and observed the characteristics of the fungal hyphae from each treated medium. The growth inhibition was calculated as the percentage of inhibition of radial growth relative to the control using the formula:

$$IA = \left[\frac{T2 - T1}{T2}\right] \times 100\% \quad (1)$$

where: *IA* – the inhibitory activity of sodium salt; *T1* – the diameter of the hyphae in the media with the added sodium salt; *T2* – the diameter of the hyphae with no sodium salt.

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Assessment of the number of conidia. The six day old colonies were harvested by gently rubbing the aerial hyphae loaded with 1 mL of sterile water. One millilitre of conidia suspension was dissolved in 9 mL of sterile water and vortexed for 3–5 min to release the conidia from the mycelia. The conidia density was counted microscopically using haemocytometer counter via serial dilution.

The conidia density (CD) was calculated using the formula:

$$CD = \frac{x}{L \times T \times D} \times 10^3 \quad (2)$$

where: CD – the number of conidia; x – the number of counted conidia; L – counted area (0.2 mm^2); T – the counted depth (mm); D – the dilution; 10^3 – the 1 mL suspension volume (10^3 mm^3).

The sodium salt solution trials to control stem canker. The field trial was conducted on five year old dragon fruits obtained from the IP2TP Sumani orchard. The dragon fruit orchard has been severely affected by stem canker five years ago. The plants were pruned heavily during the initial experiment to obtain similar plant conditions and pruned slightly after three months (only the young infected cladodes were cut). The annual rainfall from September 2020 to March 2021 was 58–267.2 mm, and the average mid-day relative humidity and temperature in the Solok region were 85–95% and 25 °C, respectively. The study consists of four plots for the chemically rotated fungicides [based on the recommendation of the Indonesian Tropical Fruit Research Institute (ITFRI) and Emilda et al. 2016], and four plots for sodium salt solution with two rows as barriers (eight poles per row) in the middle of the fields. Each plot consisted of four poles with two plants per pole. The spacing between rows is 3 m. The treatment with sodium salt was applied intensively for seven months, two times a week with a concentration of 30 g/L water using a mist blower. The contact and systemic chemical fungicide as the used control were a rotation of propineb, 80% mancozeb, 50% carbendazim, and difenoconazole 250 g/L every two weeks. The dose of the fungicide was applied according to the product recommendation. The fertilisation regime and other cultural practices followed the recommendations by the Directorate General of Horticulture (Direktorat Jendral Hortikultura) (Noegrohati et al. 2019).

We recorded the disease severity index (DSI) every four weeks for seven months. The data on the weight and disease intensity on the fruit were calculated from two harvests in April 2021.

The DSI was determined with the following scores: 0 = minor symptoms, 1 = mild symptoms (plants showing white spots in cladodes at 1–10 location points with a length of 5 cm), 2 = moderate symptoms (one white spots on cladodes at 1–10 location points with a length of 6–10 cm or 10–20 points with a length of 5 cm) and 3 = severe symptoms (> 10 location points with a length of 20 cm). The DSI on the fruit was calculated using four scores: 0 = less symptoms, 1 = mild symptoms (spot on fruit at < 1/4 total surface area of the fruit peel), 2 = moderate symptoms (spot on fruit at 1/4–1/2 total fruit peel surface area), and 3 = severe symptoms (spots on > 1/2 of the total surface area of the fruit peel). The stem canker DSI on the cladodes and fruits is determined into three criteria, namely (*i*) mild symptoms = index 0–1; (*ii*) medium symptoms = index 1.1–1.9 and (*iii*) high/severe symptoms = index 2–2.9:

$$DSI = \frac{\Sigma\ (ni \times vi)}{z \times N} \quad (3)$$

where: DSI – the disease severity index; Σ – the sum; ni – the frequency of the cladode/fruit with the vi score; vi – the score of the symptoms; z – the total number of cladodes/fruits; N – the maximal disease index.

Data analysis. The data presented in Table 1 are the means and standard deviations of four replicates and were analysed using an analysis of variance (ANOVA) and the Statistical tool for Agricultural software (STAR, IRRI, Philippines) for the differences. The differences were further analysed for their significance using Tukey's HSD test at $P < 0.05$. The data assumption presented in Figures 4 and 5 were tested for homogeneity of variances and normal distribution. All the data presented in Figures 4 and 5 were either tested with the independent *t*-test ($P < 0.05$), plus Mann-Whitney test ($P < 0.05$) or the Wilcoxon signed rank test ($P < 0.05$) using Minitab software (Akers 2018).

RESULTS

Isolate characterisation

The growth patterns of both isolate colonies were radial with irregular edges (Figure 1B). The colony's mycelial mass was greyish-white on the third day

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of incubation and became greenish-dark grey with further incubation with an abundant and dense aerial mycelium with dark pigmentation, irregular edges, and a dense production of arthrospores (Figure 1B). The hyphae were brown in colour, septate, and branched, thus constricted into spore chains before disarticulation. The arthroconidia were ellipsoid to ovoid and hyaline to dark brown with septate arthrospores (Figure 1C and 1D). The isolates were tentatively identified as *Neoscytalidium* sp. The isolates were deposited in the culture collection of the Plant Protection Laboratory, Indonesian Tropical Fruit Research Institute, with accession numbers SMNND11 and ARPN10.

Pathogenicity test of the *Neoscytalidium* isolates

The inoculated cladode was surrounded by protruding brown round spots observed at three days after inoculation with a mycelial mass propagated on the inoculation sites at five days after inoculation (Figure 1F and 1G). The cladodes were inoculated with water only and remained healthy and symptomless (Figure 1E). The fungus re-isolated from these inoculated cladodes was morphologically like the *Neoscytalidium* isolates (Figure 1H).

Molecular identification of the pathogen

The sequencing analysis revealed that the amplification of SMNND11 and ARPN10 with the ITS region resulted in a less similar band, with 520 bp for the SMNND11 isolates and 596 bp for the ARPN10 isolates. The SMNND11 isolate showed 100% sequence homology with *N. dimidiatum* (OP 247691) from the pitaya stem canker disease in China and the ARPN10 isolate had 98.66% sequence homology with *Neoscytalidium* sp. from the pitaya stem canker disease in Guangdong, China using the BLAST search. Because the similarity of SMNND11 with *N. dimidiatum* (OP247691) is almost 100%, we subsequently aligned the sequences using EMBOSS Needle and found that the isolates have 96.3% sequence homology.

Based on the neighbour-joining analysis of the ITS region in the MEGA 11 analysis program, the isolates of SMNND11 and ARPN10 are found in different branches, and it can be interpreted that the two are related, but at the genus level (Figure 2).

The SMNND11 isolate had a genetic closeness with isolate *N. dimidiatum* (OK458559) from Keningau Malaysia, whereas ARPN10 isolate was genetically closer to *Neofusicoccum parvum* (KJ193678) from mangoes of the China isolates.

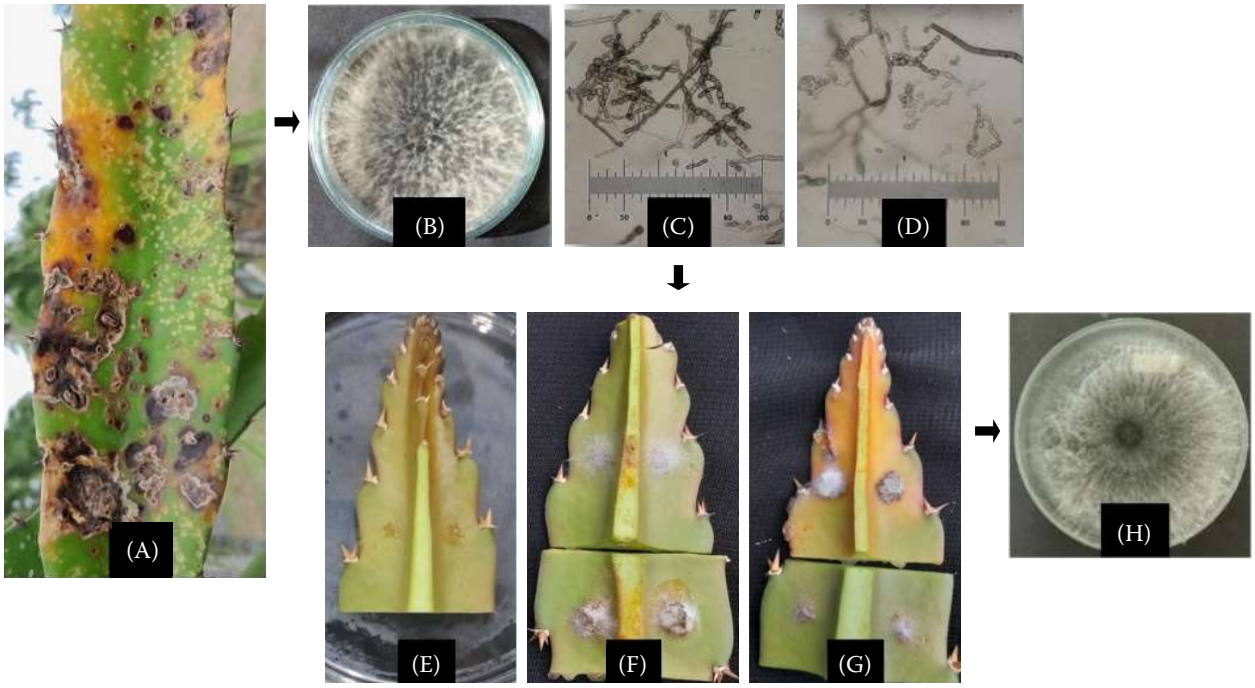

Figure 1. Pathogenicity test of two *Neoscytalidium* sp. isolates against *Hylocereus polyrhizus*
Diseased cladode (A), isolated fungus SMNND11 (B), micrograph of the arthrospore chain of the fungus SMNND11 (C), and ARPN10 (D), cladode after being treated with sterile water (E), SMNND11 isolate (F), and ARPN10 isolate (G), re-isolated from the artificially infected cladode with ARPN10 isolate (H)

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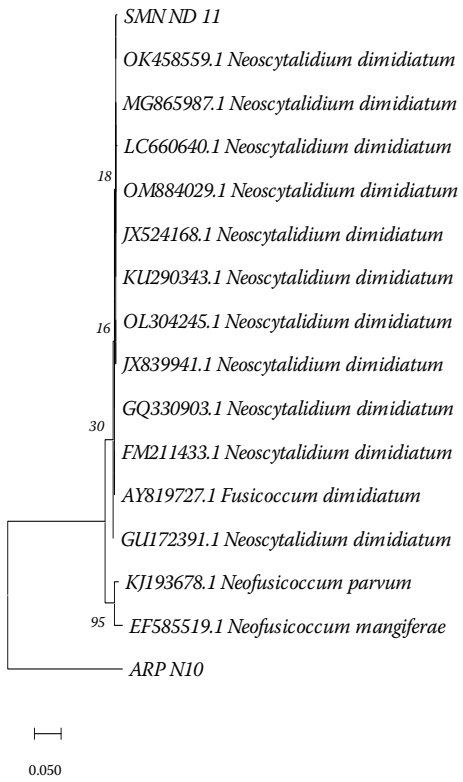

Figure 2. Phylogenetic tree by the neighbour-joining method based on the internal transcribed spacer sequences from the present isolates and other reference strains of *Neoscytalidium dimidiatum*

The pair alignment of the present isolates using Emboss Needle showed that the isolates share a homology of less than 50%. The ITS sequences of SMNND11 and ARPN10 were submitted to the National Center for Biotechnology Information (NCBI) GenBank with accession numbers LC726370 and LC726371, respectively.

In vitro assay and field trial

The efficacy of sodium salts against *N. dimidiatum* was evaluated under *in vitro* and in field conditions. In the *in vitro* test, the addition of sodium salt with a certain concentration in the growth media affected the growth and development of the fungus *N. dimidiatum*. Table 1 shows the effects of sodium salt at various concentrations on the *N. dimidiatum* mycelial growth and the number of conidia. The effectiveness of the inhibition of sodium salt on the mycelium growth was determined from the diameter of the fungal colony and calculated when the growth of the mycelium on the media without the addition of sodium salt was maximal [reached the edge of the Petri plate, i.e., four days after isolation (DAI)]. The results showed that the mycelial growth was not visually inhibited by sodium salt at 2%. This concentration reduced the mycelial growth by 0%, and the difference between this and the inhibitory effects of the media containing no sodium salt (mock) was not statistically significant ($P < 0.05$).

Table 1. Inhibitory activity of the sodium salt solution with various concentrations on the growth of *Neoscytalidium dimidiatum* up to seven DAI and the conidia density

Sodium salt (%)	Inhibitory activity (%)	Conidia density (10^6 spore/mL)
0 (mock)	0.00 ± 0.000^{d}	26.125 ± 7.598^{a}
1	0.00 ± 0.000^{d}	24.875 ± 8.062^{a}
2	0.00 ± 0.000^{d}	11.083 ± 3.374^{b}
3	16.00 ± 0.156^{c}	9.250 ± 1.998^{b}
4	63.30 ± 0.033^{b}	1.500 ± 0.470^{c}
5	73.00 ± 0.042^{a}	0.754 ± 0.287^{c}

$^{a–d}$Diferent letters in the column indicate a significant difference according to Tukey's HSD test ($P < 0.05$)

However, the 3, 4 and 5% concentration reduced the mycelial growth by 16.1%, 63.3% and 73.3%, respectively, which was significantly higher ($P < 0.05$) than the inhibitory rates of the ≤ 2% concentrations and the mock one.

The effects of the various concentrations of sodium salts on the *N. dimidiatum* sporulation show wide variations (Table 1). In this study, except for the 2% concentrations that cannot inhibit the mycelial growth of *N. dimidiatum*, sodium salt with 2–5% concentrations had inhibitory effects on the sporulation ranging 57.57–97.11% and the differences between the 2–3% and 4–5% of sodium salt were not statistically significant ($P < 0.05$), but were significantly different with the sodium salt at 1% and 0% (mock).

The result also shows that adding sodium salt to the medium affects the colour and hyphae elongation. The mycelium of the *N. dimidiatum* grown on sodium salt medium changed to dark green in the middle, whereas, at the edges, the mycelium was greyish coloured starting from three days (Figure 3A). Then, the hyphae grew short, and branched with a blunt end hyphae tip (Figure 3C). Although the *N. dimidiatum* mycelium on the media without the addition of sodium salt grew grey after threedays and turned uniformly dark green (Figure 3B), its hyphae grew straight and unbranched, and the hyphae tips were narrow (Figure 3D).

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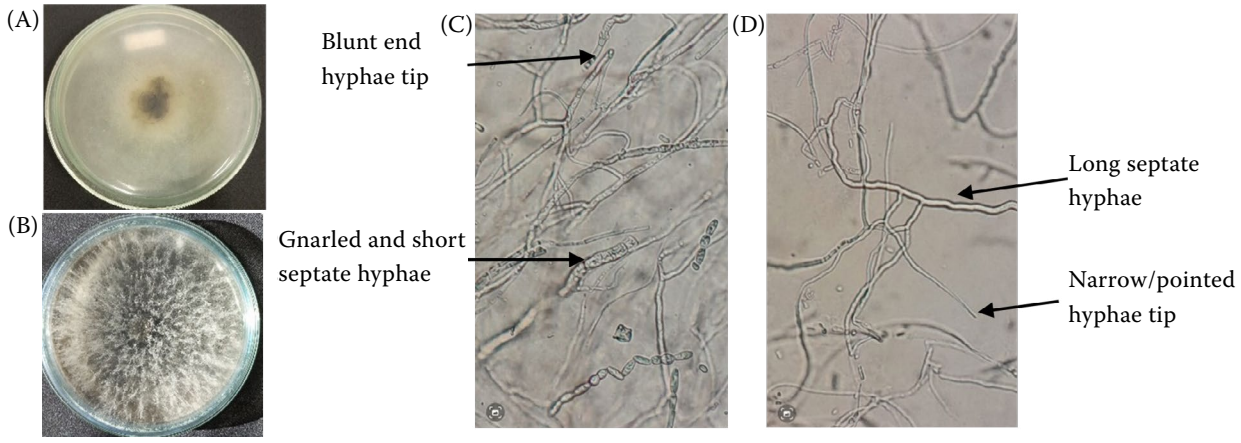

Figure 3. Morphology of the mycelium colour of the isolates in the medium containing 4% sodium salt (A) and no sodium salt (B) at 3 DAI and a micrograph of the hyphae shape of the isolates in the medium containing sodium salt (C) and no sodium salt (D)

The hyphae of the isolate grown in a medium containing sodium salt grew with short blunt end tips, short septate, and twisted and gnarled hyphae. The hyphae of the isolate grown in the medium with no sodium salt grew with an unbranched, point end tip, and with no reduced biomass of the hyphae

DSI on plants in the field trial

The disease severity in the treatment plants at the beginning of application (after pruning) was classified as mild (mean DSI < 1) (Figure 4, Wilcoxon signed rank test, $P < 0.005$). Mild symptoms of stem canker were still found on the main stem of the plant. The DSI in plants tended to decrease from either the salt or rotating fungicide application until seven months of observation [Figure 4, independent *t*-test ($P < 0.005$), Mann-Whitney test ($P < 0.05$)]. After seven months, the result showed that the DSI on the plants sprayed with the 30 g/L sodium salt solution was 0.334, which was lower than with the rotating fungicides that reached 0.758. The independent *t*-tests showed that the difference between the means of the DSI determined by the application of the sodium salt solution and the fungicide rotation was statistically significant at $P < 0.05$ ($t = -3.83$, $n = 16$, $P = 0.004$). The result implies that sodium salt is efficient in controlling the disease.

Besides observing the disease severity in the plants, we observed the disease intensity of the stem canker disease on the fruit. Observations were made twice during the harvest period (two weeks apart) after seven months of application of the sodium salt solution and rotating fungicides. Figure 5A shows no difference between the number of fruits (Wilcoxon signed rank test,

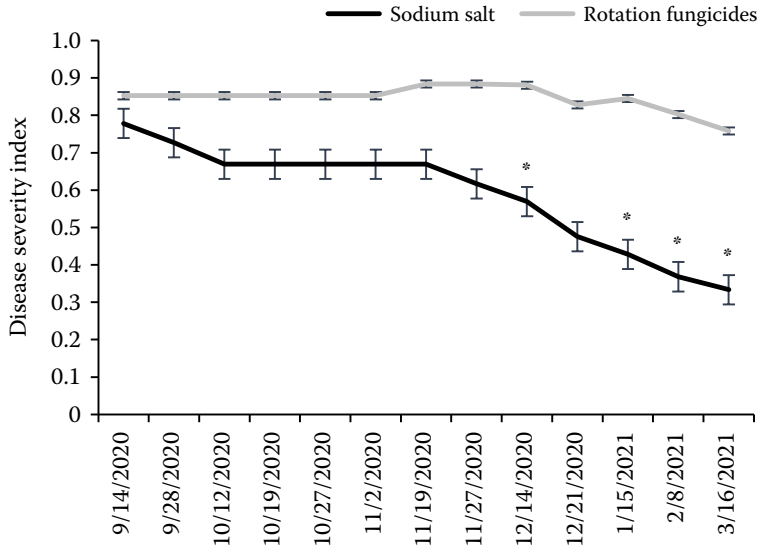

Figure 4. Disease severity index of the plants controlled with 30 g/L of the sodium salt solution and rotating fungicides (propineb, 80% mancozeb, 50% carbendazim, and difenoconazole 250 g/L) every two weeks

Bars are the mean ± SE [$n = 16$ (sodium salt) and 16 (rotated fungicide)]

*Significant differences between the treatments. Data were analysed either with the independent *t*-test ($P < 0.005$) (DSI December 14, 2020, January 15, to March 16, 2021), plus the Mann-Whitney test ($P < 0.05$) (DSI September 28 to November 27, 2020, and December 21, 2020) or the Wilcoxon signed rank test ($P < 0.05$) (DSI September 14, 2020)

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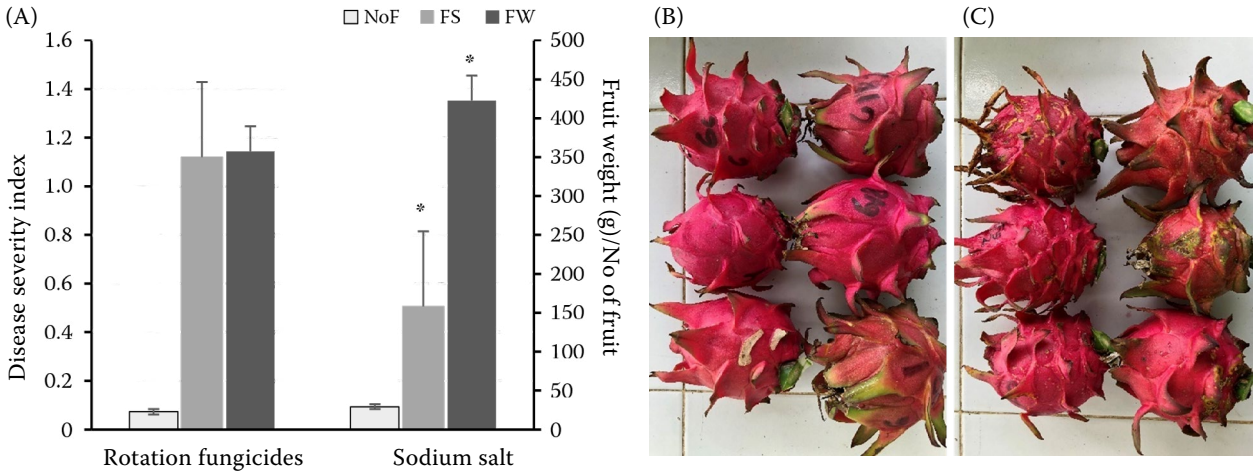

Figure 5. Number of fruits (NoF), fruit weight (FW) and disease intensity of the fruits (FS) harvested from the plants sprayed with 30 g/L of sodium salt twice a week and rotating fungicides (propineb, 80% mancozeb, 50% carbendazim, and difenoconazole 250 g/L) every two weeks (A), fruit harvested from the plant being treated with the sodium salt solution (B) and being treated with the rotating fungicide (C)

The data were the mean of the twice harvested period. Bars are the mean ± SE

*Significant differences between the treatments. The NoF was analysed with the Wilcoxon signed rank test ($P < 0.05$), the FW and FS were analysed with the independent t-test ($P < 0.005$)

$P < 0.05$), but a significant difference between the fruit weight and fruit symptoms of the two treatments (independent t-test: $P < 0.005$ and Mann-Whitney test: $P < 0.05$).

Furthermore, few symptoms on the fruits were detected after applying sodium salt solution and rotating fungicides. The fruit harvested from the plants with the sodium salt shows fewer symptoms than the rotating fungicide applications. The DSI criteria of the fruit is mild, and there were significant differences between the two treatments (independent t-test, $P < 0.005$) (Figure 5B and 5C).

Thus, the DSI of the fruit harvested from the plants with the sodium salt solution application decreased to 36.8% when compared with the rotating fungicide applications. It is assumed that the use of sodium salt can reduce the symptoms on the fruit in comparison to the application of the rotating fungicides.

DISCUSSION

Dragon fruit is a new plant that is grown in Indonesia. However, in recent years, the production of this fruit crop has drastically decreased. This plant has been destroyed by the stem canker disease, which was triggered by the large-scale monoculture planting. In a short time, the disease quickly spread throughout the development area. Identification of pathogens that cause stem canker in dragon fruit plantations in Solok West Sumatra was carried out by Jumjunidang et al. (2019). In this study, one of two fungal isolates collected from dragon fruit stems was infected with stem canker in the exact location as the Jumjunidang et al. (2019) isolates, but in different years. The two isolates of this fungus morphologically had white-grey aerial hyphae that developed rapidly on PDA media; the hyphae became brownish grey or dark grey within three days. Similarly, the microscopic character shows that this fungus has insulated hyphae and varied spore shapes, namely oval, rounded, like stems, and some are intertwined to form chains (it has a high similarity with isolates of Solok). This characteristic supports that this pathogen is *Neoscytalidium* sp. (Jumjunidang et al. 2019). The morphological and asexual phases of the *Botryosphaeriaceae*, such as *Neofusicoccum, Pseudofusicoccum* and *Neoscytalidium*, look the same. This genus shows *Fusicoccum*-type conidia similarly with narrow, ellipsoid to slightly oval and thin-walled forms, making them morphologically indistinguishable (Crous et al. 2006). Hence, a molecular analysis and phylogenetic studies must be conducted. The identification results showed that SMNND11 isolate was different from the ARPN10 isolate. Based on phylogenetic tree analysis, the ARPN10 isolate was more closely

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related to *N. parvum* causing stem canker in mango plants. Then, from the results of the pathogenesis test, this isolate was also proven to be able to attack dragon fruit (Figure 1). However, the ITS region cannot distinguish a species sufficiently, various genetic markers need to be used. Dy et al. (2022) pointed out that the ITS, nuclear large subunit, and tubulin region were three regions that can be used to distinguish *N. dimidiatum* very accurately.

In the *in vitro* test, the mycelium growth inhibition of the *N. dimidiatum* (SMNND11) isolate was more than 50%, starting from a 4% sodium salt concentration. Sodium chloride was effective in controlling the Fusarium crown and root rot caused by *Fusarium oxysporum* f. sp. *asparagi* and *F. proliferatum* than other chloride salts (calcium chloride, ammonium chloride and manganese chloride) (Reid et al. 2001). Stevenson et al. (2015) stated that the higher the concentration of sodium chloride on a substrate, the lower the water activity, which impacts the inhibition of the hyphae elongation. In the present study, however, sodium salt at 3% had a negative effect on the shape of the *N. dimidiatum* (SMDND11) isolates. As the sodium salt concentration increased, at a concentration of 3 to 5%, the hyphae shape of *N. dimidiatum* (SMNND11) were twisted and gnarled (Figure 3). This phenomenon was found in *Stereocaulon commixtum, S. japonicum*, and *Cladonia vulcani.* The hyphae of *S. commixtum, S. japonicum* and *C. vulcani* were twisted and gnarled, and the tips of hyphae were swollen, and the biomass of hyphae was decreased (Takahagi et al. 2000).

Our data indicated that adding sodium salt affects the number of conidia that develop in the media. Juniper and Abbott (2006) stated that sodium chloride inhibits spore germination and mycelium growth. The mechanisms by which sodium salts affect the number of conidia on the amended PDA are not known. Boumaaza et al. (2015) observed that the sporulation rate of *Verticillium alboatrum*, *V. dahliae* and *Botrytis cinerea* were stimulated when the NaCl concentration increased. However, our result showed a decrease in the conidia production *N. dimidiatum* with an increase in the sodium salt concentrations, which is consistent with those observed by Sanogo (2004). Boumaaza et al. (2015) found that a 300 ppm sodium salt concentration stimulated the *B. cinerea* sporulation, but an increased concentration inhibited the mycelial growth and sporulation.

In field testing, it was also seen that the application of a sodium salt solution at a dose of 30 g/L twice a week in a dragon fruit plantation (stem canker endemic site) could suppress the disease symptoms on the plants and fruits better than the fungicide applications. The potential of a sodium salt solution as a synthetic fungicide has been described by several studies. Deliopoulos et al. (2010) explained that various types of inorganic salts can reduce the disease severity of 49 types of pathogenic fungi in 34 types of plants. They emphasised that the potency of this sodium salt solution can be used as an alternative and can be integrated with more comprehensive controls than with a synthetic fungicide.

We sprayed a sodium salt solution and rotated fungicides for seven months (on the flowering stage) and the harvesting of the fruit was conducted one month later. Our results indicate that the sodium salt solution decreased the disease symptoms on the fruit better than rotating fungicidal applications. How the salt affects the disease symptoms on the fruit are not known. However, the sodium salt may affect the natural metabolism of dragon fruit which may affect the resistance of the fruit cells to the pathogen infection. A study states that several types of salt can induce resistance to grey mould in grapes, triggered by an increased superoxide dismutase, peroxidase, ascorbate peroxidase, phenolic and flavonoid activity in the fruit cells. It has been further explained that the compound plays a primary role during the synthesis of lignin, which acts as a cell wall reinforcement, improving resistance against several pathogens and altering the antioxidant ability of fruits to control the pathogen infection (Youssef et al. 2020).

In the present study, we could not explain how salt application of up to seven months affected the firmness and flavour changes in the fruit flesh. A study showed that dragon fruit sprayed with up to 4.0 g/L calcium chloride, a type of salt, once a week until 28 days after anthesis can strengthen the hardness of the peel, but does not affect the pH, solid soluble content, and acidity (Ghani et al. 2011). The quality of red dragon fruit was also not affected after soaking it with 4.0 g/L of calcium chloride for 30 min (Awang et al. 2013). In another study, Naveena and Immanuael (2017) stated that there was no difference in the quality (pH, total soluble solid, fruit weight) between tomato fruits treated with calcium chloride or sodium chloride.

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The centre of dragon fruit orchards in West Sumatra is Padang Pariaman, located mainly on the coastal area. Many plantations in this area have been drastically destroyed due to the stem canker attacks. The potential for sodium salt as an alternative fungicide opens up opportunities for farmers to use salt in the field. Farmers having plantations/orchards near the coastal area can use seawater as a source of salt for curative measures of stem canker disease on their dragon fruit plants. Furthermore, salt is easily obtained by farmers, is cheaper than the chemical fungicide prices, and is safer for one's health. In the present study, the total salt used for seven months is 50 400 g with a price of EUR 0.000 61/g, and the total money spent was EUR 30.82. In seven months, the fungicides propineb and mancozeb were applied at 1 050 g/each fungicide, 630 g of carbendazim was applied, and 210 mL of difenoconazole was applied. The price of each chemical fungicide; propineb is EUR 0.015/g, and other three are EUR 0.037/g. The total expenditure for all the fungicides was EUR 65.49. With this cost, the farmer can save at least EUR 34.67 in seven months.

CONCLUSION

In this study, *Neoscytalidium* sp. has been isolated and identified as the pathogen of stem canker of dragon fruit using morphological, molecular, pathological and culture analyses. It is the first report of *Neoscytalidium* sp. to infect dragon fruit in Indonesia. Sodium salt at > 3% displayed the highest suppression of the pathogen in the *in vitro* tests and showed more efficiency in controlling stem canker of dragon fruit than the fungicides that were rotated in the field trial. The information in this article should be used to understand the epidemiology of the disease and to find an alternative management strategy against stem canker disease caused by *N. dimidiatum.*

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