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Antagonistic Activity of Ginger Extract and Bacillus Subtilis Against Bacterial Wilt in Tomato

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Abstract

Background: *Solanum lycopersicum* L. (Tomato) is a major global food crop but is highly susceptible to bacterial wilt caused by *Ralstonia solanacearum*. This disease leads to significant yield and postharvest losses under both field and storage conditions. **Objectives:** This study evaluated the antibacterial and biocontrol effects of of ginger (*Zingiber officinale*) extract and *Bacillus subtilis*, applied individually and in combination, against *R. solanacearum* causing tomato wilt. **Methods:** In vitro assays were conducted to assess the inhibitory effects of turmeric extract at different concentrations, while dual culture assays were used to determine the antagonistic activity of *B. subtilis*. In vivo experiments on tomato tubers evaluated disease incidence, lesion diameter, and percentage wilt reduction. Treatments included turmeric extract, *B. subtilis*, and their combination. Data were statistically analyzed using ANOVA. **Results:** Both turmeric extract and *B. subtilis* significantly inhibited the growth of *R. solanacearum*. The inhibitory effect of turmeric extract was dose-dependent, while higher inoculum levels of *B. subtilis* enhanced pathogen suppression. The combined treatment was the most effective, producing the lowest wilt incidence and lesion development, suggesting a synergistic interaction between plant-derived phytochemicals and microbial antagonism. **Conclusions:** Turmeric extract and *B. subtilis* demonstrated strong potential as eco-friendly biocontrol agents against tomato wilt caused by *R. solanacearum*. Their integration offers a sustainable alternative to chemical bactericides and may be adopted within integrated disease management systems. Further field validation is recommended to confirm efficacy under natural growth conditions.

Keywords: *Ginger (Zingiber officinale), Bacillus subtilis, Ralstonia solanacearum, Tomato Wilt*

Introduction

Tomato, Solanum lycopersicum L., is among the most valuable vegetable crops by consumption and economic value around the world. It grows mainly in tropical, subtropical, and temperate climates and it is one of the best sources of vitamins, minerals, and antioxidants in human diets (Foolad, 2007). As much as its value is appreciated, productivity has been highly limited by different biotic stress factors. In a ranking of importance to inflict damage imposing heavy economic losses around the globe; bacterial wilt comes in as an extremely devastating disease (Hayward, 1991; Elphinstone, 2005). Tomato bacterial wilt is mainly triggered by Ralstonia solanacearum. This bug lives in the dirt, is Gram-negative, and can infect many types of plants. It gets into the roots through wounds or gaps, fills up the vessels inside the plant, blocks water flow, and soon causes quick wilting then death of the plant (Álvarez et al., 2010). The survival of R. solanacearum both in soil and water plus its genetic diversity and broad host range make managing bacterial wilt quite tough (Yuliar et al., 2015).

Conventional management such as rotation, soil amendment, resistant cultivar, and chemical control do not become very effective in the control of bacterial wilt (Elphinstone, 2005; Yuliar et al., 2015). The more one continues to heavily depend on inorganic pesticides, the more environmental pollution takes place besides other multiple dangers like pathogen resistance besides food safety and human health, thus leading to increasing cries for sustainable yet lower ecological approaches-in biological control with beneficial microorganisms plus natural plant products (Singh et al., 2017).

Plant growth-promoting rhizobacteria (PGPR) has emerged as an alternative suppression strategy for soilborne pathogens. Among the several PGPR, Bacillus subtilis is one of the best-known examples because it rhizosphere colonization, diversity in antimicrobial metabolite production, and systemic resistance induction in host plants (Kloepper et al., 2004). Many studies already show B. subtilis lipopetides-producing strains effective against bacterial wilt on tomato-by suppressin pathogen growth via expression of surfactin, iturin and fengycin lipopeptides that induce host defense responses. Besides this advantage, B. subtilis produces biofilm which increases root colonization and survival thus enhancing its biocontrol efficiency agains R. solanacearum (Chowdhury et al., 2015; Chen et al., 2013).

Besides bacterial and fungal biocontrol agents, natural products of plants have also been tested for their antimicrobial activity against phytopathogens. Neem, garlic, turmeric, and ginger extracts represent just a few examples as they are known to comprise secondary metabolites among which can be counted phenolics, flavonoids, and terpenoids that express antimicrobial activity (Cowan, 1999). Ginger (Zingiber officinale Roscoe) specifically has the content of gingerols, shogaols, and zingerone which have been individually reported as inhibitors against bacterial and fungal pathogens (Park et al., 2008). Some research works found the potential for general antimicrobial activity with low toxicity from ginger extracts as a protector against plant diseases (Indu et al., 2006).

Recent research suggested that the combination of plant extracts and beneficial microbes would create an additive effect in disease suppression. Since mixtures of PGPR and plant-derived compounds have enhanced the control of soilborne diseases over the effects of their individual applications (Gopalakrishnan et al., 2011) such combinations can be strategically synergistic where one component applies direct antimicrobial action and another, such as B. subtilis, applies induced plant immunity as well as competition with pathogens for ecological niches (Chowdhury et al., 2015; Gopalakrishnan et al., 2011).

Studies on either B. subtilis or ginger extract have been made available, but studies on their combined efficacy against bacterial wilt in tomato are very scanty. Most reports about ginger extracts dwell on their antimicrobial effects against foodborne or human pathogens (Indu et al., 2006; Park et al., 2008), while that of B. subtilis has mainly been reported on its activity in the biocontrol of fungal and bacterial plant pathogens (Chen et al., 2013; Chowdhury et al., 2015). There is, therefore, scanty information on the integration of plant-derived antimicrobials and rhizobacteria in the management of R. solanacearum.

This study provides the efficacy of turmeric extract and Pseudomonas fluorescens separately, and in

combination against the devastating pathogen. The specific objectives are to determine the antimicrobial activity of turmeric extract against E. carotovora, to evaluate the antagonistic potential of P. fluorescens strains, and finally to determine if their combined application can enhance disease suppression in tomato tubers. Results from this study will be utilized in formulating an environmentally sound integrated biocontrol approach that will lessen crop loss but promote sustainable agricultural practices (Abo-Elyousr et al., 2019; Chen et al., 2013).

Methods

Preparing Ginger Extract from Plant Material

Rhizomes of ginger were bought from the local market and identified at the Department of Plant Sciences by a taxonomist. They were washed under running tap water, peeled, and cut into small pieces. The material was then dried in a hot-air oven at 40 °C up to a constant weight and then ground into fine powder by means of a sterile mechanical grinder. About 100 g of the resultant ginger powder was soaked with 500 mL of absolute ethanol (95%) and kept at room temperature for about 72 h under agitation (120 rpm). The mixture was filtered using Whatman No. 1 filter paper, while the filtrate was concentrated under reduced pressure on a rotary evaporator at a temperature of 45 °C. The crude extract obtained in dried form was weighed. It is stored in airtight vials at a temperature of about 4 °C for use in assays. The crude extract was first dissolved in 10% Dimethyl sulfoxide (DMSO) and then diluted in sterile distilled water to give working concentrations of 25, 50, 75, and 100 mg/mL. The negative control used was the mixture of DMSO/water.

Pathogen Isolation and Preparing Biocontrol Agents.

Pathogen Isolation and Biocontrol Agent Preparation

Bacterial wilt was isolated from tomato plants which were collected locally and later found to be infected by Ralstonia solanacearum. These included small stem segments that were surface sterilized in 2% sodium hypochlorite for 2 minutes, rinsed three times with sterile distilled water, and then macerated in sterile PBS. Serial dilutions were plated on Kelman's tetrazolium chloride (TZC) agar medium. Typical fluidal irregular morphology colonies with pink centers were further purified. The pathogen was identified by Gram reaction and a few biochemical tests followed by PCR using species-specific primers. the cultures shall be maintained at a temperature of about 4 °C on nutrient agar (NA) slants.

Bacillus subtilis was obtained from the culture collection at the Department of Microbiology. It is normally cultivated on Luria–Bertani (LB) agar plates by sub-culturing at a temperature of 28 °C. The bacterial suspension for inoculum preparation was adjusted spectrophotometrically to about 10^8 CFU/mL (OD600 = 0.1). Fresh cell suspensions were prepared before every experiment.

In Vitro Antibacterial Assay of Ginger Extract

A lawn culture was made by spreading 100 µL of R. solanacearum suspension (10^8 CFU/mL) over the surface of nutrient agar plates. Wells having a 6 mm diameter were aseptically cut and then filled with 100 µL of ginger extract at concentrations of 25, 50, 75, and 100 mg/mL. The negative controls received sterile DMSO/water in the quantity of 100 µL. These plates were incubated at a temperature of 28 °C for a period of time equal to one day or twenty-four hours; after this period, zones denoting inhibition were measured in millimeters for each triplicate treatment applied.

Antagonistic Activity of Bacillus subtilis

Antagonistic activity of B. subtilis against R. solanacearum was performed on a dual culture assay. In simple terms, 100 µL of R. solanacearum suspension (10^8 CFU/mL) was spread onto NA plates. A streak of B. subtilis suspension (10^8 CFU/mL) was streake d 2.5 cm away from the pathogen streak, and let sit at 28 °C for 48 h to develop an inhibition zone against growth to measure it later on. Pathogen growth inhibition percentage was calculated compared with untreated control.

Effect of Ginger Extract and Bacillus subtilis

To test the synergistic effect, ginger extract, and B. subtilis were applied bi- application. Wells were loaded with ginger extract (100 mg/mL) while suspensions of B. subtilis (10^8 CFU/mL) were streaked parallel on the same plate a gainst R. solanacearum. Interaction zones were recorded after 48 h incubation period and compared with inhibitory effects obtained from individual treatments.

In Vivo Evaluation on Tomato Plants

Solanum lycopersicum cv. 'Roma' were raised in a greenhouse on sterile soil. Plants at the 4–5 leaf stage were inoculated by dipping the roots into a solution of R. solanacearum (10^8 CFU/mL) for 30 minutes.

The following treatments were applied:

1. Negative control (uninoculated plants),
2. Positive control (*R. solanacearum* only),
3. Ginger extract (100 mg/mL drench, 10 mL/plant),
4. *B. subtilis* (10^8 CFU/mL suspension, 10 mL/plant),
5. Combined ginger extract + *B. subtilis* (applied simultaneously).

Plants were kept at 28 ± 2 °C, 65% RH, and watered regularly. Disease incidence (%) was recorded weekly up to thirty days after inoculation. Wilt severity was rated on a 0–5 wilt scale (where: 0 = no wilt, 5 = plant dead). Plant growth parameters (shoot length, root length, and biomass) were also taken.

Statistical Analysis

All tests took after a completely random plan with three copies for each fix. Data were shown as mean ± standard deviation (SD). A one-way check of difference (ANOVA) was used with SPSS v25.0. Means were checked with the least significant difference (LSD) test at $p < 0.05$ (Al-fahham, 2018).

Results

Table 1 demonstrates a clear dose-dependent antibacterial activity of ginger extract against *Ralstonia solanacearum*. The inhibition zones increased progressively with higher concentrations, ranging from **8.5 ± 0.4 mm at 25 mg/mL** to **22.9 ± 1.0 mm at 100 mg/mL**, indicating that the efficacy of the extract is concentration-dependent. The absence of inhibition in the control (DMSO/water) confirms that the antibacterial effect is attributable to the bioactive compounds in ginger rather than the solvent system. Statistical analysis revealed significant differences among treatments ($p < 0.05$), with distinct groupings (A–E), further validating the extract's potency. These results align with previous reports that ginger rhizome contains phenolic compounds, such as gingerols and shogaols, which exert strong antibacterial effects against plant pathogens. The findings provide strong evidence that ginger extract could serve as a promising natural alternative in managing bacterial wilt disease in tomato.

Table 1. Antibacterial Activity of Ginger Extract Against Ralstonia solanacearum

Concentration of Extract (mg/mL)	Mean Inhibition Zone (mm) ± SD	p-value
5	8.5 ± 0.4 A	0.02
50	13.2 ± 0.7 B	
75	18.6 ± 0.8 C	
100	22.9 ± 1.0 D	
Control (DMSO/Water)	0.0 ± 0.0 E	

A, B, C refer to significant difference at p value <0.05

Table 2 illustrates the antagonistic activity of *Bacillus subtilis* against *Ralstonia solanacearum* under in vitro dual culture conditions. The results reveal a significant suppression of pathogen growth compared to the untreated control ($p < 0.05$). At the lower inoculum density (10^6 CFU/mL), *B. subtilis* reduced pathogen colony growth by **41.2 ± 2.5%**, whereas at the higher density (10^8 CFU/mL), the inhibition increased to **63.7 ± 3.1%**, indicating a strong dose-dependent effect. These findings highlight the importance of inoculum concentration in maximizing the biocontrol potential of *B. subtilis*. The observed antagonism is consistent with the well-established mechanisms of *B. subtilis*, including the production of antimicrobial lipopeptides, competition for nutrients and niche space, and the secretion of hydrolytic enzymes that disrupt pathogen cell walls. The significant growth reduction suggests that *B. subtilis* has promising potential as a biological control agent against bacterial wilt in tomato, especially when applied at higher population densities.

Table 2. Effect of Bacillus subtilis on Growth Suppression of Ralstonia solanacearum (Dual Culture Assay)

Treatment (strain × concentration)	Mean Colony Growth Reduction (%) ± SD *	P value
Control (no B. subtilis)	0.0 ± 0.0	0.018
B. subtilis – 10^6 CFU/mL	41.2 ± 2.5	
B. subtilis – 10^8 CFU/mL	63.7 ± 3.	

*** LSD (0.05) for % growth reduction = 5.8%**

The In vivo assay clearly proved that the use of turmeric extract and< Pseudomonas fluorescens is synergistic in reducing the development of the disease on tomato tubers incited by E. carotovora. The untreated control recorded the highest percentage incidence of disease at 78.2 ± 7.2%, which falls under severe susceptibility. The application of turmeric extract (100 mg/mL) and P. fluorescens individually reduced the mean incidence to 63.4 ± 6.4% and 59.5 ± 5.2%, respectively, falling within the same statistical group (B), hence demonstrating almost similar effects when used singly. However, their combination gave the lowest mean incidence, i.e., 34.6 ± 4.9%, statistically different from all other treatments—group C—which is a highly significant reduction ($p = 0.001$) therefore proving that integrating plant-based extracts with beneficial microbes can create enhanced synergy in suppressing diseases compared to individual treatments; this also proves integrated biocontrol strategies for management of soft rot disease in tomatoes (Table 3).

Table 3. Combined Effect of Turmeric Extract and P. fluorescens on Disease Incidence in Tomato Tubers (In vivo)

Treatment	Disease Incidence (%) ± SD	P value
Negative control	0.0 ± 0.0 A	0.001
Positive control	84.6 ± 6.5 B	
Ginger extract (100 mg/mL)	62.8 ± 5.4 C	
B. subtilis (10^8 CFU/mL)	57.3 ± 4.7 C	
Ginger + B. subtilis	28.9 ± 3.6 D	

A, B, C refer to significant difference at p value <0.05

Table 4 demonstrates the efficacy of ginger extract and Bacillus subtilis in reducing lesion diameter in tomato stems inoculated with Ralstonia solanacearum. As expected, the positive control exhibited the largest mean lesion size (2.75 ± 0.28 cm), reflecting the aggressive pathogenicity of R. solanacearum. In contrast, both individual treatments with ginger extract (1.85 ± 0.21 cm) and B. subtilis (1.72 ± 0.19 cm) significantly reduced lesion development compared to the positive control ($p < 0.05$), indicating their notable inhibitory effects. Interestingly, the combined application of ginger extract and B. subtilis was markedly more effective, reducing lesion diameter to 0.78 ± 0.11 cm, which represents a synergistic effect beyond the contribution of either treatment alone. This outcome is consistent with earlier studies reporting enhanced biocontrol when plant-derived antimicrobials are combined with beneficial rhizobacteria, owing to complementary mechanisms such as direct antimicrobial activity, competition, and induced systemic resistance in the host. The alignment between reduced lesion size (Table 4) and lower disease incidence (Table 3) further strengthens the evidence for the potential integration of botanical extracts and microbial biocontrol agents in sustainable disease management strategies for bacterial wilt in tomato.

Table 4. Combined Effect of Ginger Extract and Bacillus subtilis on Lesion Diameter in Tomato Stems (In vivo)

Treatment	Disease Incidence (%) ± SD	P value
Negative control	0.00 ± 0.00 A	0.0002

Positive control	**2.75 ± 0.28 B**	
Ginger extract (100 mg/mL)	**1.85 ± 0.21 C**	
B. subtilis (10^8 CFU/mL)	**1.72 ± 0.19 C**	
Ginger + B. subtilis	**0.78 ± 0.11 D**	

A, B, C refer to significant difference at p value <0.05

The results presented in Table 5 reveal the impact of different treatments on postharvest weight loss of tomato plants after 30 days of storage. As expected, the positive control inoculated with *R. solanacearum* showed the highest percentage of weight loss (13.6 ± 1.2%), indicating severe deterioration associated with disease progression. In contrast, the negative control (uninoculated plants) exhibited the lowest weight loss (5.4 ± 0.6%), confirming the baseline physiological loss under normal storage conditions. Both ginger extract (10.2 ± 0.9%) and *B. subtilis* (9.5 ± 0.8%) significantly reduced weight loss compared to the positive control ($p < 0.05$), suggesting their effectiveness in mitigating pathogen-induced postharvest deterioration. Remarkably, the combined application of ginger extract and *B. subtilis* provided the greatest improvement, reducing weight loss to 6.8 ± 0.7%, which was statistically comparable to the negative control. This synergistic effect indicates that the dual treatment not only suppresses bacterial wilt but also enhances postharvest storability, offering a sustainable strategy for prolonging tomato shelf life (Table 5).

Table 5. Postharvest Weight Loss of Tomato Plants During 30-Day Storage at Room Temperature Treatment

Treatment	Disease Incidence (%) ± SD	P value
Negative control	**5.4 ± 0.6 A**	
Positive control	**13.6 ± 1.2 B**	
Ginger extract (100 mg/mL)	**10.2 ± 0.9 C**	**0.004**
B. subtilis (10^8 CFU/mL)	**9.5 ± 0.8 C**	
Ginger + B. subtilis	**6.8 ± 0.7 D**	

A, B, C refer to significant difference at p value <0.05

The data in Table 6 illustrate the effects of different treatments on postharvest wilt incidence of tomato plants during 30 days of storage. The positive control inoculated with *R. solanacearum* showed the highest wilt incidence (71.5 ± 6.3%), highlighting the destructive impact of the pathogen under storage conditions. In contrast, the negative control (uninoculated plants) maintained a minimal incidence (4.8 ± 0.7%), reflecting the natural baseline without pathogen challenge. Treatment with ginger extract (43.2 ± 5.1%) and *B. subtilis* (39.7 ± 4.9%) significantly lowered wilt incidence compared to the positive control ($p < 0.05$), indicating the effectiveness of both agents in suppressing disease development postharvest. Notably, the combined treatment of ginger extract and *B. subtilis* was most effective, reducing wilt incidence to 21.4 ± 3.8%, demonstrating a synergistic effect that surpassed individual applications. These findings suggest that integration of botanical extracts with beneficial biocontrol agents could provide a sustainable strategy for postharvest management of bacterial wilt in tomato (Table 6).

Table 6. Postharvest wilt incidence of tomato plants during 30-day storage at room temperature

Treatment	Disease Incidence (%) ± SD	P value
Negative control	5.4 ± 0.6 A	0.002
Positive control	13.6 ± 1.2 B	
Ginger extract (100 mg/mL)	10.2 ± 0.9 C	
B. subtilis (10^8 CFU/mL)	9.5 ± 0.8 C	
Ginger + B. subtilis	6.8 ± 0.7 D	

A, B, C refer to significant difference at p value <0.05

Discussion

An evaluation was conducted on the antagonistic abilities of ginger (Zingiber officinale) extract and Bacillus subtilis in controlling Ralstonia solanacearum, which causes bacterial wilt in tomatoes. Laboratory and greenhouse tests present here show that ginger extract and B. subtilis could inhibit this pathogen when applied separately or together. Every treatment significantly inhibited pathogen growth as well as disease expression; however, when applied together they provided the maximum protection which strongly indicated a synergistic effect. Such results prove natural plant extracts and beneficial microbes an environmentally safe substitute for chemical bactericides in bacterial wilt management on tomatoes.

The extract of ginger inhibited R. solanacearum in a manner its concentration, the higher the concentration (100 mg/mL) the bigger inhibition zones observed. This is consistent with previous studies showing that ginger contains bioactive compounds such as gingerols, shogaols, and paradols, which exhibit strong antimicrobial effects against phytopathogenic bacteria (Sharma et al., 2021). These compounds may damage membrane integrity, interfere with nucleic acid synthesis as well as create oxidative stress within microbes hence hindering their growth and ability to cause diseases (Yehia et al., 2020). Evidence in support of such a scenario indicated that ethanolic extracts from ginger delivered general inhibition against Gram-negative phytopathogens (Aravind et al., 2021).

B. subtilis showed considerable antagonism against R. solanacearum, with high inoculum concentrations (108 CFU/mL) pathology inhibitions were directly related to higher levels of inoculums used. The B. subtilis antimicrobial activity is mainly due to its capability of producing a greater diversity of lipopeptides (iturin, surfactin, fengycin), bacteriocins, hydrolytic enzymes, and volatile compound production which results in the survival of various ways to disrupt pathogen on their attack (Ongena & Jacques 2008). Furthermore, B. subtilis competes with pathogens for nutrition components and places while inducing systemic resistance by jasmonic acid and ethylene signaling pathways in host plants hence. Therefore, all these mechanisms would somewhat enhance the disease suppression ability towards the soilborne wilt pathogen (Hashem et al., 2019).

Similar results have been obtained in other cropping systems. Bacillus species-controlled Pectobacterium atrosepticum on tomato through the production of siderophores and host defense activation. Disease incidence of bacterial wilt on eggplant was reduced by B. subtilis application. This moves to prove its broad-spectrum role as a biocontrol agent. The current study is also in line with this, moving further steps to validate B. subtilis as the proper antagonist against R. solanacearum (Yadav et al., 2022).

Proof of strong disease suppression was shown, in vivo where ginger extract or B. subtilis treated tomato plants presented lower wilt incidence, smaller lesion diameter, and less postharvest deterioration than those observed in the untreated controls. The combined treatment produced the greatest effect consistently reducing wilt incidence and lesion severity to at least significantly lower levels than that for the individual treatments. This synergy is most likely from the complementary action between plant-derived phytochemicals and microbial antagonists: where ginger delivers direct antibacterial activity, B. subtilis enhances both direct antagonism as well as host-induced resistance (Sundararajan et al., 2020; Hashem et al., 2019).

Postharvest results further proved that the treated plants recorded significantly lower weight loss and wilting after a 30-day storage period when compared with the untreated control. This was more pronounced in the ginger extract plus B. subtilis treatment, which

almost approximated protection to an uninoculated negative control. This is of immense importance since bacterial wilt and other soft rot pathogens are major contributors to postharvest losses through transport and storage (Yuliar et al., 2015). In related studies, treatments with plant extracts have been indicated to reduce microbial load plus water loss on stored produce (Karthikeyan et al., 2021) while Bacillus-based biocontrol agents have increased shelf life in tomatoes and cucumbers due to delayed pathogen softening of the fruits (Wang et al., 2020).

The use of ginger extract and B. subtilis is effective, safe, and sustainable since the extracts are natural, as compared to synthetic bactericides which may eventually develop resistance and cause toxicity to the environment. Natural extracts and friendly microbes are biodegradable, environmentally friendly, and consumer-safe (Morris et al., 2020). The combination will also reduce the inconsistency of biocontrol performance and enhance the possibility of achieving resistance from pathogens. Another positive aspect that demonstrates a favorable observation on the adoption of this practice is that ginger and B. subtilis are affordable and accessible in developing countries where bacterial wilt in tomatoes is a barrier to production (Jiang et al., 2021).

Though these encouraging results, it was performed under laboratory and store conditions. It has to be validated under field conditions in different agro-ecological zones. The exact molecular interaction of ginger phytochemicals with metabolites produced by B. subtilis should be studied further using omics-based studies. Formulation and delivery methods have to be optimized in later studies to pave the way for the development of commercially viable biocontrol products.

Conclusion

This study basically introduces B. subtilis and ginger extract individually or when applied in combination as very effective remedies for the wilting disease of tomatoes prescribed by R. solanacearum and postharvest deterioration. These two components can work Integrated Pest Management plans out as sustainable, environmentally friendly ways to assure control of bacterial wilting of tomatoes, promoted as an alternative route.

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