Bulgarian Journal of Agricultural Science, 30 (No 1) 2024, 57–66

Inhibitory mechanism of *Citrus sinensis* L. osbeck peel extract against the growth of *Sclerotium rolfsii* Sacc. pathogen causing stem rot disease in peanut plants

Janeka Safitri[1*], Abdul Latief Abadi[2], Anton Muhibuddin[2] and Liliek Sulistyowati[2]

[1] *Brawijaya University, Faculty of Agriculture, Program of Plant Pathology, Jl. Veteran, Malang, East Java, Indonesia*
[2] *Brawijaya University, Faculty of Agriculture, Department of Plant Protection, Malang 65145, East Java, Indonesia*
[*]*Corresponding author:* janekasafitri9@gmail.com

Abstract

Safitri, J., Abadi, A. L., Muhibuddin, A. & Sulistyowati, L. (2024). Inhibitory mechanism of *Citrus sinensis* L. osbeck peel extract against the growth of *Sclerotium rolfsii* Sacc. pathogen causing stem rot disease in peanut plants. *Bulg. J. Agric. Sci., 30*(1), 57–66

Sweet orange is a tropical fruit that is widely cultivated in Indonesia. Oranges are one of the most popular types of fruit. Besides being consumed directly, several types of citrus fruit can be processed into beverages and other processed foods. Sweet citrus fruits are usually eaten whole or processed into drinks after peeling the outer skin (flavedo). This peeling process causes a lot of orange peel waste. Orange peel waste is one of the wastes that can be processed to produce new products that have a high value. Orange peel contains essential oils that can be used as a vegetable fungicide to control plant diseases. One of the most common pathogens is *Sclerotium rolfsii*. These pathogens are soil pathogens that can survive in the soil for a long time. Currently, synthetic pesticides are the main control of the fungus *S. rolfsii.* The use of synthetic fungicides can cause resistance and environmental pollution. For this reason, alternative control of *S. Rolfsii* is needed. This study aims to (i) determine the content of compounds in *Citrus sinensis* peel extract with GC-MS (ii) to determine the inhibition on the growth of *S. Rolfsii* (iii) to determine the antifungal mechanism of *Citrus sinensis* peel extract. The results showed that *Citrus sinensis* peel extract can be antifungal in suppressing the growth of *S. Rolfsii* and possibly as a plant-based fungicide. *Citrus sinensis* peel extract is fungicidal which causes the death of the fungus *S. Rolfsii* in the treatment of 4.5% and 5% concentrations of phenol, flavonoid, and terpenoid compounds contained in *Citrus sinensis* peel extract can play a role in antifungal activity against *S. Rolfsii* by affecting the morphology of hyphae, causing cell walls, and there is no difference in hyphae.

Keywords: antifungal; *Citrus sinensis*; fungicide; peel extract; *Sclerotium*

Introduction

Sweet orange (*Citrus sinensis* L. Osbeck) is a tropical fruit that is widely cultivated and commercialized in Indonesia. Sweet oranges are one of the most popular fruits in the world. Besides being consumed directly, several types of citrus fruit can be processed into beverages and other processed foods. Sweet citrus fruits are usually eaten whole or processed into drinks after peeling the outer skin (flavedo). This peeling process causes a lot of orange peel waste. Sweet citrus fruits are widely consumed worldwide as fresh produce or juices and comprise about 70% of the total production and consumption of large quantities of citrus (Liu et al., 2012; Sharma et al., 2017; Hernandez et al., 2021). The higher the consumption of lime the more.

Orange peel waste is one of the wastes that can be processed to produce useful new products. Orange peel contains essential oils. Sweet orange peel essential oil contains

ascorbic acid, which causes a sour taste (Jwanny et al., 2012; Handayani et al., 2019). The main content of orange peel essential oil is generally limonene (Rezende et al., 2020). In addition, this essential oil also contains bioactive compounds such as phenolic compounds, alkaloids, flavonoids, and limonene, which are terpenoid derivative compounds and the most abundant. Orange peel oil is also reported to contain tannins, reducing sugars, flavonoids, and phenols (Rauf et al., 2014). Potential antimicrobial components in the orange peel are D-limonene, terpenes, sesquiterpenes, oxygenated monoterpenes, linalool, acid esters, aliphatic invites, and other unknowns (Singh et al., 2021). These volatile oil compounds can be used to turn orange peel waste into a viable product. One of them is a plant-based fungicide that can be used to control pathogenic fungi that cause disease in plants.

The rest of the orange peel, which is considered waste, can be a source of antioxidants and antimicrobials (Hanafy et al., 2021). Some research results show that essential oils can be antifungal. Previous research that sweet orange peel extract can inhibit the growth of fungi *Candida albicans, Aspergillus niger* and *Penicillium notatum* (Oikeh et al., 2020). Another study also showed the crude ethanolic extract of orange peel as a significant inhibition of *Aspergillus flavus* when compared to controls (Liu et al., 2021). Orange peel extract can inhibit the growth of pathogenic fungi on plants such as *Alternaria alternata, Botrytis cinerea*, and *Monilinia fructicola* (Hernandez et al., 2021).

One of the pathogenic fungi that often attacks plants is the pathogen *Sclerotium rolfsii*. *S. rolfsii* (Telemorph: *Athelia rolfsii*), which can cause stem rot, is a serious threat to peanut production, and occurs worldwide. *S. rolfsii* is a soil-borne disease that can persist in the soil for a long time. During the period of infection, *S. rolfsii* forms a large number of white hyphae and old sclerotia, which spread rapidly in the field, reducing production soil reductions by 10%–80% (Xie et al., 2014; Chen et al., 2018; Yan et al., 2021; He et al., 2022). *S. rolfsii* is also a broad host-pathogen that can infect more than 400 plants, and new hosts are continuously being identified (Coulibaly et al., 2022). This pathogen can also survive in extreme soil conditions because it has sclerotia, which can survive for more than 10 years.

Currently, pesticides are the main control of the fungus *S. rolfsii.* The use of synthetic fungicides can cause resistance and environmental pollution. Overuse of synthetic fungicides leads to the development of resistant mutants and has consequent harmful effects on humans and the environment (Coulibaly et al., 2022). Therefore, it is necessary to control the fungus *S. rolfsii* such as *Citrus sinensis* peel extract. The content of natural ingredients such as essential oils in vegetable fungicides makes plant fungicides easier to decompose in the environment compared to synthetic functions. This study aims to determine the potential of *Citrus sinensis* peel extract in suppressing the growth of the fungus *S. rolfsii* and to determine the mechanism of *Citrus sinensis* peel extract as an antifungal agent in its effect on the morphology of *S. rolfsii.* So far, no studies have been conducted regarding the precise targeting of *Citrus sinensis* peel extract on *S. rolfsii.*

Materials and Methods

Study area

A pathogenic fungus was isolated from peanut plants with stem rot symptoms found in Ngerjo Village, Tanggung Gunung Sub-District, Tulungagung Regency, East Java. The fungus was then brought to the plant disease laboratory of the Faculty of Agriculture, Brawijaya University (Figure 1.)

Fig. 1. Location of isolated pathogenic fungi (8.25493384S, 111.86887338E)

Pathogen isolation

The fungus was isolated from diseased plants by taking the diseased plant parts. The diseased plant parts were cut by ± 1 cm, and the healthy plant parts were also cut by ± 1 cm. Next, the plant parts were grown on PDA media and incubated at room temperature. Then, the obtained fungi were identified by observing their morphology macroscopically and microscopically. The macroscopic morphological observation of the fungus included examining the color and type of colony growth, as well as the surface texture of the colonies on the PDA medium. Observations of the microscopic structure of the fungus included examining for the presence or absence of branching hyphae, hyphal bulkheads, sandwich joints, and conidium shape. If the results of the macroscopic and microscopic tests showed the characteristics of the *S. rolfsii* fungus, the pathogenesis test then followed. A pathogenicity test was carried out to ensure that the isolates that were found were of *S. rolfsii*, as the cause of stem rot disease in peanut plants.

Extraction

Extraction of *Citrus sinensis* was carried out by the maceration method, using 96% ethanol as solvent. Peeled oranges were cleaned and cut into small pieces before drying in an oven at 40°C for 36 hours. The dried *Citrus sinensis* peels were then mashed with a blender. Then, solvent was added to a ratio of 1:4 and the mixture was stirred using a shaker for 5 × 24 h. The peel extract was evaporated using a rotary evaporator at a temperature of 55°C with a rotation of 100 rpm for 1 h (until the solvent did not drip). The obtained viscous extract was then put into a sterile container and stored in a refrigerator. The extract was then analyzed using GCMS GC8890-MS5977B. 1 l of the extract sample solution was injected into the GCMS, which has a J&W Scientific, HP-5MS capillary column with dimensions of 30 m × 250 m × 0.25 m. Helium carrier gas was given at a flow rate of 1 ml/min (constant) with a 1:50 split ratio; the rate of increase was 10°C/min and the temperature was increased to 280°C for 24 minutes. The injector port temperature was 300°C and the mass spectrometer interface was 280°C. Identification of phytochemical compounds used the Willey and NIST databases.

Antifungal activity test

Antifungal activity test of *Citrus sinensis* peel extract against the *Sclerotium sp. fungus* was performed with the food poisoning method. The test was carried out by adding *Citrus sinensis* peel extract to PDA media. The concentration of extracts were 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, and 5%, and the test also involved synthetic pesticides as fungicides containing the active ingredients of difenoconazole, azoxystrobin, and propineb. The diameters of the fungal colonies were measured every day until the fungal colonies in the control treatment filled the Petri dishes. Fungal diameters were measured with a ruler and calculated with the following formula:

$$D = \frac{D1 + D2}{2},$$

where D = Diameter of pathogenic fungus grown on PDA medium;

$D1$ = Vertical diameter of fungal colonies grown on PDA medium;

$D2$ = Horizontal diameter of fungal colonies grown on PDA medium.

The value of inhibition percentage was calculated by comparing the diameters of the fungal colonies that grew on each petri dish in each treatment with the fungal colonies on the control petri dish. The inhibition percentage of *Citrus sinensis* peel extract was calculated with the following formula (Nweke, 2015):

$$\%\ \text{inhibitoty} = \frac{Dc + Dt}{Dc} \times \frac{100}{1},$$

where Dc = Diameter of fungal colonies in the control;

Dt = Diameter of fungal colonies in the treatment.

Mechanism of inhibition of fungal growth

The mechanism of inhibition of fungal growth by *Citrus sinensis* peel extract was observed using a light microscope. Observations were made on the fungus grown on PDA media that had been dripped with extract on glass slides. Observations were made from 1 day after inoculation to 5 days after inoculation.

Mode of antifungal action

Observation of the mode of antifungal action of *Citrus sinensis* peel extract was carried out using an electron microscope. Scanning Electron Microscopy (SEM) observations were carried out at the Bioscience Laboratory of Brawijaya University. Observations were made using samples of the *S. rolfsii* fungus in the treatment without the extract (the control) and the treatments with the extract. In the observations using SEM, the fungal hyphae were prepared on a glass cover and glued with carbon tape. The samples were also conductively coated with a layer of platinum using an ion-sputtering machine. The samples were then inserted into the TM3000 electron microscope chamber. Observations were made at a magnification of 2500x.

Data analysis

The obtained data were analyzed by ANOVA with a level of 5%. If the test results were significantly different, the Duncan Multiple Range Test (DMRT) was then performed. All obtained data were processed using Microsoft Excel 2019 software and presented in the form of graphs and tables.

Results and Discussion

Pathogen isolation

Pathogenic fungi were isolated from symptomatic plants found on agricultural land. In field observations, diseased peanut plants showed symptoms such as wilting and falling of plant leaves, as well as stem rot (Figure 2A). On the stem of the plant, there is a white fungal mycelium from the base of the stem to the root of the plant, and there are brown granules (sclerotia) around the roots of the plant (Figure 2B). The results of the isolation of pathogenic fungi on PDA media showed fungal colonies that grew white with a smooth surface and spread throughout the surface of the petri dish. On the 10th day after isolation, there were small granules on the surface of the mycelium. The granules were at first yellowish-white, before changing to light brown to dark brown with increasing age of the fungal colony (Figure 2C). Microscopic observation showed that the fungal hyphae were elongated and branched. In the fungal hyphae, there was a clamp connection and no conidia (Figure 2D). Kumar et al. (2014) stated that the mycelium of the *Sclerotium rolfsii* Sacc. fungus is at first silky white before changing to a dull white with a fan-like spread.

A pathogenicity test was carried out to prove that the isolated fungus is a pathogen that can attack plants. The results of the pathogenicity test showed that the discovered fungus was able to attack the peanut plant of the Tasya 2 variety. On the seventh day after inoculation, there were sclerotia around the base of the peanut plant stem and the soil surface around the base of the stem (Figure 3B). The peanut plants died 28 days after planting. In the peanut plants, there was rotting of the roots and the base of the stem, and root growth was not optimal (Figure 3C). Pathogen *Sclerotium rolfsii* Sacc. can attack on all plant tissues but generally attack plant rootstocks near or the soil surface. The fungus can survive for a

A B C D

Fig. 2. Pathogen isolation:
A – Appearance of diseased plant stems; B – Plant roots affected by disease; C – Macroscopic isolation of pathogenic fungi; D – Macroscopic isolates of pathogenic fungi

A B C

Figure 3. Pathogenicity of pathogenic fungi:
A – Germination phase; B – Fungus sclerotia on soil surface; C – Plants dying at 28 days after planting

long time because it produces a lot of sclerotia on the infected plant tissue from the remains of the infected plant (Mahadevakumar et al., 2016).

Antifungal Activity

The results on the antifungal activity of *Citrus sinensis* peel extract on the growth of *S. rolfsii* are presented in Table 1.

Table 1. Average Growth of *Sclerotium rolfsii* Sacc. with Different Concentrations of *Citrus sinensis* Peel Extract at 5 Days after Inoculation

Treatment	Diameter, cm	Inhibition percentage, %
Control	8.25±0.09 h	0
0.5%	7.85±0.18 gh	5
1%	6.65±0.27 gh	19
1.5%	4.44±0.49 fg	47
2%	2.05±0.32 def	75
2.5%	1.87±0.20 cdef	77
3%	1.39±0.27 bcde	83
3.5%	0.77±0.13 abcd	91
4%	0.25±0.11 abc	97
4.5%	0.00±0.00 ab	100
5%	0.00±0.00 a	100
Propineb	2.84±0.11 ef	68
Difenoconazole and Azoxystrobin	0.78±0.08 abcde	91

Note: Numbers followed by the same letter in the same column show that they are not significantly different based on the Duncan Multiple Range Test (α = 5%) after being transformed into √x+0.5

The inhibition of fungal colony growth was directly proportional to the increase in the concentration of *Citrus sinensis* peel extract. This indicates that the concentration of the extract can affect the effectiveness of the antifungal compounds contained in the *Citrus sinensis* peel extract. At a concentration of 2.5%, the *Citrus sinensis* peel extract began to show antifungal activity by stopping the growth of the *S. rolfsii* fungus; the classification is very strong when compared to without the addition of the extract, and the percentage of inhibition is higher than the fungicidal active ingredient of propineb (contact fungicide). The concentration was found to be fungicidal. The highest inhibition was found in extract concentrations of 4.5% and 5% with an inhibition percentage of 100% at 5 days after inoculation, which is a higher inhibitory value compared to fungicides with the active ingredients of Difenoconazole and Azoxystrobin (systemic fungicide).

Compound content of Citrus sinensis peel extract

Citrus sinensis peel extract was analyzed using GC-MS to determine the essential oil content in the extract. GC-MS is a technique used to separate the volatile compounds contained in an extract. The compounds separated by GC analysis will come out of the column and flow into the MS, at which point these compounds are identified based on their molecular weights. Based on the results of the GC-MS analysis of *Citrus sinensis* peel extract, 25 compounds were obtained with different retention times according to the type of compound analyzed (Figure 4). Retention time (RT) is the time (minutes) the compound is read by GM-MS. The peaks that appear indicate different compounds with different retention times. Figure 4 shows the compound with the concentration contained in RT 15.299, which is the element 2-Furancarboxaldehyde, 5-(hydroxymethyl) with a percentage area of 29.22%. The second peak is benzoic acid, which is seen in RT 12 753 with an area percentage of 13.16%, and Phloroglucinol, which is a phenol group is found in RT 8 942 with an area percentage of 6.15%.

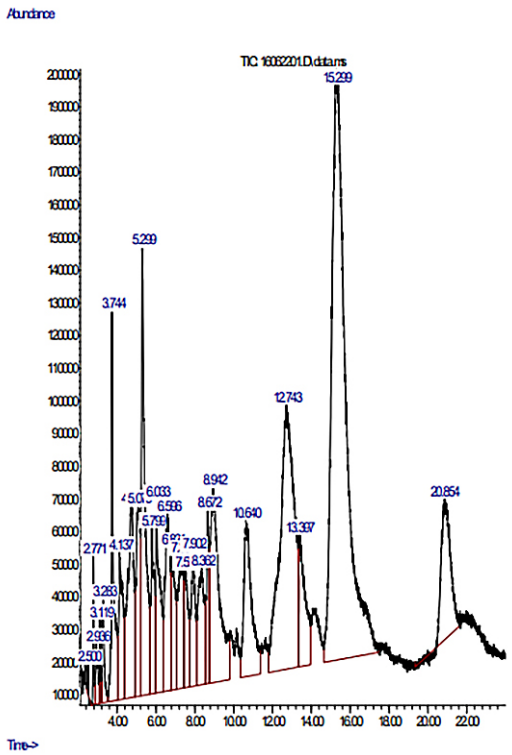

Fig. 4. GCMS chromatogram of *Citrus sinensis* peel extract

The major compound contained in cinnamon extract in this study was 2-Furancarboxaldehyde, 5-(hydroxymethyl). Ramalakshmi & Muthuchelian (2011) stated that the compound 2-Furancarboxaldehyde, 5-(hydroxymethyl) belongs to the aldehyde group, and the compound 4H-Pyran-4-one, 2.3-di-

Table 2. Content of compounds in *Citrus sinensis* peel extract

No	Retention Time	% Content	Compound	Qual.	Group
1	2.500	0.07	2-Butanethiol	25	Flavonoid
2	2.771	0.53	3-methylbutanal-Isopentanal	7	Another compound
3	2.936	0.42	N-Methyl-Proplylamine-1-Propanamine	14	Another compound
4	3.119	0.33	Ethylene Thiourea	38	Organic acid
5	3.283	0.80	L-lysine	38	Organic acid
6	3.744	2.81	Furfural	86	Aldehyde
7	4.137	2.09	Furfurylic alcohol	46	Aldehyde
8	4.742	4.26	Butanamide, N-(aminocarbonyl)-3-methyl	25	Organic acid
9	5.079	2.70	L-Valinol	22	Organic acid
10	5.299	5.75	2(3H)-Furanone, 5-methyl-	58	Aldehyde
11	5.799	2.04	1-methoxy-1,3-cyclohexadiene	78	Terpenoid
12	6.033	2.75	2-n-propylthiacyclohexane 2H-Thiopyran	47	Flavonoid
13	6.596	3.17	1,3-Cyclohexanedione	43	Terpenoid
14	6.821	1,89	1-(2-propenyl)tricylo	18	Fenol
15	7.308	2.43	Butyric acid	47	Aromatic compound
16	7.522	1.71	2H-Pyran Tetrahydro-2-methoxy	25	Flavonoid
17	7.902	2.01	2-Hexanethiol	47	Another compound
18	8.362	2.50	5-methyl-2-pyrazinymethanol	30	Another compound
19	8.672	1.54	Linalool	58	Terpenoid
20	8.942	6.15	Phloroglucinol	59	Fenol
21	10.640	4.33	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	72	Flavonoid
22	12.743	13.16	Benzoic acid	30	Aromatic compound
23	13.397	2.91	Phenol	43	Fenol
24	15.299	29.22	2-Furancarboxaldehyde, 5-(hydroxymethyl)	87	Aldehyde
25	20.854	4.43	2-Methoxy-4-vinyphenol	93	Fenol

hydro-3.5-dihydroxy-6-methyl which belongs to the Flavonoid compounds play a role in antimicrobial activity. Zabkan & Pavela (2013) stated that natural phenolic compounds are one of the most abundant antifungal active substances in plant essential oils which are characterized by the presence of a hydroxy group (–OH), which is bound to a benzene ring or a complex aromatic ring structure. Flavonoids are compounds that belong to the phenolic group and are the largest group of polyphenolic compounds that have hydroxyl groups and are lipophilic which can change membrane function and can act as oxidative phosphorylation partners that allow them to penetrate cell membranes (Hernandez et al., 2021).

Mechanism of inhibition of fungal growth

The mechanism of antifungal activity of *Citrus sinensis* peel extract was observed using a light microscope. There were morphological changes in the *S. rolfsii* fungus due to the effect of *Citrus sinensis* peel extract when compared to control hyphae. At the beginning of the first day of observation, it was seen that the hyphae began to bend (Figure 5B), and then the hyphae began to roll (Figure 5C). On the next day, the hyphae began to thin and shrink at 3 days after inoculation (Figure 5D), and the tips of the hyphae swelled 4 days after inoculation (Figure 5E). The hyphae branch began to lyse, causing the hyphae to break (Figure 5F) on the observation at 5 days after inoculation.

Scanning electron microscopy

Observations using SEM were carried out 3 days after inoculation. The mycelium of *S. rolfsii* in the control treatment showed a characteristic morphology with elongated and regularly branched hyphae with a smooth outer surface (Figure 6A). Treatment with *Citrus sinensis* peel extract clearly showed morphological changes in the hyphae of *Sclerotium rolfsii* Sacc. The hyphae in the treatment showed curling and wrinkling (Figure 6B), and the hyphae branches looked flat and empty (Figure 6C). In addition, there were also severed hyphae branches (Figure 6D and 6E).

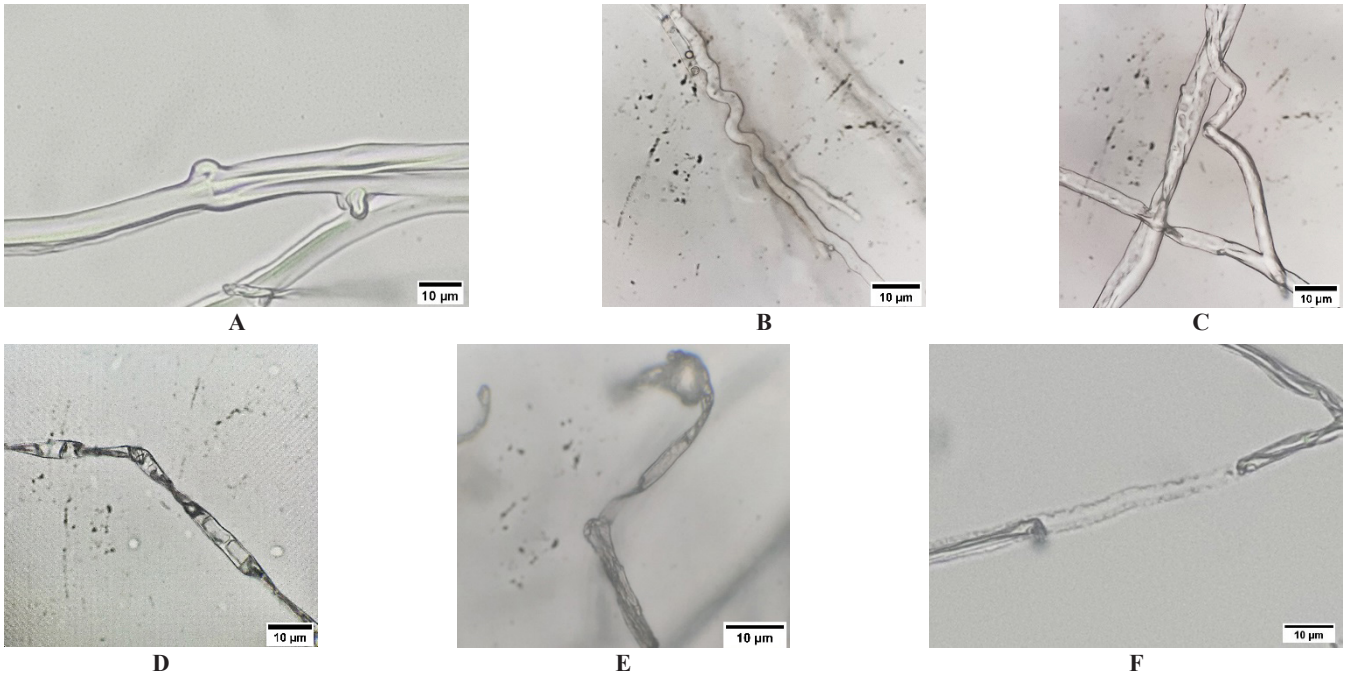

Fig. 5. Damage to the fungal hyphae of *S. rolfsii* due to the effect of *Citrus sinensis* peel extract seen through a light microscope:
A – control; B – bent hyphae; C – hyphae curling; D – hyphae curling and shrinkage; E – swollen hyphae tips; F – lysed hyphae

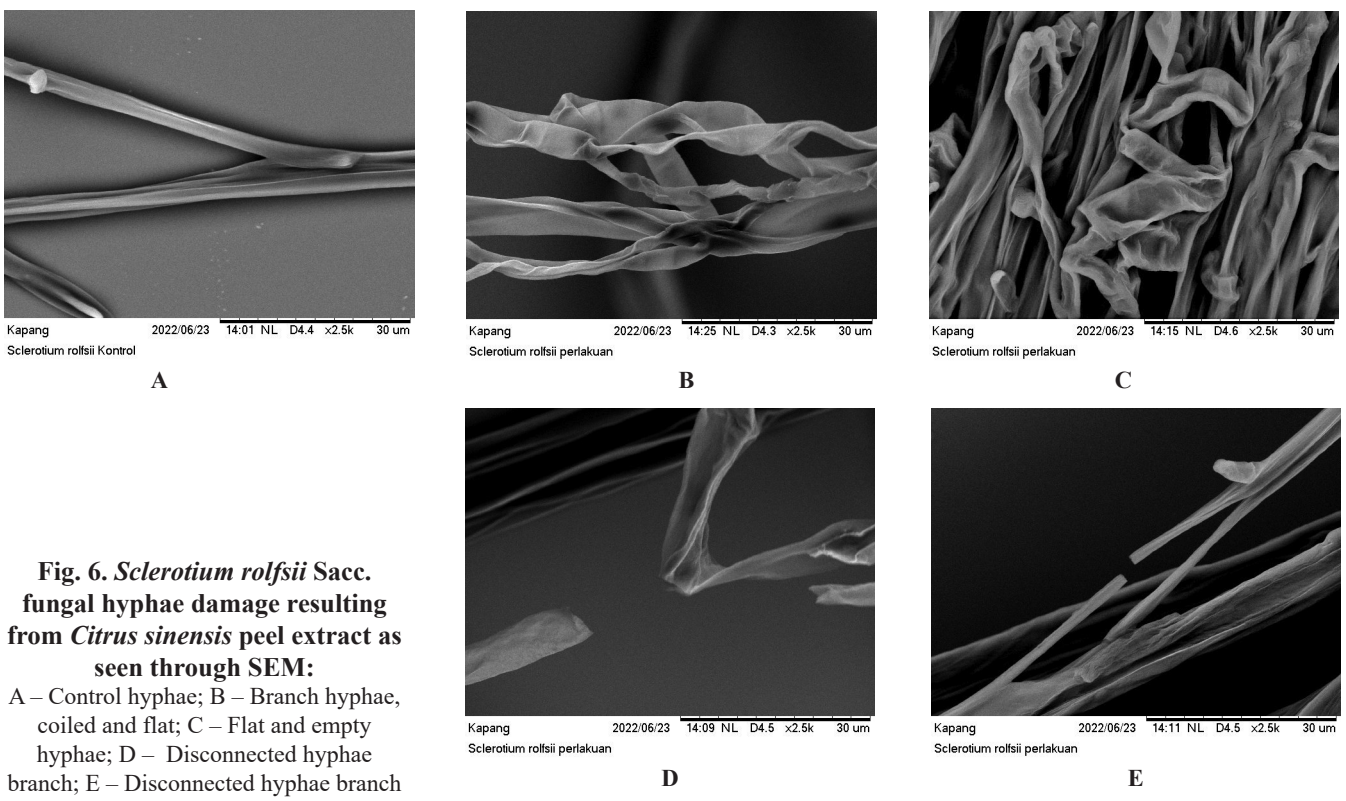

Fig. 6. *Sclerotium rolfsii* Sacc. fungal hyphae damage resulting from *Citrus sinensis* peel extract as seen through SEM:
A – Control hyphae; B – Branch hyphae, coiled and flat; C – Flat and empty hyphae; D – Disconnected hyphae branch; E – Disconnected hyphae branch

Discussion

The inhibition of fungal colony growth was directly proportional to the increase in the concentration of *Citrus sinensis* peel extract. This indicates that the concentration of *Citrus sinensis* peel extract can affect the effectiveness of the antifungal compounds contained in the extract. At a concentration of 2.5%, *Citrus sinensis* peel extract began to show antifungal activity by stopping the growth of the *S. rolfsii* fungus; this is classified as very strong when compared to without the addition of the extract, and the percentage of inhibition is higher than the fungicidal active ingredient of propineb (contact fungicide). *Citrus sinensis* peel extract showed antifungal activity at an extract concentration of 2.5% with approximately 77% growth conidiation compared to the growth of *S. rolfsii*. *Citrus sinensis* peel extract caused complete growth inhibition of *S. rolfsii* at extract concentrations of 4.5% and 5% with an inhibition percentage of 100% at 5 days after inoculation (Table 1), for which the inhibitory value was higher compared to fungicides with the active ingredients of Difenoconazole and Azoxystrobin (systemic fungicides). Jing et al. (2014) stated that the results of their study showed that essential oil from citrus can be antifungal and have a broad effect spectrum on *Aspergillus* spp., including *A. niger, A. flavus, A. fumigatus, A. terreus,* and *A. parasiticus*. The results from another research showed that the essential oils extracted by hydrodistillation from *Citrus sinensis*, *C. reticulata*, and *C. deliciosa* fruit peels exhibit antifungal activity against the *Sclerotinia sclerotiorum* phytopathogen by inhibiting fungal growth by approximately 50% at 300 µL (Dias et al., 2020).

The mechanism of the inhibitory antifungal activity of *Citrus sinensis* peel extract is not certain, which compounds play an active role in inhibiting the growth of the pathogenic fungus *S. rolfsii* because it is needed in further molecular research. According to Handayani et al. (2019), the antifungal activity of several combinations of antifungal compounds can be more effective than the work of each compound itself. On the other hand, the combined activity of several antifungal compounds is less effective than the work of each compound. Judging from the antifungal activity of the active compound of *Citrus sinensis* peel against the growth of the pathogenic fungus *S. rolfsii,* several citrus compounds contained in the extract of the *Citrus sinensis* peel could do.

The content of compounds in sweet *Citrus sinensis* extract has the potential as a fungicide that inhibits the growth of pathogenic fungi *S. rolfsii.* These compounds can be related to the enzymatic reaction of cell wall synthesis, which affects the morphogenesis and growth of fungi. Each compound contained in *Citrus sinensis* peel extract has a different mechanism of antifungal activity and may act as a fungicide. Yong et al. (2022) stated that furfural compounds were applied in agriculture as fungicides. Furfural is very effective in inhibiting the growth of the fungus *Tilletis foctens* on wheat plants. Seed immersion treatment showed that furfural and formaldehyde solution could kill fungi within a certain immersion period (Zeitsch, 2000; Yong et al., 2022). However, the antifungal mechanism of furfural compounds and aldehyde groups is not known. Another compound that can affect the antifungal activity of *Citrus sinensis* peel extract is benzoic acid. Berne et al. (2015) stated that benzoic acid and its derivatives can form undissociated lipophilic acids that can penetrate membranes and cause intracellular acidification and cause oxidative stress, protein aggression, lipid peroxidation, and inhibit the entry of substances into the membrane.

The mechanism of antifungal activity of *Citrus sinensis* peel extract was indicated by the inhibition of the growth of *S. rolfsii* and changes in hyphal morphology, which caused damage to the hyphal morphology to cause death. Morphological damage to the hyphae of the pathogenic fungus *S. rolfsii* due to administration of *Citrus sinensis* peel extract was seen from the changes in hyphae morphology such as loss of rigidity in the cell wall (hyphae wrinkled and curled), leakage of hyphae (hyphae looked flat and empty) and broken hyphae connections. Sharma & Tripati (2008) stated that the antifungal activity of the essential oil of *Citrus sinenis* occurs on the cell wall and cytoplasmic withdrawal in the hyphae and eventually causes the death of the fungus.

Changes in the morphology of the fungus *S.rolfsii* can be caused by compounds contained in *Citrus sinensis* peel extracts such as phenolic compounds, flavonoids, and terpenoids. The flavonoid compounds can play a role in antifungal activity by disrupting mitochondrial homeostasis and also by disrupting the integrity of cell membranes in fungi. While the terpenoid group has an inhibitory mechanism against fungi by causing changes in the permeability of fungal cell membranes (Zabka & Pavela, 2013). The results showed that linalool damaged the cell membrane of the *Trichophyton rubrum* by detecting intracellular components released into the external environment (Lima et al., 2017). Linalool, which belongs to the terpene group, can affect the integrity and function of the plasma membrane, and therefore, can inhibit growth and induce significant morphological changes. The fungal cell membrane is a dynamic structure consisting of enzymes and proteins embedded in a lipid bilayer. Often, the proper function of cellular systems interacts closely with fungal morphogenesis and plays a key role in virulence and pathogenicity (Lima et al., 2017). Another study stated that linalool damages the cell membrane of *Aspergillus flavus*

and causes leakage and shows a loss of membrane integrity (Li et al., 2022). All fungal cell activities are linked to the cell membrane. Once the cell barrier is destroyed, the mass of cellular contents leaks out, affecting several important functions in the cell (Liao et al., 2022). Other compounds that can play a role in the antifungal activity of *Citrus sinensis* peel extract are phenol compounds. According to Liu et al. (2021), several mechanisms underlying the antifungal activity of phenolic compounds include 1) inhibition of glycan and chitin biosynthesis which results in deformation of fungal cell walls, 2) disruption of plasma membranes and their biosynthesis which causes leakage of intracellular components, 3) suppression of fungal nucleic acid metabolism through inhibition of processes in mitochondria, 4) inhibition of metabolic enzymes. Based on the results of the study, it can be said that *Citrus sinensis* peel extract has fungi toxic activity that inhibits the growth of *S.rolfsii*, causes damaging morphological changes, and causes death, so *Citrus sinensis* peel extract is suitable as an alternative in controlling *S. rolfsii.* The present study also confirmed the antifungal activity of citrus oil already described in literature, but the modes of action and morphological alterations have not yet been studied.

Based on the present study, it can be said that *Citrus sinensis* peel extract has toxic activity to fungi that inhibits the growth of *S. rolfsii* and causes damaging morphological changes and death, and thus *Citrus sinensis* peel extract is suitable as an alternative in controlling *S. rolfsii*. Further molecular research is needed to ascertain which compounds play an active role in inhibiting the growth of the *S. rolfsii* pathogenic fungus, in relation to the inhibitory antifungal activity mechanism of *Citrus sinensis* peel extract.

Acknowledgments

The authors would like to thank the supervisors who have provided direction and support. Gratitude is also given to parents, who have supported morally and materially, as well as to friends who have helped and contributed to this research.

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Received: **September, 11, 2022**; *Approved*: **December, 19, 2022;** *Published:* **February, 2024**