

Inducible resistance of *Falcataria falcata* (L.) Greuter & R.Rankin for gall rust disease using plant extracts as elicitors

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Abstract

Falcataria falcata is valuable species for timber production in Indonesia. Yields from this species, however, are reduced by gall rust caused by *Uromycladium falcatarium*. The aim of this research was to identify whether extracts of *Salix babylonica* and *Clerodendrum paniculatum* could induce systemic resistance of *F. falcata* to gall rust. Experiments were conducted at the nursery of Vocational College, Universitas Gadjah Mada. Three factors were tested, i.e. plant extract (*S. babylonica* or *C. paniculatum*), extract concentration (diluted or undiluted), and time of application (inoculated one day or seven days after elicitor application). Parameters measure were total phenolic compounds and the symptom of gall rust. The total phenolic compounds produced by seedlings treated with *C. paniculatum* extract were significantly lower than other treatments. After 5 months of observation, gall rust symptoms were only found on seedlings treated with *C. paniculatum* extract, i.e. stiff and bent shoot, stiff and slightly bent with a white stripe on branch, stiff and slightly bent leaf stalk, and gall formation.

Key word: *Clerodendrum paniculatum*, *Salix babylonica*, total phenolic, *Uromycladium falcatarium*.

Introduction

Falcataria falcata (L.) Greuter & R.Rankin has been planted widely in Indonesia both at industrial plantation forest and community forest with an aim to provide wood for industry (Dendang and Sudomo 2020, Nandini et al. 2023, Yunita et al. 2023). Growth of this important tree, however, can be severely affected by gall rust

(Suharti et al. 2019). This disease was identified causing damage to *F. falcata* in Indonesia since 1993 (Rahayu et al. 2010). It is also present in other countries of Southeast Asia, including Malaysia and Philippines (Rahayu et al. 2010, 2018; Lacandula et al. 2017; Palma et al. 2020; Calunsag et al. 2022).

The pathogen causing gall rust of *F. falcata* is *Uromycladium falcatarium* Doung-

sa-ard, McTaggart & Shivas (Dounsa-ard et al. 2014, Suharti et al. 2019, Rahayu et al. 2020). Dounsa-ard et al. (2014) mentioned that the morphology of *U. falcatarium* and *U. tepperianum* (Sacc.) McAlpine, which is previously reported as caused of gall rust, was similar, but *U. falcatarium* had fewer striae (25–32) than *U. tepperianum* (30–45). In addition, Rahayu et al. (2020) reported that *U. falcatarium* from areas surrounding volcano mountain was more virulent than others.

Gall rust can attack plants at all stages of growth, from seedlings in the nursery to mature stands in the field (Rahayu et al. 2020). Corryanti and Novitasari (2015) suggested that gall rust may cause up to 90 % mortality of *F. falcata*. Moreover, Lelana et al. (2024) mentioned that disease prevalence in *F. falcata*, which were planted around 100 – 500 m from inoculum source, reached 100 % seventh year after planted. According to Rahayu et al. (2018) development of gall rust is more intensive in environments with high relative humidity (≥ 90 %), low wind speed (about 30 to 50 km·h⁻¹), frequent fogs, usually in forest growing on steep slopes and surrounded by high and dense vegetation.

Several studies have been conducted on innovative methods for the control of gall rust. Provenance tests were carried out to determine whether high resistance to *U. falcatarium* occur in different populations of *F. falcata* (Charomaini and Ismail 2008, Baskorowati et al. 2012). Plant growth promoting rhizobacteria (PGPR) and mycorrhiza were tested to improve the vigour of *F. falcata* (Putri and Bramas-to 2017, Baskorowati et al. 2024). Silviculture practices, such as removing gall followed by tar smearing treatment, were conducted to control gall reformation (Cip-

ta et al. 2017). Furthermore, mix-planted forests were undertaken to manipulate the environment to reduce favourability for pathogen development (Lestari et al. 2013, Dendang and Sudomo 2020). Despite some effort looking for suitable management methods, inducible resistance of *F. falcata* against gall rust using plant extract elicitors has not been examined.

Induced resistance is a phenomenon that stimulates plant to activate resistance through the application of elicitors. It is wide-ranging and effective in managing infections, as numerous resistance triggers disease control ranging from 20 % to 85 % (Walters et al. 2005). In response to elicitors, plants release chemical compounds, such as phenolics, anthocyanins, and flavonoids as a defence mechanism against pathogen attack (Aguilar et al. 2023). Furthermore, Putri et al. (2014) reported that *F. falcata* seedlings attacked by pathogens produced higher concentrations of total phenolics than those that were uninfected.

Hanudin et al. (2016) showed that extract of *Salix* sp. effectively suppressed white rust disease on *Chrysanthemum* sp. flowers caused by *Puccinia horiana* Henn. Besides, Pandawani et al. (2022) reported that leaf extract of *Clerodendrum paniculatum* L. successfully inhibited zucchini yellow mosaic virus in *Cucurbita pepo* L. These extracts, therefore, can be examined in eliciting systemic resistance of *F. falcata* seedlings against gall rust, particularly at the seedling stage, where *F. falcata* is more susceptible to *U. falcatarium* infection. Therefore, the aim of the research was to determine the effects of plant-derived extracts of *Salix babylonica* L. and *Clerodendrum paniculatum* when applied to *F. falcata* exposed to gall rust.

Material and Methods

Experimental design

Experiments were conducted for seven months in the nursery of the Vocational College, Universitas Gadjah Mada. The experiment was laid out in a completely randomized design (CRD) with three factors, elicitor (*S. babylonica* or *C. paniculatum*), extract concentration (diluted or undiluted), and inoculation time of *U. falcatarium*'s teliospores (inoculated one day or seven days after elicitor application). In total, there were 8 treatment combinations, i.e. diluted *S. babylonica* extract, inoculated one day after extract application (DS1); undiluted *S. babylonica* extract, inoculated one day after extract application (US1); diluted *S. babylonica* extract, inoculated seven days after extract application (DS7); undiluted *S. babylonica* extract, inoculated seven days after extract application (US7); diluted *C. paniculatum* extract, inoculated one day after application extract (DC1); undiluted *C. paniculatum* extract, applied one day after application (UC1); diluted *C. paniculatum* extract, inoculated seven days after application extract (DC7); undiluted *C. paniculatum* extract, inoculated seven days after application (UC7). Each treatment and control (control, without extract application) were replicated 28 times, 25 plants for disease observation and 3 plants for total phenolic content measurement.

Extraction of plant

Extractions were carried out following the methods of Duriat (2008) and Hanudin et al. (2016). Briefly, *S. babylonica* and *C. paniculatum* fresh mature leaves were crushed into 0.01 M phosphate buffer pH 7.0. The leaves to buffer ratio for diluted

extract was 1: 1 (w/v, 100 g per 100 ml), while for undiluted 2: 1 (w/v, 200 g per 100ml). Leaf extracts were stored at < 10 °C until required.

Inoculation of seedlings

Teliospores of *U. falcatarium* were collected from the community forest of Yogyakarta (7°37'06.6" S 110°27'30.5" E). Six weeks old healthy seedlings of *F. falcata* were inoculated with teliospores of *U. falcatarium* using a suspension-drop technique where 3 mL of spore suspension was applied onto each seedling at terminal shoot and leaves using a pipette. Inoculum density was $2.46 \cdot 10^6 \cdot \text{mL}^{-1}$. Treated seedlings were later placed in plastic shade for one month to maintain high humidity.

Analysis of total phenolic contents

To understanding changes in total phenolic contents after application of elicitor extracts, host plant tissues were analysed at 1, 3, 5, and 7 days after application. The leaf of host plant was separated from the twigs and was air dried for about seven days at room temperature. As much as 1 g air dried sample was extracted with methanol (30 mL) for three days at room temperature. The solution was then filtered using Whatman No. 2. Prior the analysis, 0.5 mL of methanol extract was reacted with 2.5 mL of Folin-Ciocalteu reagent diluted tenfold. The sample then was allowed to stand for 2 min at room temperature. To the solution, 2 mL of Na_2CO_3 solution (7.5 %) was added and the reaction was stood for 30 min. The absorbance of the solution was recorded at 765 nm using a visible spectrophotometer (model SP-3000 Nano, Optima, Tokyo, Japan). Total phenolic contents were calculated using

gallic acid standard as curve calibration in unit of mg gallic acid equivalents (GAE) per g air dried sample (Diouf et al. 2009).

Statistical analyses

Data were subjected to multi-way analysis of variance (ANOVA) followed by HSD tests to determine significant treatments effects. All analyses were conducted in R with agricolae package.

Result and Discussion

Total phenolic content differed significantly with type of elicitor treatment ($P < 0.01$), whereas concentration of elicitor and time of application had no significant effect. The highest concentration of total phenolics was found in control seedlings. Total phenolic content in seedlings treated with *C. paniculatum* extract were significantly lower compared to other treatments ($P < 0.01$). Phenolic concentration in seedlings control, treated by extracts of *S. babylonica* and *C. paniculatum*, were 4.71, 4.25, and 3.44 mg GAE·g⁻¹, respectively (Fig. 1). The higher concentration of phenolics in control seedlings compared to those treated with *C. paniculatum* and *S. babylonica* extracts suggests that the untreated plants may rely more on phenolic-based defences as a general stress response (Putri et al. 2014), while the elicitor treatments likely activate alternative defence pathways, potentially resulting in different resistance or susceptibility to gall rust disease. Previously, it was reported that addition the elicitor to the seedlings or plants could affect to the number of bioactive compounds or secondary metabolites, such as phenolics (Shein et al. 2003, Al-Khayri and Naik 2020, Santos et al. 2023). For example, free proanthocyani-

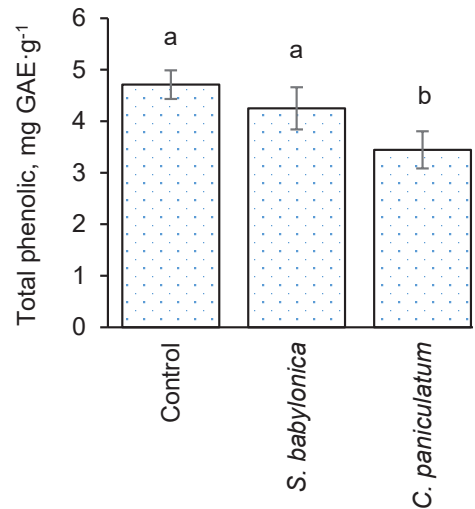


Fig. 1. Total phenolic of *F. falcata* treated by extract of *S. babylonica* and *C. paniculatum*.

din decreased in *Pinus sylvestris* L. seedling after treating by mycelium extract of *Fusarium* (Shein et al. 2003), total phenolic and total flavonoid contents of *Phoenix dactylifera* cell decreased after the addition of pectin, yeast extract, salicylic acid, cadmium chloride, and silver nitrate elicitor (Al-Khayri and Naik 2020), and total phenolic content of *Cereus hildmannianus* K.Schum. callus cultures decreased after 8% sucrose treatment (Santos et al. 2023). Hence, the decreasing of the total phenolic contents in this study were in agreement with those reported in previous studies. In the phenylpropanoid pathway, phenolics and flavonoids are made, starting an important enzyme process that produce bioactive compounds (Manivanan et al. 2016). However, the addition of elicitor with high concentration might inhibit the production of these bioactive compounds (Ali et al. 2015). Furthermore, the addition of elicitor that rich in phenolics or antioxidants could inhibit the secretion of phenolic compounds (Titov et al. 2006).

These facts suggest that the reduction of total phenolic content in this study might be due to high concentration of secondary metabolites, such as saponins, alkaloids, flavonoids, and terpenoids in *C. paniculatum* extracts (Hafiz et al. 2019, Hebbar et al. 2022, Khairuddin et al. 2022).

Total phenolic contents in *F. falcata* seedlings fluctuated over the first week after treatment with elicitor extracts (Fig. 2). Generally, total phenolic contents decreased from the first to fifth days after application of extracts but increased thereafter. Application of both diluted and undiluted extracts of *C. paniculatum* led to lower phenolic compound concentrations than found in other treatments ($P < 0.01$).

Antibiotic compounds in plants take an important role for defence mechanisms against pathogen attack (Chester 1933). Phenolic compounds may be amongst the preformed chemicals responsible for defence (Putri et al. 2014, Mahawer et al. 2022). Based on the result of this study, it appeared that total phenolic contents of *F. falcata* seedlings reduced following

treatment with *C. paniculatum* extracts, compared to other treatments. This reduction in phenolic concentrations may be a reason for earlier development of gall rust symptoms compared to plants in other treatments. Singh et al. (1998) mentioned that germination of *Ascochyta rabiei* (Pass.) Labr., pathogen that causes blight disease at chickpea, is decelerated at higher concentration of malic acid, one form of phenolic compounds. Likewise, Cagirgan et al. (2011) reported that the resistant genotype of chickpea contains higher concentration of malic acid than the susceptible one. Therefore, the response of total phenol content against disease in this study agreed with previous studies (Singh et al. 1998, Cagirgan et al. 2011, Putri et al. 2014, Mahawer et al. 2022, Misra et al. 2023, Khanday et al. 2024).

Gall rust symptoms appeared 5 months after inoculation (figs 3 and 4). The symptoms only emerged at seedling, which were applied by diluted and undiluted extract of *C. paniculatum*. The part of seedlings infected by *U. falcatarium* were stiff

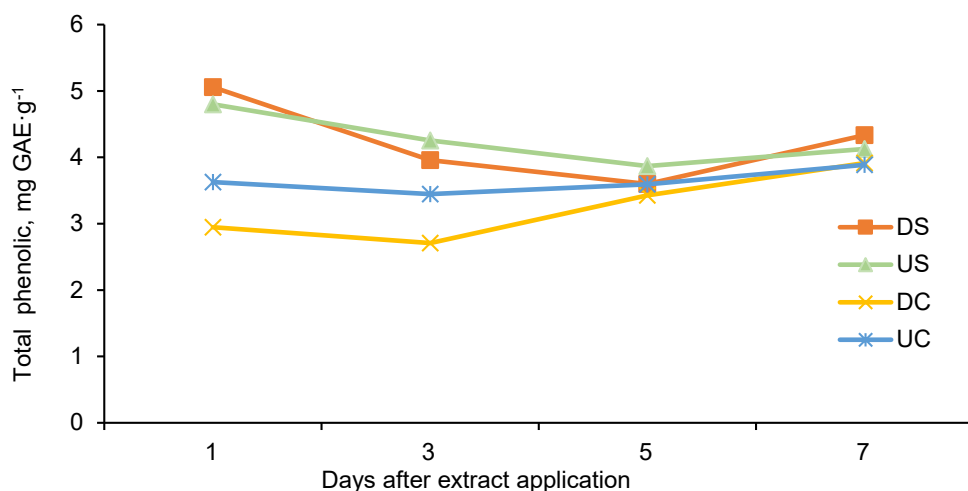


Fig. 2. Trend total phenolic of *F. falcata* after application of elicitors extract.

Note: DS – diluted extract of *S. babylonica*, US – undiluted extract of *S. babylonica*, DC – diluted extract of *C. paniculatum*, UC – undiluted extract of *C. paniculatum*.



Fig. 3. Early symptom at seedling which treated by diluted extract of *C. paniculatum*: (a) stiff and bent shoot, (b) stiff and slightly bent branch and white stripe on the infected branch surface.

and bent. A white strip appeared on the surface of infected branches. Small galls formed on the main stem. The symptoms observed in this study were similar to those reported by Rahayu et al. (2010). Moreover, Rahayu et al. (2010) explained that infection was indicated by cellular changes including misshapen periderm and phloem and distorted xylem cells. The gall that formed in our research has a green colour, and teliospores of *U. falcatarium* did not appear, indicating that the infection is in the early stage. In the next stage, the gall colour and shape might change due to the tumour surface being covered with active reddish-brown spores (Rahmawati et al. 2019) and varying from round to irregular shapes (Anggraeni 2009). In addition, we found that fungal hyphae were also visible between the wood cells of *F. falcata* seedlings. According to Rahmawati et al.

(2019), microscopic observation revealed that the structure of wood tissue affected by tumour rust disease was scattered and irregular.

Conclusions

In the present study, the effects of plant-derived extracts of *S. babylonica* and *C. paniculatum* to *F. falcata* seedling resistance against gall rust disease were evaluated. The total phenol content and gall rust growth were also discussed. Total phenol content of *F. falcata* control ($4.71 \text{ mg GAE} \cdot \text{g}^{-1}$) and treated by extracts of *S. babylonica* ($4.25 \text{ mg GAE} \cdot \text{g}^{-1}$) were significantly higher than treated by extracts of *C. paniculatum* ($3.44 \text{ mg GAE} \cdot \text{g}^{-1}$). In addition, the gall rust symptoms including stiff and bent shoots, stiff and slightly

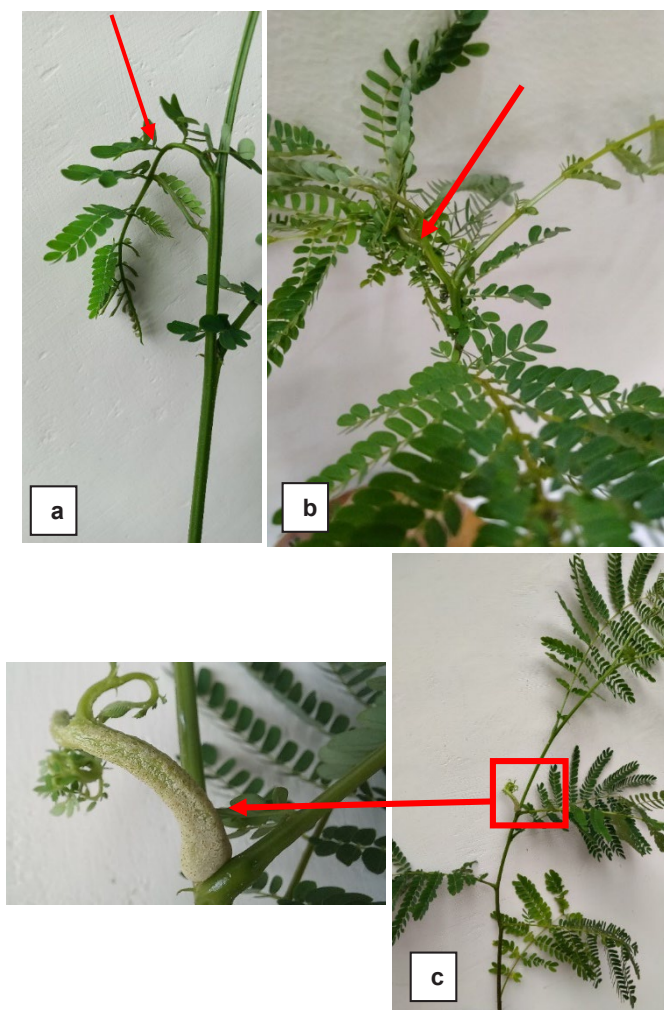


Fig. 4. Early symptom at seedling treated by undiluted extract of *C. paniculatum*: (a) stiff and slightly bent leaf stalk, (b) stiff and slightly bent shoot, (c) gall formation.

bent branches with white stripes, stiff and slightly bent leaf stalks, and gall formation, were observed only in seedlings treated with *C. paniculatum* extract five months after infection. In conclusion, the use of plant extract elicitors in this study did not appear to induce resistance in *F. falcata*, particularly with *C. paniculatum* extract.

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