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Comparative Evaluation of the Allelopathic Activity and Total Phenolic Content of Aqueous Leaf Extracts of *Cassia fistula* L. and *Caesalpinia pulcherrima* (L.) Sw. against *Phaseolus vulgaris* L.

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Abstract

Allelopathy plays an important role in plant–plant interactions through the release of bioactive compounds that influence the germination and growth of neighbouring plants. The present study comparatively evaluated the allelopathic activity and total phenolic content of aqueous leaf extracts of *Cassia fistula* L. and *Caesalpinia pulcherrima* (L.) Sw. using *Phaseolus vulgaris* L. as the test species. Fresh leaf extracts were prepared in distilled water to obtain 50% and 100% concentrations and assessed through laboratory germination bioassays. Germination percentage, radicle length and radicle growth inhibition were recorded on the seventh day after treatment. Total phenolic content of the aqueous extracts was determined using the Folin–Ciocalteu colorimetric method, and the data were analysed by one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) test at $P < 0.05$. *Cassia fistula* significantly inhibited seed germination at both extract concentrations, whereas *C. pulcherrima* exhibited only a concentration-dependent but statistically non-significant reduction in germination. In contrast, radicle elongation was more sensitive to allelopathic stress than seed germination, with the 100% aqueous extract of *C. pulcherrima* producing greater inhibition of radicle growth than the corresponding extract of *C. fistula*. Total phenolic content of the aqueous extract of *C. pulcherrima* was approximately three-fold higher than that of *C. fistula*. However, the greater phenolic content was associated with stronger inhibition of radicle elongation but not with greater inhibition of seed germination, indicating that total phenolic content alone does not fully explain the observed allelopathic effects. The findings demonstrate that both species possess allelopathic potential and provide a basis for future studies aimed at identifying the bioactive allelochemicals responsible for their phytotoxic activity and evaluating their potential applications in sustainable weed management.

Keywords: Allelopathy, Allelochemicals, Cassia fistula, Caesalpinia pulcherrima, Phaseolus vulgaris, Total Phenolic Content, Radicle Elongation.

INTRODUCTION

Allelopathy is defined as the effect one plant has on another plant (including microorganisms) through the release of chemical compounds in the environment (Rice, 1984; Cheng and Cheng, 2015). These allelochemicals are found in different concentrations in several parts of plants *viz*., leaves, stems, roots, rhizomes, seeds, flowers and even pollen (Bertin *et al.,* 2003) and they are released into the environment through different pathways as leaf exudates leached into soil or volatile exudates from green leaves, decay of plant residues and root exudations (Chon *et al.,* 2006). The allelochemicals, are mostly secondary metabolites, which are produced as by products during different physiological processes in plants (Farooq *et al.,* 2006). Important secondary metabolites identified as allelochemicals are phenolics, alkaloids, flavonoids, terpenoids, momilactone, hydroxamic acids, brassinosteroids, jasmonates, salicylates, glucosinolates, carbohydrates and amino acids (Jabran *et al.,* 2010). Different types of biotic and abiotic stresses like drought, temperatures, pathogen attacks may alter the production of these allelochemicals (Cseke and Kaufman, 2006). Numerous studies have demonstrated that allelochemicals released at higher concentrations adversely affects the neighbouring plants at physiological, cytological and biochemical levels, thereby impairing their overall growth and development

(Rizvi *et al.,* 1992; Harper and Balke, 1981; Jafariehyazdi and Javidfar, 2011).

Phenolic compounds are major groups of plant allelochemicals in ecosystem (Jacob and Sarada, 2012; Inderjit, 1996). Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups. In allelopathy, phenolic compounds include simple aromatic phenols, hydroxy and substituted benzoic acids and aldehydes, hydroxy and substituted cinnamic acids, coumarins, tannins and perhaps few flavonoids (Zeng *et al.,* 2008). Phenolic allelochemicals affect membrane integrity, nutrient uptake, photosynthesis, respiration, oxidative balance and enzyme activities, thereby inhibiting seed germination and seedling growth (Devi, 1992; Politycka, 1997; Batish *et al.,* 2008). Phenolic compounds have been reported from the root exudates of several trees (ZhiQun *et al.,* 2000). Among the numerous classes of allelochemicals, phenolic compounds have received considerable attention because of their abundance in plants and their documented role in plant–plant interactions. Several medicinal plants rich in phenolics have therefore been investigated for their allelopathic potential (Singh *et al.,* 2001; Espinosa *et al.,* 2008).

Cassia fistula Linn. also called golden shower is a medium-sized tree, growing to 10–20 m. It is aboriginal to India, Sri Lanka and diffused in various countries. *Cassia fistula* has been reported to inhibit the germination of radish seeds and the weed field bindweed, suggesting its potential allelopathic activity. The species is also known for its rich phytochemical composition and diverse medicinal properties as hepatoprotective, antipyretic, anti-inflammatory, antimicrobial and antifungal activities (Rizvi *et al.,* 2009) and its parts, such as bark, fruit, leaves and seeds, are used traditionally to cure diseases.

Caesalpinia pulcherrima is a shrub in the pea family Fabaceae, growing to 3 m tall and is native to the tropics and subtropics of the Americas. It is an ornamental medicinal perennial shrub plant which are widely distributed throughout India. The phytochemical screening of the plant clearly showed the presence of alkaloids, glycosides, phenolics, tannins and flavonoids. Despite its rich phytochemical composition, information regarding the allelopathic potential of *C. pulcherrima*, particularly against economically important crop species, remains limited. The plant is a having varying medicinal properties, which are widely used for the treatment of inflammation, ulcers, fevers, tumors etc. (Venu and Zachariah, 2013).

In allelopathic studies, radicle elongation is regarded as one of the most sensitive indicators of allelopathic stress because the radicle is the first organ to emerge from the germinating seed and remains in direct contact with phytotoxic compounds in the surrounding environment. Consequently, inhibition of radicle growth is widely used to evaluate allelopathic potential. *Vigna radiata (L.)* Wilczek is an economically important pulse crop and is widely employed as a model species in allelopathic bioassays because of its rapid and uniform germination and sensitivity to phytotoxic compounds. The present study was conducted to evaluate the comparative allelopathic effect of the aqueous leaf extracts of *Cassia fistula* and *Caesalpinia pulcherrima* on seed germination and radicle growth of *Phaseolus vulgaris* and to determine whether the phenolic compounds in the extracts were associated with the observed allelopathic effects. It will be beneficial to understand the effect of phenolic rich species on inhibition of seed germination and early seedling growth as it may contribute to identification of natural sources of bioactive compounds for sustainable weed management and environmentally friendly crop production.

MATERIAL AND METHODS

- **Plant materials**: The study was conducted in the months of February and March in the year 2026. Fresh, healthy and disease-free leaves of *Cassia fistula* L. and *Caesalpinia pulcherrima* (L.) Sw. were collected from the college campus. Fresh healthy seeds of *Phaseolus vulgaris* L. were procured from the Chengannur market, Kerala, India, and used as the test species.
- **Preparation of aqueous leaf extracts**: Freshly collected leaves were washed thoroughly with distilled water followed by 70% ethanol and air-dried at room temperature. The dried leaves were powdered and ten grams of leaf powder from each species were extracted separately with 100 mL of distilled water by continuous shaking for 24 h at room temperature. The extracts were first filtered and the filtrates were considered as the 100% aqueous stock extracts and stored at 4°C until use. A 50% extract was prepared by diluting the stock extract with distilled water immediately before the experiment.
- **Germination bioassay:** The germination bioassay was conducted in 9-cm diameter Petri dishes lined with filter paper. The experiment consisted of five treatments: distilled water (control), 50% and 100% aqueous leaf extracts of *Cassia fistula*, and 50% and 100% aqueous leaf extracts of *Caesalpinia pulcherrima*. Seven healthy seeds of *Phaseolus vulgaris* were placed in each Petri dish, and 10 mL of either 50% or 100% aqueous leaf extract was added. Distilled water served as the control treatment. Each treatment consisted of three independent replicates arranged in a completely randomized design (CRD). From the third day onwards, moisture was maintained by spraying uniform amount of distilled water in the control petriplates and respective extracts in the treatment plates. A seed was considered germinated when the radicle protruded at least 2 mm from the seed coat.

The experiment was continued for seven days till the emergence of two leaves in the control plate and observations were recorded on the seventh day.

- **Germination percentage:** The number of germinated seeds in each treatment was recorded and the germination percentage was calculated using the following equation:

  Germination percentage= $\frac{G}{N}$ x 100

  Where, G = number of germinated seeds, and N = total number of seeds sown.
- **Radicle length:** On the seventh day, all germinated seedlings were carefully removed from the filter paper, and the radicle length of each seedling was measured in centimetres (cm).
- **Radicle growth inhibition:** The percentage inhibition of radicle growth was calculated using the following equation:

  Radicle inhibition = $\frac{C-T}{C}$ x 100

  Where C= mean radicle length of control

  T= Mean radicle length of treatment
- **Determination of total phenolic content:** The aqueous extracts were evaporated to dryness in a water bath maintained at 45°C to obtain the dry extracts. Total phenolic content was determined using the Folin–Ciocalteu colorimetric method described by Slinkard and Singleton (1977) with slight modifications.
- **Preparation of gallic acid standard:** A stock solution of gallic acid (1 mg mL^{-1}) was prepared by dissolving 10 mg of gallic acid in a small volume of ethanol and making the final volume to 10 mL with distilled water. Aliquots corresponding to 10, 25, 50, 75 and 100 µg of gallic acid were transferred to separate test tubes and the volume adjusted to 1 mL with distilled water. Five millilitres of 10% Folin–Ciocalteu reagent followed by 4 mL of 7.5% sodium carbonate solution were added to each tube to obtain a final reaction volume of 10 mL. After thorough mixing, the reaction mixtures were incubated at 40°C for 60 min and the absorbance was measured at 760 nm against a reagent blank. All measurements were carried out in triplicate, and a gallic acid calibration curve was constructed.
- **Preparation of sample:** Five hundred micrograms of each dried aqueous extract were dissolved in 500 µL of distilled water. Five millilitres of 10% Folin–Ciocalteu reagent and 4 mL of 7.5% Sodium carbonate solution were added to obtain a final reaction volume of 10 mL The mixtures were incubated under the same conditions as the standards and absorbance was recorded at 760 nm. Total phenolic content was calculated from the gallic acid calibration curve and expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE g^{-1}) using the following equation: C = cV/m; where **C** is the total phenolic content (mg GAE g^{-1} dry extract), **c** is the concentration obtained from the calibration curve (mg mL^{-1}), **V** is the volume of extract (mL), and **m** is the mass of extract (g).

- **Statistical Analysis:**

All experiments were performed in triplicate. Data are expressed as mean ± standard deviation (SD). Differences among treatments for germination percentage and radicle length were analysed using one-way analysis of variance (ANOVA). When significant differences were detected, treatment means were separated using Tukey's Honestly Significant Difference (HSD) post hoc test at the 5% level of significance ($P < 0.05$).

RESULTS

- **Effect of aqueous extract on seed germination**

Table 1: Effect of aqueous extract on seed germination

Treatment	Germination %	Tukey group
Control	100±0.00	a
Cf(50)	80.33±8.08	b
Cf(100)	80.33±8.08	b
Cp(50)	90.67±0.08	ab
CP(100)	85.67±0.58	ab

Values are expressed as mean ± SD (n = 3). Means followed by different superscripts differ significantly according to Tukey's Honestly Significant Difference (HSD) test at P < 0.05. Means sharing at least one common letter are not significantly different.

Cassia fistula aqueous extract at 50% and 100 % concentration exhibited 80 .33 % germination which was nearly 20 % less than the control. The difference in the germination percentage between the control seeds and the treatment here was determined to be significant according to Tukeys HSD test. *Caesalpinia pulcherima* (CP100), showed 85.67% germination of seeds while *Caesalpinia pulcherima* at 50% concentration (CP 50) showed 91% of germination of the seeds. The difference in the germination percentages between the control and *C. pulcherima* leaf extracts was not found to be significant according to Tukey's Honestly Significant Difference (HSD). Thus, the study showed that the seed germination was more effectively inhibited by *Cassia fistula* leaf extracts (20%) than *Caesalpinia pulcherima leaf* extracts both at lower as well as at higher percentages. Yet the aqueous extracts failed to give more than 20% inhibition to the seeds, which suggests that the extracts only had a mild allelopathic effect on the seed germination.

- **Effect of aqueous extract on radicle length of *Phaseolus vulgaris***

Table 2: Effect of aqueous extract on radicle length of *Phaseolus vulgaris*

Treatment	Radicle length	Radicle inhibition (%)	Tukey group
Control	5.03±2.05	0	a
Cf (50)	3.68±2.91	26.84	ab
Cf (100)	3.12±1.83	37.97	b
Cp (50)	3.64±2.02	27.63	ab
CP (100)	2.85±1.75	43.34	b

Values are expressed as mean ± SD (n = 3). Means followed by different superscripts differ significantly according to Tukey's Honestly Significant Difference (HSD) test at P < 0.05. Means sharing at least one common letter are not significantly different.

The inhibitory effects of leaf extracts of the taken plants on root elongation were assessed. The aqueous leaf extracts significantly affected the radicle growth of *Phaseolus vulgaris* seedlings. The mean radicle length was highest in the control (5.03 ± 2.05 cm), followed by CF50 (3.68 ± 2.91 cm), CP50 (3.64 ± 2.02 cm), CF100 (3.12 ± 1.83 cm), and CP100 (2.85 ± 1.75 cm). Tukey's HSD test revealed that the radicle lengths of seedlings treated with CF100 and CP100 were significantly lower than those of the control ($P < 0.05$), whereas the remaining pairwise comparisons were not statistically significant. The Results showed that unlike in the seed germination, the radicle growth was more suppressed by *Caesalpinia pulcherima* leaf extract at 100% (radicle length 2.84cm) than *Cassia fistula* leaf extract at 100% (radicle length 3.12cm). The *post hoc* test revealed that the inhibition of the germinated seedling by *Caesalpinia pulcherima* leaf extract at 100% was significant at $p<0.05$ significance level in comparison to the control but was insignificant in comparison to all the other extracts. The CF(50) and CF(100) inhibited the radicle by 27.63% and 37.97% respectively. The effect of *Cassia fistula* leaf extract on root length was contrary to the result obtained by *Hong et al* (2003) who in their experiments had revealed a 50% inhibition of raddish root elongation by the leaf extracts of *C. fistula*.

- **Phenol estimation:**

The result of phenol estimation showed that the leaf extract of *Caesalpinia pulcherima*. contained more than three-fold higher total phenolic content than *Cassia fistula*.

Table 3: Total phenolic content of aqueous leaf extracts

Plant Species	Total phenolic content (mg GAE/g dry weight)
Cassia fistula	36.101±3.71
Caesalpinia pulcherima	117.88±7.2

Values are expressed as Mean ± SD (n=3)

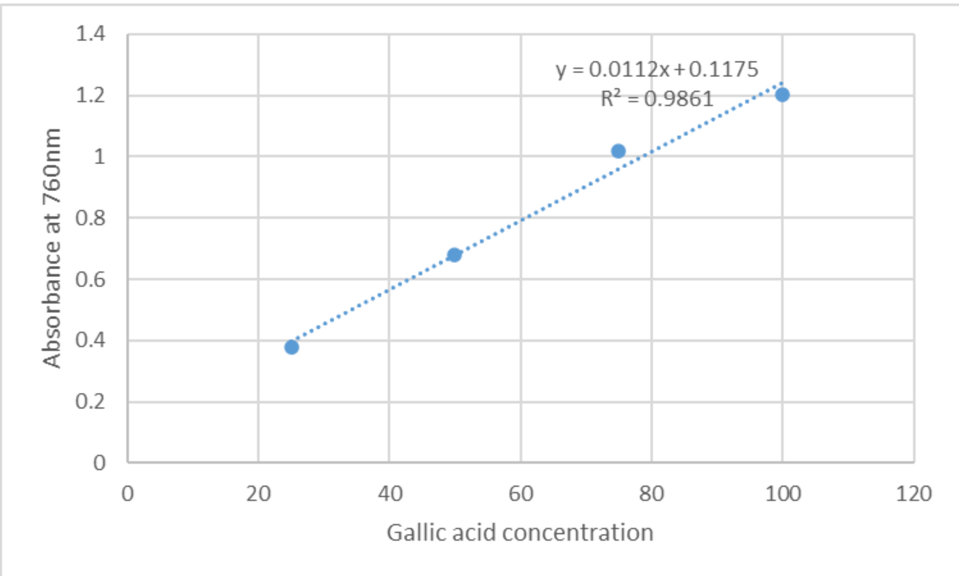

Fig. 1: Calibration curve of gallic acid used for the determination of total phenolic content by the Folin–Ciocalcateu method. Values represent the mean absorbance of three independent determinations

The gallic acid calibration curve exhibited good linearity over the concentration range of 25–100 µg, with the regression equation y = 0.0112x + 0.1175 and a coefficient of determination (R^2) of 0.9861. Although *Caesalpinia pulcherrima* possessed considerably higher total phenolic content than *Cassia fistula*, the allelopathic responses observed in the germination bioassay did not increase proportionally. While *C. pulcherrima* produced stronger inhibition of radicle elongation, both species exerted only moderate effects on seed germination, suggesting that factors other than total phenolic content may also contribute to allelopathic activity.

DISCUSSION

Plant produces a wide range of phytochemicals that can be extracted both using water and various organic solvents. Water is the natural medium through which allelochemicals leach into the soil, thereby affecting neighbouring plants. The present study, therefore, employed aqueous extracts to evaluate their effects on seed germination and radicle growth of the crop plant *Phaseolus vulgaris.* Both concentrations of the aqueous leaf extract of *Cassia fistula* produced similar inhibition of seed germination, suggesting that sufficient biologically active constituents were already present in the 50% extract to significantly inhibit seed germination of *Phaseolus vulgaris*. The present findings are consistent with previous reports demonstrating the allelopathic activity of *C. fistula* against field bindweed (*Convolvulus* spp.) (Maqbool *et al.,* 2024) Although the aqueous extract of *Caesalpinia pulcherrima* exhibited a concentration-dependent reduction in seed germination, the inhibition was not statistically significant, suggesting a comparatively weaker effect on seed germination under the present experimental conditions. Interestingly, the total phenolic content of *C. pulcherrima* was more than three-fold higher than that of *C. fistula*. This indicates that total phenolic content alone does not fully explain the observed inhibition of seed germination, and that other water-soluble allelochemicals or synergistic interactions among phytochemicals may also contribute to the observed allelopathic activity. While the inhibitory effects on seed germination were relatively modest, the aqueous leaf extracts produced a much stronger response in radicle elongation, indicating that post-germination growth was more sensitive to allelopathic stress.

The aqueous leaf extracts of both *Caesalpinia pulcherrima* and *Cassia fistula*, particularly at the 100% concentration, produced significant inhibition of radicle elongation, indicating that radicle growth was more sensitive to the aqueous extracts than seed germination. Among the treatments, the 100% extract of *C. pulcherrima* produced greater inhibition than the corresponding extract of *C. fistula*, suggesting stronger phytotoxic activity against early seedling growth. The phenol estimation study revealed a three-fold higher phenol content in *C. pulcherima* aqueous extract than *C. fistula* aqueous leaf extract. The higher phenolic constitution of leaf aqueous extracts of *C. pulcherrima* may account for the higher inhibition of radicle elongation than *C. fistula* leaf extracts in this study. The present findings differ from the findings of Hong *et al.,* (2003) who demonstrated a 50% reduction in the root length of raddish treated with aqueous leaf extracts of *Cassia fistula*. This could be due to the variations in the target species, extraction procedure, extract concentration, or experimental conditions. This supports the view that both the donor and target species control the response of recipient plants (Aasifa and Siddiqui, 2014). To the best of our knowledge, reports describing the allelopathic activity of aqueous leaf extracts of *C. pulcherrima* against crop or weed species remain limited.

Previous phytochemical investigations have reported the presence of alkaloids, sterols and tannins in *Cassia fistula* leaves (Chaves *et al.,* 2023). These compounds, together with phenolics, may contribute to the observed allelopathic activity. The present study demonstrated substantially higher total phenolic content in *C. pulcherrima* and although the higher phenolic content was not associated with stronger inhibition of seed germination, it coincided with greater inhibition of radicle elongation. Overall, the results indicate that radicle elongation is a more sensitive indicator of allelopathic activity than seed germination in *Phaseolus vulgaris*. Although *Caesalpinia pulcherrima* contained substantially higher total phenolic content than *Cassia fistula*, the relationship between phenolic concentration and allelopathic activity differed between germination and radicle growth, suggesting that additional allelochemicals or synergistic interactions among phytochemicals contribute to the overall allelopathic response.

The present study was conducted under controlled laboratory conditions using aqueous leaf extracts and a single test species. Therefore, further studies involving additional crop and weed species, characterization of individual allelochemicals, and validation under field conditions are required to better understand the ecological significance and practical applications of the observed allelopathic effects. The present study demonstrated that aqueous leaf extracts of *Cassia fistula* and *Caesalpinia pulcherrima* possess allelopathic activity against *Phaseolus vulgaris*, although their effects differed between seed germination and early seedling growth. *Cassia fistula* exhibited a significantly greater inhibitory effect on seed germination, whereas *C. pulcherrima* produced stronger inhibition of radicle elongation. The substantially higher total phenolic content observed in the aqueous leaf extract of *C. pulcherrima* may have contributed to its greater inhibition of radicle growth; however, the comparatively weaker effect on seed germination indicates that total phenolic content alone does not fully account for the allelopathic activity observed. The results further demonstrate that radicle elongation is a more sensitive indicator of allelopathic effects than seed germination in *Phaseolus vulgaris*. Overall, the findings highlight the allelopathic potential of both species and provide a scientific basis for further investigations aimed at identifying the specific bioactive compounds responsible for their phytotoxic effects and evaluating their potential applications in sustainable weed management under field conditions.

CONCLUSION

The present study provides comparative evidence of the allelopathic potential of aqueous leaf extracts of *Cassia fistula* L. and *Caesalpinia pulcherrima* (L.) Sw. against the *Phaseolus vulgaris* L., demonstrating that both species influence seed germination and early seedling growth, albeit through distinct patterns of inhibition. *Cassia fistula* exerted a significantly stronger suppressive effect on seed germination at both 50% and 100% concentrations, whereas *Caesalpinia pulcherrima* showed only a mild, statistically non-significant, concentration-dependent decline in germination. In contrast, radicle elongation emerged as a more sensitive parameter of allelopathic stress than germination itself, with the 100% aqueous extract of *C. pulcherrima* producing a greater reduction in radicle length than the corresponding *C. fistula* extract. This divergence between the two growth parameters underscores those different physiological processes, germination versus post-germination radicle development, respond differentially to phytotoxic compounds, reinforcing the value of assessing multiple endpoints when characterizing allelopathic activity.

A particularly noteworthy finding of this study is the disconnect between total phenolic content and the extent of allelopathic inhibition. Although the aqueous extract of *C. pulcherrima* contained more than three times the total phenolic content of *C. fistula*, this higher phenolic concentration corresponded with stronger inhibition of radicle elongation but not with greater suppression of seed germination. This suggests that phenolic compounds alone do not fully account for the allelopathic effects observed and that other water-soluble allelochemicals, including alkaloids, tannins and possibly synergistic interactions among multiple phytochemical classes, likely contribute to the overall phytotoxic response. This finding aligns with the broader understanding in allelopathy research that a single class of secondary metabolites rarely explains the totality of an extract's inhibitory activity, and that the biological effect often results from complex additive or synergistic interactions among diverse compounds present in plant tissues.

The comparative design of this study, examining two co-occurring ornamental and medicinally significant species against a common agriculturally important test species, also carries practical implications. Both *Cassia fistula* and *Caesalpinia pulcherrima* are widely planted as ornamental and avenue trees across Kerala and other tropical regions and the demonstrated phytotoxicity of their leaf leachates raises important considerations for intercropping systems, agroforestry planning and weed management strategies in areas where these species are prevalent. The differential sensitivity of germination versus radicle growth further suggests that the ecological impact of leaf litter or leachate from these species on neighbouring vegetation may depend heavily on the developmental stage of the affected plants at the time of exposure. Thus, the findings demonstrate that both species possess allelopathic potential and provide a basis for future studies aimed at identifying the bioactive allelochemicals responsible for their phytotoxic activity and evaluating their potential applications in sustainable weed management.

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