LenteraBio
p-ISSN: 2252-3979 e-ISSN: 2685-7871

Isolation of Fungi from Leaves Siam (*Citrus nobilis* var. microcarpa) with Zig-zag Symptoms in Citrus Plantation in Singkawang City

Isolasi Jamur dari Daun Jeruk Siam (Citrus nobilis var. microcarpa) Bergejala Zig-Zag di Perkebunan Jeruk Kota Singkawang

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Abstract. Various disease symptoms can be found on the leaves of the Siamese orange (Citrus nobilis var. microcarpa), one of which is a disease that has zig-zag symptoms. The aim of this research was to isolate fungi from Siamese lime leaves with zig-zag symptoms. This research was conducted at the Microbiology Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences, Tanjungpura University, Pontianak, from January to December 2021. Sampling was carried out in one of the orange plantations in Singkawang City. Isolation is carried out using the direct plating method (direct planting). Morphological characteristics were observed by looking at macromorphological characteristics, namely the color of the upper and lower colonies of the fungus and observing micromorphologically, namely the type of hyphae and spores. This research obtained three fungal isolates which are thought to be members of the genera Diplosporium, Mucor and Penicillium. The isolates obtained are thought to cause disease in citrus plants with zig-zag symptoms.
Key word: identification; leaf disease; fungi

Abstrak. *Berbagai gejala penyakit dapat ditemukan di daun jeruk siam (Citrus nobilis var. microcarpa), salah satunya yaitu penyakit yang bergejala zig-zag. Tujuan penelitian ini untuk mengisolasi jamur dari daun jeruk siam bergejala zig-zag. Penelitian ini dilakukan di Laboratorium Mikrobiologi, Jurusan Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Tanjungpura Pontianak, pada bulan Januari sampai Desember 2021. Pengambilan sampel dilaksanakan di salah satu perkebunan jeruk Kota Singkawang. Isolasi dilakukan dengan menggunakan metode direct plating (tanam langsung). Karakteristik morfologis dilakukan pengamatan dengan cara melihat karakteristik secara makromorfologis yaitu warna koloni atas dan bawah jamur dan mengamati secara mikromorfologi yaitu jenis hifa dan spora. Penelitian ini memperoleh tiga isolat jamur yang diduga anggota genus Diplosporium, Mucor dan Penicillium. Isolat yang diperoleh diduga dapat menyebabkan penyakit pada tanaman jeruk dengan gejala zig-zag.*
Kata kunci: *identifikasi; penyakit daun; jamur*

INTRODUCTION

Diseases on citrus leaves with zigzag symptoms can be mediated by insect vectors, one of which is mediated by the insect *Phyllocnistis citrella*. These symptoms are known as citrus canker disease. Infection with this pathogen usually occurs through stomata, lenticels and wounds, and is very susceptible to young growing tissues or young plant leaves. Citrus canker becomes a serious disease when wet weather conditions occur during the period of bud emergence and development. Zig-zag leaf disease is one of the symptoms of leaf cancer, which is generally caused by the bacteria *Xanthomonas* sp., (Polek, 2007). This cancer disease that attacks citrus leaves, twigs and fruit can affect the growth, quality and quantity of citrus fruit. Severe citrus cancer infections cause the fruit to remain small and often experience growth failure. Shoots or branches that are still green can also be infected and growth disrupted (Semangun, 2004).

Research on fungi isolated from the leaves of Siamese orange plants which showed signs of illness was carried out by Ningsih et al. (2012) shows that there are three types of fungi, namely *Basidiophora* sp., *Capnodium* sp. and *Sphaceloma* sp. *Basidiophora* sp. The cause of melanosis disease on citrus leaves which has symptoms in the form of spots with small indentations that are yellow to

blackish brown. *Capnodium* sp. causes sooty dew disease which has symptoms in the form of black spots on the outer layer of the leaf organs. *Capnodium* Sp. the cause of anthracnose disease which has symptoms in the form of plant shoots turning dark so they appear burnt. *Sphaceloma* sp. causes scab disease which has symptoms in the form of yellow warts found on the leaves. Data that informs about fungus from Siamese lime leaves with zig-zag symptoms is currently not available. Therefore, this research is important to determine the fungus found on the leaves of Siamese orange plants (*Citrus nobilis* var microcarpa) with zig-zag symptoms.

MATERIALS AND METHODS

Samples of Siamese lime leaves with Zig-zag symptoms (Figure 1) were taken and then stored in sterile plastic. Samples are placed in a plastic bag and then put into a cooler box. The samples were then taken to the Microbiology laboratory, Faculty of Mathematics and Natural Sciences, Tanjungpura University, Pontianak.

Figure 1. Zig-zag symptomatic leaves

Isolation is carried out using the direct planting method (direct platting). *Citrus* leaves that have disease symptoms are cleaned with liquid detergent, then use running water to clean. Cut squares of lime leaves measuring 1x1 cm on the part of the leaf with cancer symptoms and the healthy part. The leaf cuttings were sterilized for 30 seconds using 1% NaOCl solution then removed with tweezers and washed for five minutes using sterile distilled water with three repetitions, then placed on filter paper to air dry. The leaf cuttings are placed in 3 points in the PDA medium and then allowed to grow in the culture medium for the fungal mycelium. Re-purify any mushrooms that grow using new PDA media (Harahap et al., 2013).

Identification of fungi on leaves with zig-zag symptoms obtained in Siamese orange (*Citrus nobilis*) plantations by paying attention to macromorphological and micromorphological characteristics. Microscopic characteristics were observed by taking a fungal culture using a sterilized tube needle and placing the fungal culture on a sterilized glass object, then to observe the fungus under the microscope, a drop of Lactophenol was added. The fungal characters were matched with the book Illustrated Genera of Imperfect Fungi Fourth Edition (Barnet & Hunter, 1998), A Guide To Tropical Fungi (Tan, 1990) and the book Descriptions of Medical Fungi (Sarah et al., 2016). Data obtained from the results of isolation and identification were analyzed descriptively and presented in the form of visuals (photos) macroscopic and microscopic.

RESULTS

Based on the research results, it was found that three genera of fungi on the leaves of Siamese orange plants had zig-zag symptoms. The fungus that was isolated from the leaves of the Siamese orange plant is thought to be a member of the genus *Diplosporium*, *Mucor* and *Penicillium*. The *Diplosporium* fungus (Figure 2) has brown colored colonies with a slightly rough hyphae texture, round conidia in one phialid, with erect, hyaline conidiophores. *Mucor* (Figure 3) has the character of white colonies on rounded edges and a rough texture. The micromorphological characteristics of this fungus have non-septated hyphae, imperfectly round sporangium, erect hyaline sporangiophores. *Penicillium* (Figure 4) has a dark green upper colony color with white sides around it, with a slightly

rough texture. This fungus has micromorphological characteristics in the form of long chain conidia, round shape, single phialid shaped like a brush, single branched metula (biverticillate-symmetric).

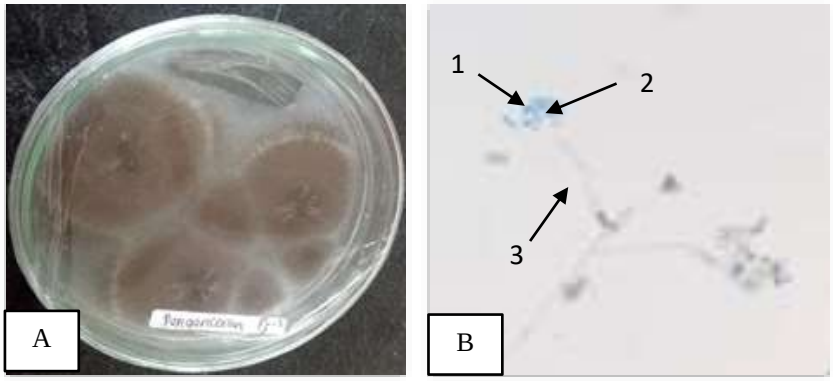

Figure 2. *Diplosporium,* A. Macromorphological *Diplosporium,* B. Micromorphological *Diplosporium* at magnification 40x : 1. Conidia 2. Phialid 3.Conidiophore

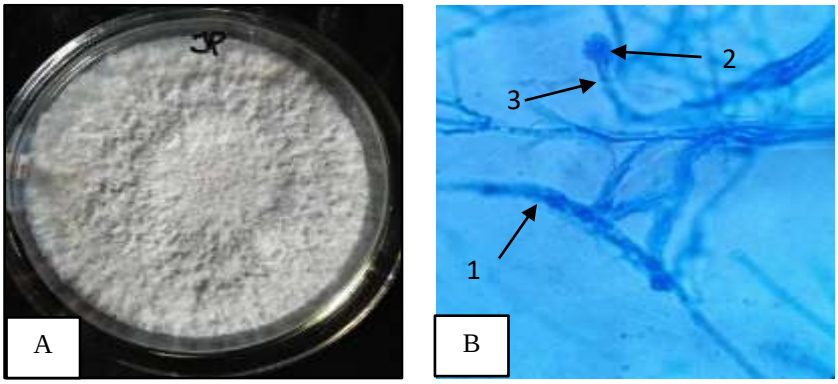

Figura 3. *Mucor* A. Macromorphological *Mucor* . B. Micromorphological *Mucor* at magnification 40x 1. Hyphae 2. Sporangium 3. Sporangiophore

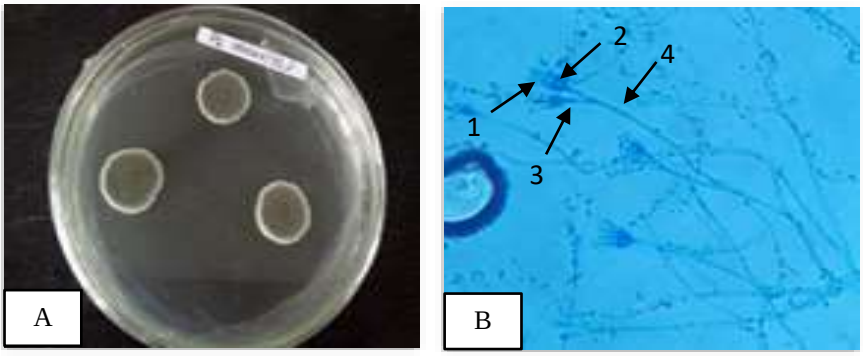

Figura 4. *Penicillium* A. Macromorphological *Penicillium* B. Micromorphological *Penicillium* at magnification 40x 1. Conidia 2. Phialid 3.Metulae 4. Conidiphore

DISCUSSION

Based on the results of the isolation and identification of fungi from leaves with zig zag symptoms, it was found that the genus *Diplosporium* has dark brown macromorphological characteristics with a slightly rough hyphae texture, round conidia in one phialid, with erect, hyaline conidiophores. This character is in line with the statement by Barnet and Hunter (1998) that *Diplosporium* has erect, hyaline conidiophores with irregular branches. According to Tortora et al.,

(2002) *Diplosporium* fungi are found in the rhizosphere soil. The *Diplosporium* fungus found on orange leaves is thought to be passively inoculated onto the plant host. This is in accordance with the opinion of Sopialena (2017), namely that inoculum comes from the soil on the land where plants grow. Pathogenic inoculum is generally carried to the plant host passively by wind, water and insects.

The *Mucor* fungus has the characteristic of a white upper colony with rounded edges and a rough texture, the back of the colony is initially white and then yellow. Colony growth filled the growth medium by 9 cm on day 7. Micromorphological characters had non-septated hyphae, round sporangium, long sporangiophores. Based on Sarah (2016), the *Mucor* genus has a fast growing colony morphology, cotton-like to hairy, white to yellow, to dark gray, with sporangium development. Sporangiophores are erect, single or branched, forming large (60-300 mm in diameter), terminal, round sporangia, multispores, without apophyses (widening structures at the tip of the sporangiophore) and with good development. Sporangiospores are hyaline, gray or brownish, round to elliptical, and have smooth walls. Apart from being found on Siamese lime leaves, *Mucor* fungus is also found on other plants. The *Mucor* fungus causes disease on long bean leaves with disease symptoms in the form of slightly yellow leaves with irregular spots and symptoms of wilting and yellowing of the leaves caused by the *Mucor* racemosus fungus. The *Mucor* sp fungus was also found from the isolation of endophytic fungi on the leaves of the mangosteen plant (*Garcinia mangostana* L.) ((Suciatmih et al., 2011). Apart from that, the *Mucor* sp fungus was also found on the leaves of Javanese Ginseng (*Talinum paniculatum*) which lives on the tissue. leaves (Sugiharti et al., 2016). *Mucor* is a fungus that can attack phanerogamae plants and is often found in post-harvest products because of its very fast colony growth (Susilowati & Listyawati, 2001).

The *Penicillium* fungus found on Siamese orange leaves has a macromorphological character, the color of the upper colony is dark green with white around the sides, with a slightly rough texture. This fungus has micromorphological characteristics in the form of long chain conidia, round shape, single phialid shaped like a brush, single branched metula (biverticillate-symmetric). According to Tan (1990), *Penicillium* has chain-shaped conidia on brush-like phialids. Fungal colonies are green, grayish green or bluish green. *Penicillium* fungus can attack various fruits, foods and materials such as leather and fabric. The *Penicillium* fungus also attacks the leaves of long bean plants. *Penicillium* fungus can cause disease on rubber leaves, this is in accordance with research from Veronica, et al., (2015), that Penicillium was found on leaves aged 0 - 9 months which had symptoms of disease, namely the leaf blade had white circles, as well as on the tips of the leaves look slightly wrinkled. Research by Andriani et al., (2019) shows that in pineapple the results of isolation and identification of pathogenic fungi were obtained in the form of fungi from the genus *Penicillium* which cause disease symptoms in the form of regular leaf spots. According to Yadav et al., (2018) the *Penicillium* genus can be found in various habitats, including plant parts such as the phyllosphere, rhizosphere, and endophyte, and from various rotting fruits. Thus, it can be said that the *Penicillium* fungus is thought to cause Siamese orange leaf disease.

CONCLUSION

Based on the research that has been carried out, the results obtained are three fungal isolates found in the isolation of the leaves of Siamese orange plants (*Citrus nobilis* var. microcarpa) with zig-zag symptoms, namely members of the genus *Diplosporium*, *Mucor* and *Penicillium,* each of which has different characteristics both macromorphologically and micromorphology. The isolates obtained are thought to cause disease in citrus plants with zig-zag symptoms.

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Article History:
Received: 13 March 2023
Revised: 7 August 2024
Available online: 21 August 2024
Published: 30 September 2024

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How to cite this article:
Lestari N., Rahmawati, Mukarlina. 2024. Isolation of Fungi from Leaves Siam (*Citrus nobilis* var. microcarpa) with Zig-zag Symptoms in Citrus Plantation in Singkawang City. LenteraBio; 13(3): 437-441.