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Eco-Friendly Antifungal from Seven Botanical Extracts and Its Effect To Plant Pathogenic Fungi

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Fusarium oxysporum **has been the enemy in agriculture due to the wide range of infections in the whole crop and postharvest. Botanical extracts in traditional medicine systems were considered valuable sources for discovering new antifungals. Thus, exploration to get antifungals from eco-friendly botanical extracts as an alternative to synthetic fungicides needs to be expanded. Therefore, this study aims to test the antifungal potential of seven eco-friendly botanical aquadest extracts against *F. oxysporum in vitro*. The antifungal potential test was done on PDA media and the mycelial growth data was collected every two days for seven days. There were three antifungal phenomena were observed. The pandan leaf extract had a 4% fungal inhibition zone (phenomenon I). The extract of banana leaf, Hibiscus flower, papaya leaf, guava twig (phenomenon II), orange peels, and betel leaf (phenomenon III) had no values of fungal inhibition zone. Thus, pandan leaves extract revealed a fungal inhibition while other six botanical extracts had not enough antifungal potential *in vitro*.**

Key words: agriculture, antifungal phenomena, crops, phytopathogenic, potential inhibition

INTRODUCTION

Fusarium oxysporum Schlecht (Hypocreales: Nectriaceae) (Michielse and Rep 2009) is a phytopathogenic fungus that caused seedling blights, root and crown rots, bulb and corm rots, and vascular wilt diseases of a wide range agronomically important crops (Zemánková and Lebeda 2001). This fungus species has several toxins, i.e., Moniliformin 130-270 mg/kg (Chelkowski *et al.* 1990), Sambutoxin 150 mg/kg (Kim and Lee 1994), and trans-Zearalenon 12.80 µg/g of rice (Richardson *et al.* 1985). The *Fusarium* species complex causes *Fusarium* diseases cycle on the crops and postharvest through its conidia infection to the stems, leaf, fruit, vascular system, and inflorescence of major tropical fruit crops (Zakaria 2023). The symptoms of *Fusarium* infection are discoloration of stems, roots, and leaf; tracheids and vessels are anastomosed early; and vascular tissues are folded and degreened (Buddenhagen 2009). Furthermore, the colony pigmentation of *F. oxysporum* on peanut sucrose agar (PSA) medium is a white, pink, or dark purple pigment of floccose aerial mycelium with a growth rate of 80–100 mm/10 days (Zemanková and Lebeda 2001).

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Botanical extracts of *Azadirachta indica* (leaf), *Capsicum frutescence* (fruit), *C. frutescence* (leaf), *Zingiber officinale* (leaf), and *Cymbopogon nardus* (tuber) dried powders dissolved in water showed the inhibitor zone range were lowest 52.5% (500 ppm) and highest 100% (3,000 ppm) referred to *C. frutescence* fruit *in vitro* against *Penicillium digitatum* (Al-Samarrai *et al.* 2012). Furthermore, the spraying extracts of *A. indica* (leaf), *C. frutescence* (fruit), and *C. frutescence* (leaf) showed considerable statistical significance when applied to fruits against *P. digitatum* (Al-Samarrai *et al.* 2012). Surprisingly, the water extract of *Punica granatum* peels can be an alternative against the synthetic fungicide because its antifungal activity was higher than the Marisan 50 PB fungicide and highest than the other 23 botanical water extracts against *F. oxysporum* (Rongai *et al.* 2015).

A natural plant-derived commercial product, cinnamaldehyde, is widely used as an antifungal (Copping and Duke 2007). Cinnamaldehyde disrupts the fungal membranes of particular genera and its strong odor is used as a repellent and attractant (Copping and Duke 2007). However, botanical extracts in traditional medicine systems were considered valuable sources for discovering new antifungals (Mishra *et al.* 2020). Thus, an exploration to get antifungal from eco-friendly botanical extracts needs to be expanded. Therefore, this study aims to test the antifungal potential of eco-friendly botanical aquadest extracts, i.e., pandan leaf, banana leaf, *Hibiscus* flower, papaya leaf, guava twig, orange peels, and betel leaf against *F. oxysporum in vitro*.

MATERIALS AND METHODS

Materials. *Fusarium oxysporum* isolates (obtained from Laboratory of Mycology, Department of Biology, IPB University), Potato Dextrose Agar, petri dishes with nine cm diameter, paper disc with six mm diameter for the extract test, glass materials, ruler, stationery, oven , and the seven botanical aquadest extracts (Table 1).

Preparation of Botanical Extracts. Seven fresh botanical materials (Table 1) were dried at 70°C for 3 x 24 hours. Following the ratio, the dried materials were blended with aquadest (Table 1) and filtered with calico fabric. The extracts were saved at 4°C and ready to use.

Preparation of Potato Dextrose Agar (PDA) as Culture Medium of *Fusarium oxysporum*. All the compositions following manufacture instruction. The media were sterilized at 121°C, 1 atm, and 15 min. The sterilized media were poured into petri dishes and saved at room temperature to inoculate *F. oxysporum* isolate.

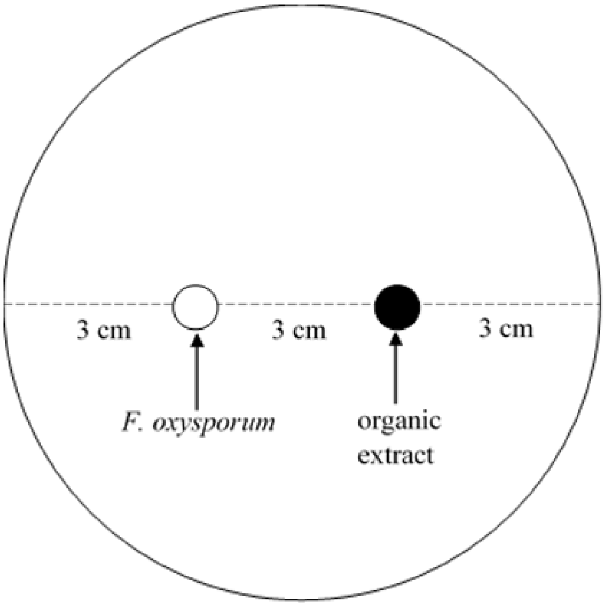
Fungal Propagation and Characteristic Assessment. *Fusarium oxysporum* was grown and propagated on the PDA medium. The characteristics assessment of *F. oxysporum* was done by observing the fungus sample under a light compound microscope. One week-incubated *Fusarium oxysporum* that has approximately 1-2 cm diameters were ready to be tested for the antifungal potential.

Mycelial Growth Inhibition Test Design. The antifungal potential test was done on PDA media in petri dishes. A colony of *F. oxysporum* and a disc soaked into botanical extract were placed on the PDA medium at the distance of three cm between them (Figure 1). The mycelial growth observation was done in seven days by collecting the growth data (R1 and R2) every two days. The inhibition zone was counted by a formula in Figure 1. The colony colour of *Fusarium* was noted and recorded during the span.

Table 1. The ratio of seven botanical material dry mass against aquadest solvent

Samples	Class: order (itis.gov)	Ratio (g:ml)*
Pandan leaf	Magnoliopsida: Pandanales	1:4
Banana leaf	Monocotyledonae: Zingiberales	1:4
Hibiscus flower	Magnoliopsida: Malvales	1:9
Papaya leaf	Magnoliopsida: Brassicales	1:4
Guava twig	Magnoliopsida: Myrtales	1:4
Orange peels	Magnoliopsida: Sapindales	1:2
Betel leaf	Magnoliopsida: Piperales	1:9

*The ratio of dried samples and aquadest to obtain the aquadest botanical extracts



$$\text{Inhibition zone (\%)} = \frac{R1 - R2}{R1} \times 100\%$$

R1: the mycelium lenght to the petri dish edge

R2: the mycelium lenght to the botanical extract dish

Figure 1. The design of anti-*Fusarium oxysporum* potential test against botanical extract on PSA medium and the inhibition zone formula

RESULTS

The *Fusarium oxysporum* observation under a light microscope showed matured characteristics (Figure 2A-E). Furthermore, seven primary colonies of *F. oxysporum* mycelium were placed on the first day in separated PDA media against seven botanical extracts and grown in seven days (Figure 3). The mycelial growth was observed by measuring the R1 (colony radial to the petri dish edge) and R2 (colony radial to the botanical extract disc) (Figure 3). The R2 must be shorter than R1 to calculate a positive antifungal inhibition zone percentage on the seventh day (Figure 1). On the first day, the shortest R2 length was from *F. oxysporum* that tested against papaya leaf extract (1.15 cm), banana leaf, pandan leaf, *Hibiscus* flower, guava twig, and orange peels and the longest was betel leaf extracts (1.6 cm) (Figure 3). Meanwhile, on the seventh day, the shortest R2 was from *F. oxysporum* that tested against pandan leaf (2.4 cm), banana leaf, orange peels, papaya leaf, guava twig, betel leaf, and the longest was *Hibiscus* flower (4.9 cm) (Figure 3).

The inhibition zone measurement was done using the colony radial data in Figure 3, with the highest zone from pandan leaf extract (4%) (Table 2). The other six extracts showed no inhibition zone percentages, revealing no fungal inhibition (Table 2). Although the other six extracts showed negative inhibition zone percentage, we found three antifungal

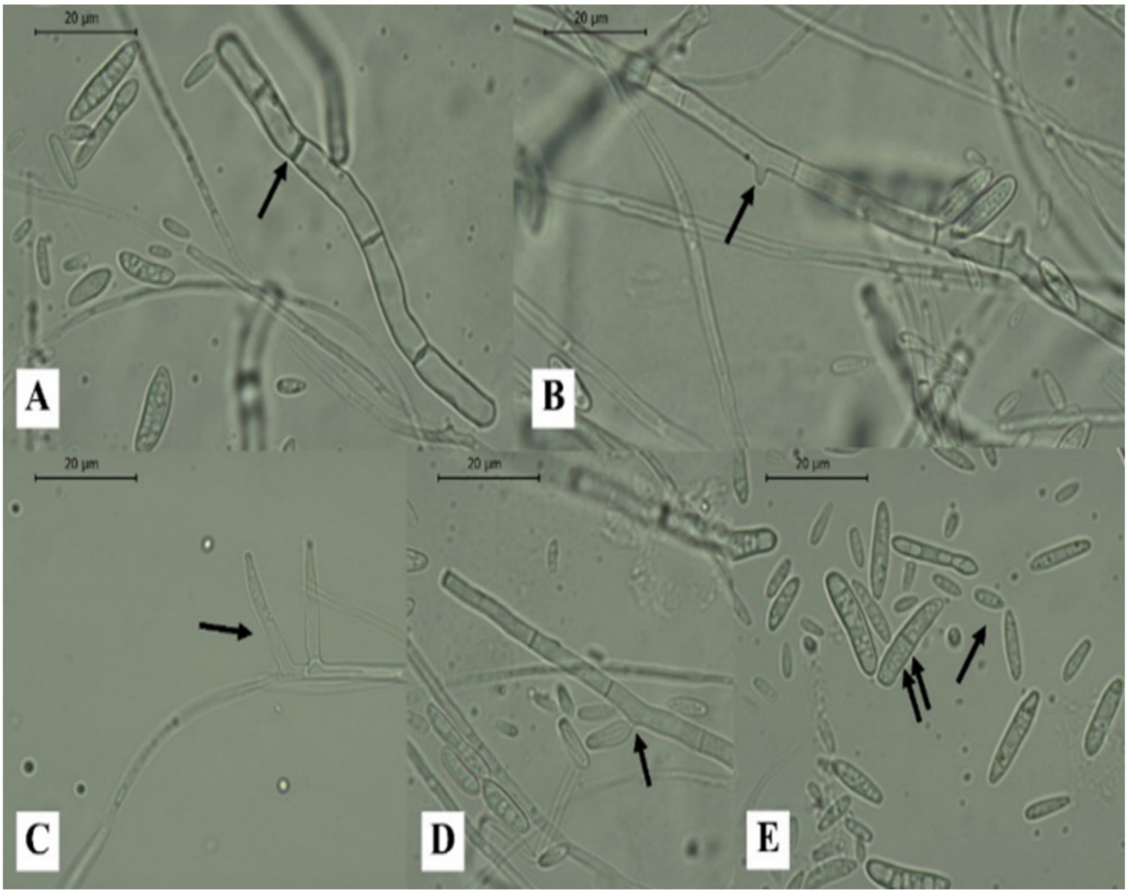


Figure 2. Microscopic characters of *Fusarium oxysporum* used in this study. (A) Septate hyphae (arrow), (B) conidiophore bud (arrow), (C) mature conidiophore (arrow), (D) conidiogenous cell with conidia (arrow), (E) micro- (arrow) and macroscopic conidia (double arrow). Magnification 400x

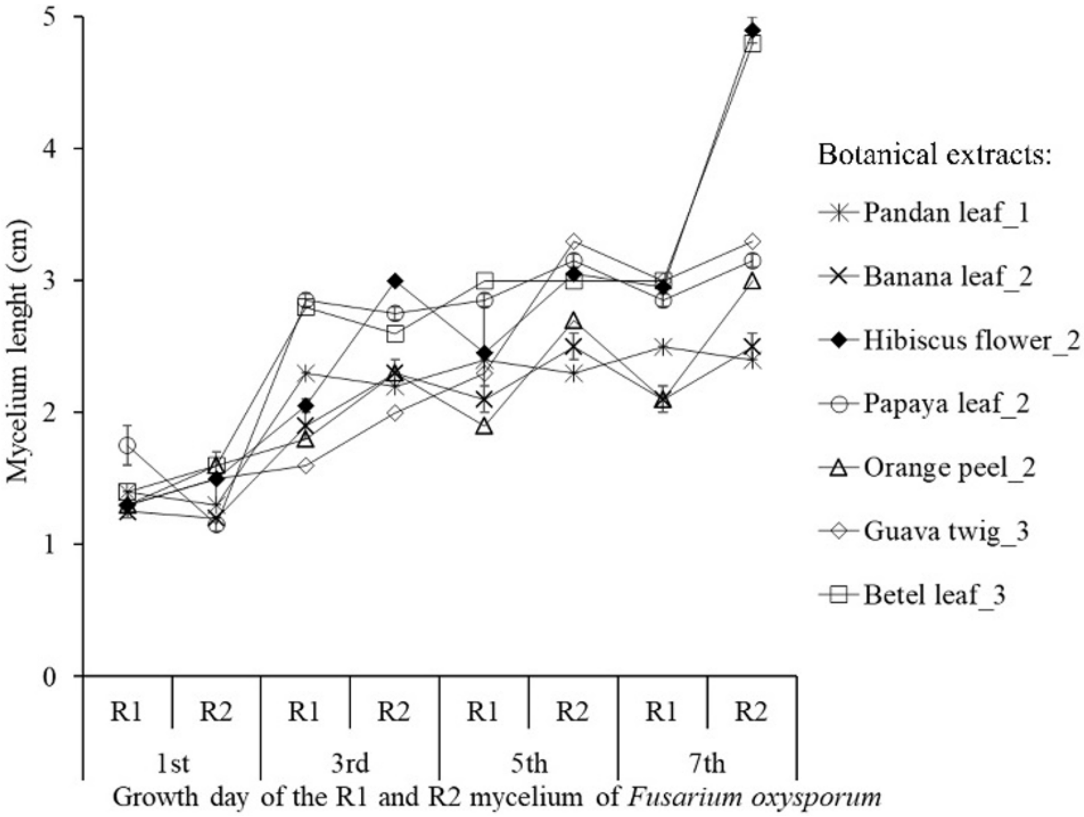


Figure 3. The mycelial growth of R1 and R2 *Fusarium oxysporum* mycelium under the test against botanical extracts. R1: the length to the petri dish edge, R2: the length to the botanical extract disc. Number 1–3 of the botanical extracts refers to the anti-fungal phenomena in Table 2

phenomena, surprisingly (Table 2). Phenomenon I occurred in pandan leaf extract only, which showed a positive inhibition zone (4%) (Table 2). Phenomenon II occurred in banana leaf, *Hibiscus* flower, papaya leaf, and guava twig extract (Table 2, Figure 4B-E. Moreover, phenomenon III occurred in orange peels and betel leaf extracts (Table 2, Figure 4F-G). Phenomenon II and III showed that the botanical extracts had not enough antifungal potential (Table 2, Figure 4B-G) . In addition, we observed that the pigmentations of *F. oxysporum* were white, black, yellow/orange, and purple (Figure 4A-G).

Table 2. Inhibition zone and antifungal phenomena of the seven botanical extracts

Botanical extract	Inhibition zone on the 7th day (%)	Antifungal phenomena*
Pandan leaf	4	I
Banana leaf	0	II
Hibiscus flower	0	II
Papaya leaf	0	II
Guava twig	0	II
Orange peels	0	III
Betel leaf	0	III

*three types of observed antifungal phenomena, i.e., phenomena I: R2 < R1 (positive inhibition zone), II: mycelium grew by avoiding the extract disc, III: mycelium grew by not piercing the extract disc. R1 and R2 refer to Figure 1. The *F. oxysporum* mycelial growth data of R1 and R2 in seven days under the antifungal potential test can be seen on Figure 3. The phenomena documentations are shown in Figure 4A–G

DISCUSSION

The morphology characters of *Fusarium oxysporum* used in this study are shown in Figure 2. The seven botanical extracts were prepared from one Monocotyledonae plant, while the rest were Magnoliopsida plants, revealing three antifungal phenomena (Table 2). The botanical extract of pandan leaf showed a positive antifungal ability, while the rest had negative inhibition percentage values (Table 2). Pandan leaf simplicial powder contains chemical compounds, i.e., alkaloid, flavonoid, saponin, and tannin compound groups (Sinaga *et al.* 2021). The 4% inhibition of the pandan leaf (Table 2) was presumably due to its chemical compound. The alkaloids compound

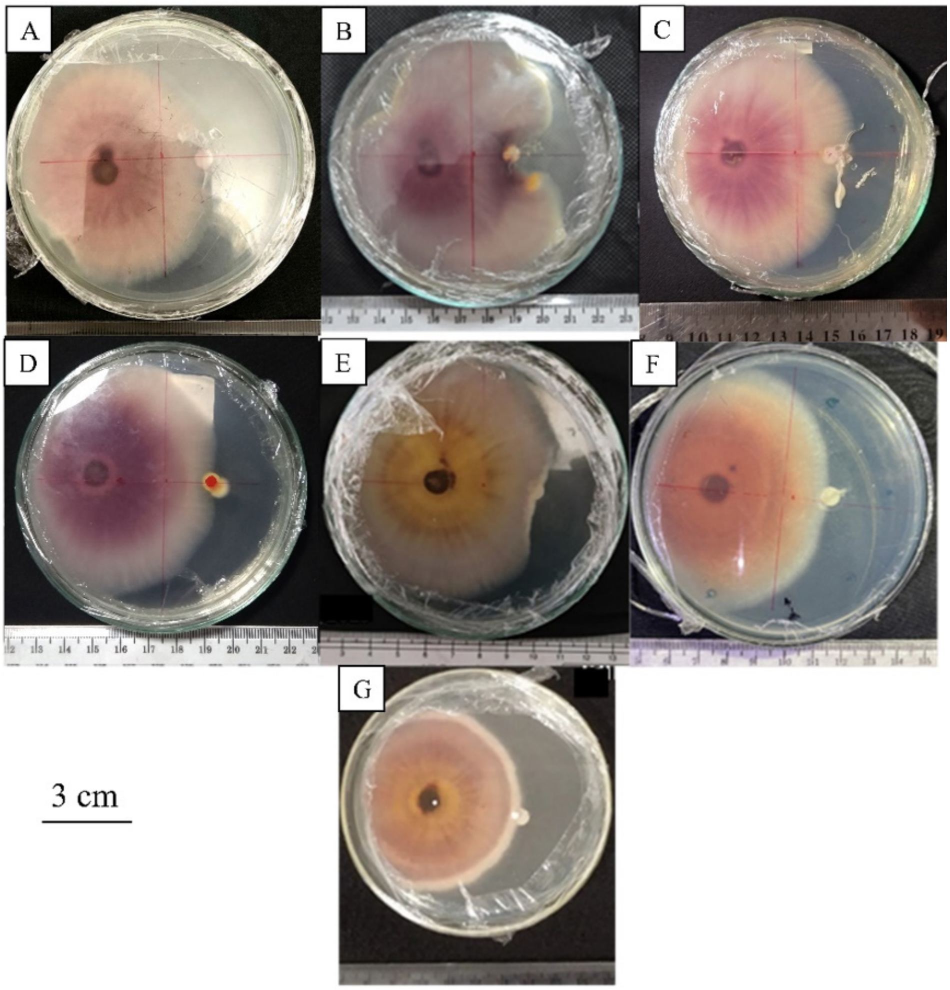


Figure 4. The *Fusarium oxysporum* mycelium condition after seven days under the antifungal potential test against the seven botanical extracts. The botanical extracts: (A) pandan leaf, (B) banana leaf, (C) *Hibiscus* flower, (D) papaya leaf, (E) guava twig, (F) orange peels, (G) betel leaf. Antifungal phenomena, I: A, II: B–E, III: F–G. Antifungal phenomena refer to the note of Table 1

group has been proven to have antibacterial, antiviral, and antifungal properties (Thawabteh *et al.* 2019). The alkylphenol compound group, i.e., thymol, methyl-thymol, eugenol, methyl-eugenol, anethole, and estragole in 10,000 µg/ml can inhibit *Microsporum canis* and *Candida albicans* mycelial growth as 16–40 mm and 7–18 mm, respectively (Fontenelle *et al.* 2011). Only the pandan leaf extract showed antifungal phenomenon I (Table 2). The pandan leaf ethanol extract of 10%, 20%, 30%, and 40% had clear zones, i.e., 7.86, 8.53, 8.76, and 9.43 mm, respectively, showed lower inhibition than Ketoconazole 2% (9.76 mm) against *Pityrosporum ovale* (Sinaga *et al.* 2021). However, a positive correlation had been established between mycelial growth inhibitory and total phenol content of botanical extracts (Rongai *et al.* 2015), confirming that the phenomenon I of pandan leaf (Table 2) was caused by its chemical compound.

Although the other six extracts had negative value of inhibition percentage, they showed antifungal phenomenon II and III (Table 2, Figure 4B–G). There were 12 out of 14 botanical extracts did not show antifungal activity to *F. solani*, but could inhibit non-*Fusarium* fungi (Webster *et al.* 2008). This suggesting that the extract with a negative value of inhibition percentage might have antifungal potential to other fungi. Papaya leaf aquadest extract showed antifungal phenomenon II on the seventh day (Table 2, Figure 4D). Furthermore, the Ethiopian mustard leaf (*Brassica carinata*) in the same Brassicales Order as Papaya leaf had an inhibitory activity of 14.7% of *F. oxysporum* mycelium on the sixth day (Rongai *et al.* 2015). All the Magnoliopsida plant sample showed those three phenomena, while the Monocotyledonae (banana leaf) extract showed only phenomenon II (Table 2, Figure 4A–G).

In addition, the mycelium of *F. oxysporum* showed ranged of pigment (Figure 4A–G). The phenomenon I and II have purple pigment, but the phenomenon III does not (Figure 4A–G). All samples of Phenomenon III and two samples of phenomenon II (*Hibiscus* flower and guava twig) extracts have dark orange-yellow pigment (Figure 4C, E, F, G). The mycelium color of black, yellow/orange, and purple might was caused by 5-deoxybostrycoidin-based melanin (Frandsen *et al.* 2016), hydroxyanthraquinone (Baker and Tatum 1998), and naphthoquinone (Lebeau *et al.* 2018), respectively. The *F. oxysporum* mycelium grew well-spread circularly with the white mycelium on the outer side (Figure 3). Furthermore, the mycelial pigment of *F. graminearum* changed from white, light yellow, and darker into brown during the 20 days of growth (Cambaza *et al.* 2018).

In conclusion, the seven botanical extracts showed three phenomena against *F. oxysporum*. Pandan leaf extract showed an antifungal activity certainty with 4% inhibition zone presumably due to its chemical compound. The extract of banana leaf, *Hibiscus* flower, papaya leaf, and guava twig inhibited *F. oxysporum* mycelial growth that avoiding the extract disc. The extract of orange peels and betel leaf inhibits *F. oxysporum* mycelial growth that not piercing the extract disc. Six out of seven botanical extracts had no potential to inhibit the mycelial growth of *F. oxysporum* and might have non-*Fusarium* antifungal potential. Future research needs to test the alcohol extracts of these samples as a comparison and explore more antifungal potential botanical eco-friendly extracts.

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