

Biotechnological Communication

Control of Fusarium Wilt Using *Streptomyces griseus* with Plant Growth-Promoting Effect on Tomato

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ABSTRACT

The efficacy of antagonistic *Streptomyces griseus* was evaluated against tomato wilt disease incited by *Fusarium oxysporum*. Among the different formulations, *Streptomyces griseus* with chitin amended formulation showed effective increase in seed germination and seedling vigour. Further, talc-based formulations of *S. griseus* mixed with or without chitin was developed and tested under greenhouse conditions. Lowest disease severity of 19.1% was observed in plants treated with self fusant (SFSg 5) *S. griseus* suspension (root dipping - 9×10^8 cfu / mL) followed by 19.5% in treatment of chitin amended *S. griseus* (root dipping - 9×10^8 cfu/mL) was recorded over control. Plant growth of the treated traits were analyzed and compared with control. The shoot length, root length, leaf area was increased significantly over the controls by the treatment of self fusant (SFSg 5) *S. griseus* suspension followed by nearby values were reached in chitin amended *S. griseus* was recorded. The chemical treatments had less effect compared with these formulations. Histochemical studies showed that cambium layers, xylem vessels per bundle, and the vessel diameter decreased in the plants inoculated with *F. oxysporum* over control and changes in variables were observed in infected plants treated with *S. griseus*. In conclusion, *S. griseus* can be a potential biocontrol agent against *F. oxysporum* for better crop production practices.

KEY WORDS: DISEASE SEVERITY, *S. GRISEUS*, SEED/ROOT INOCULATION, TOMATO GROWTH PROMOTION, WILT.

INTRODUCTION

Plants in their environment face potential deleterious organisms such as fungi, bacteria, viruses, nematodes, etc., in soil and air are responsible for losses in crop yield worldwide. Soil-borne pathogens are saprophytic in nature. They reside in the soil for brief or extended periods; enter via roots and cause disease of the roots or stem, disrupting the uptake and translocation of water and nutrients from the soil (McGovern 2015). Plant disease incidence and prevalence varies from season to season depending on the nature of the pathogen, environmental factors, and cultivars and varieties. Acquired resistance of pests & pathogens towards the chemical fertilizers, which have detrimental effects on the environment is the major concern (Abbasi et al. 2019). The use of microorganisms to control plant pathogens is now in

practice (Al-Ani and Adhab 2012, Al-Ani et al. 2012; Al-Ani and Adhab 2013; Passari et al. 2019).

The potential use of plant associated bacteria as agents for stimulating plant growth and managing soil and plant health has been well described (Compant et al. 2005). The growth-promoting bacteria colonizing the root surfaces and closely adhering soil interface (rhizosphere) and enters root interior and established as endophytic populations. The general mechanisms involved in biocontrol are to reduce the population of pathogens at their entry level and to provide strong growth promoting activity during its symbiosis (Chen et al. 2000; Naik et al. 2008). Many plants growth promoting rhizobacteria, endophytic and actinobacteria specifically belonged to the genus *Streptomyces* sp., were used to improve crop fertility by enabling the plant to get nutrients from the soil (Nejad and Johnson 2000; Araujo et al. 2000; Bloembergen et al. 2001; Kanini et al. 2013; Kamal and Sharma 2014; Abbasi et al. 2019; Passari et al. 2019).

Microbial endophytic communities are currently the focal point to explain their function as plant growth promoters

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and their involvement in plant protection. In consideration of the above, the study was aimed to evaluate growth characteristics of tomato with respect to antagonistic *Streptomyces griseus* against wilt disease induced by *Fusarium oxysporum* under greenhouse conditions.

MATERIAL AND METHODS

For the plant material, Tomato variety Co-4 susceptible to wilt disease was employed during the investigation. For the preparation of antagonist suspension inoculum, the antagonists *S. griseus* was grown on MS broth (with/without chitin) with constant shaking for 7 days at 30°C and at 125 shakes/min. (Remi – C 24). The bacterial cells were harvested by centrifugation at 8000 rpm for 15 min and resuspended in phosphate buffer (0.01M, pH 7.0) and used as inoculum (Bharathi et al. 2004). To determine the efficacy of antagonists on seed germination and seedlings vigour, the mycelial suspension of the *S. griseus* prepared was tested for their plant growth promoting activity, which was carried out by the standard roll towel method ISTA (1996). The seeds were soaked in 10 mL of the bacterial suspension (10^8 cfu/mL) for 2 h and blot dried, placed in wet blotters and incubated at room temperature for 10 days. The seeds soaked in sterile water served as control. The germination percentage of seeds was recorded and the vigour index was calculated using the following formula: Vigour index = per cent Germination x seedling length (shoot length + root length).

To determine the efficacy of *S. griseus* against fusarium wilt under greenhouse conditions, the tomato variety Co-4 was used for *in vitro* and the greenhouse experiments in the entire period of investigation. All seeds were surface-disinfected with 1% sodium hypochlorite for 5 min and rinsed three times in sterile distilled water prior to sowing. Tomato seeds for glasshouse experiments were sown in trays (27 x 42 x 7 cm³) containing an autoclaved mixture of 1:1 (v:v) ratio of vermiculite and sand. Trays were maintained on benches in a glasshouse without artificial light for 4–5 weeks before transplanting. After some days, when the seedlings had 2–4 leaves, they were carefully transplanted into the pots.

To assess the effect of *S. griseus* on disease severity, various formulations were assessed for their efficiency in controlling *F. oxysporum f. sp. lycopersici* induced wilt incidence in greenhouse conditions. A pot culture study was undertaken with the following treatments by using completely randomized design (CRD) with three replications. The treatments were imposed as seed treatment and seedlings dip (Kamal and Sharma 2014). Seeds treated with formulation was sowed in the trays and 60 days old seedlings were transferred to the pot and marked as T₁, T₂. Tomato seeds for glasshouse experiments were sown in trays (27 x 42 x 7 cm³) containing an autoclaved mixture of 1:1(v: v) ratio of vermiculite and sand. After 60 days, the seedlings were pulled out from the trays and roots were immersed in various formulation. Various methods of treatment details include, were T₁ - the plants raised from seeds treated with talk formulation of *S. griseus* (10 g/kg

of seeds), T₂ - the plants raised from seeds treated with chitin amended talk formulation of *S. griseus* (10 g/kg of seeds), T₃ - dipped in water containing talc formulation (20 g/L) for 2 h.

The roots alone were immersed in the chitin amended suspension of *S. griseus* (9×10^8 cfu/mL) formulation, T₄ - dipped in water containing talc formulation (20 g/L) for 2 h. The roots alone were immersed in the suspension of self fusant (SFSg 5) *S. griseus* (9×10^8 cfu/mL) formulation, T₅ - dipped in crude chitinase enzyme prepared from *S. griseus* with enzyme activity of 113.3 IU/mL, T₆ - dipped in partially purified endo chitinase enzyme of *S. griseus* with enzyme activity of 1000 IU/mL, T₇ - the plants raised from carbendazim-treated seeds (2 g/ kg of seeds), served as chemical check and T₈ - Healthy control. After treatment, all the treated seeding was transplanted in to the pathogen infested (substrate at 5 percent (w/w) was mixed with sterilized soil) pots at the rate single seedlings per pot. Plants in the greenhouse were watered daily.

Beginning a week after inoculation, external symptoms of Fusarium wilt (wilting and yellowing leaves) were assessed. And the disease severity was assessed as DS = (Proportion of leaves with symptoms / Total number of leaves) X 100. To assess the effect of *S. griseus* on growth attributes, the efficacy of the formulations on various growth parameters viz., root length, shoot length, fresh & dry root weight, fresh & dry shoot weight were recorded in all the treatments at monthly intervals. All the experiments were analyzed independently. The treatment means were compared by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez 1994).

For the microscopic sections and histological studies of tomato tissues, samples from greenhouse were used for histological studies. In all of the experiments, hypocotyls were cut into 1-cm-long segments and stored in FAA (Formalin-Acetic-Alcohol) at least a week before continuing the processing for embedding. Plant tissue was transferred to aluminum weighing dishes and filled with molten paraffin and placed in a warm oven (60°C) overnight. Segments were transversally cut into 25- to 50-µm-thick sections with a freezing microtome. The sections were affixed in the slides and stained with toluidine blue for 5 min, rinsed with sterile water, and mounted with 50% glycerin. Then viewed with microscope and photomicrographs were obtained from the microscope (Cal et al. 2000).

RESULTS AND DISCUSSION

Efficacy of antagonist on seed germination: The use of chemical fertilizers has become common which has had a harmful impact on the environment (Fravel et al., 2005). Hence, it is essential to identify endophytic microorganisms that can be used as a method for increasing plant host resistance to disease and the improvement of soil health (Barnawal et al. 2017). In this regard, a number of actinobacteria and endophytic *Streptomyces* sp. have been reported to stimulate plant growth, function as biocontrol agents against diverse pathogenic fungi; and in general,

increase abiotic and biotic stress tolerance in plants (Kanini et al. 2013; Kamal and Sharma 2014; Passari et al. 2017; Abbasi et al. 2019; Passari et al. 2019).

In the present study, the antagonistic strain *S. griseus* revealed the seedlings vigour (1679) and germination

percentage (92.00). The seeds, which were not treated with any PGPR strain, showed poor performance and from this screening the strain *S. griseus* was selected for developing formulation. The *S. griseus* with chitin showed 96.10% germination and the highest seedling vigour of 3954 and it was improved over control (Table 1).

Table 1. Efficacy of antagonists on seed germination and vigour index of seedling

Treatments	Germination %	Shoot length (cm)	Root length (cm)	Vigor index
<i>S. griseus</i>	92.00 ^c	7.65 ^d	10.40 ^a	1679
<i>S. griseus</i> with chitin	96.10 ^d	6.25 ^{bc}	14.06 ^d	3954
Carbendazim	85.00 ^{ab}	5.25 ^{ab}	12.00 ^c	1331
Control	80.59 ^a	3.95 ^a	9.00 ^a	1044

Values are the mean of three replications. Means followed by a common letter are not significantly different at 5% level by DMRT. (% - percentage; Cm – Centimeter)

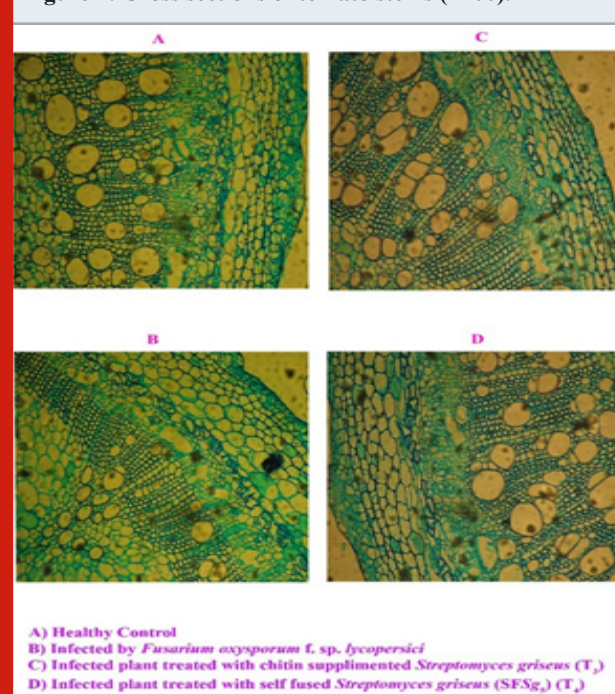
With an increased number of colonies, improved performance on seed germination and seedling vigour was observed. The studies on growth promotion indicated that antagonistic strain *S. griseus* promote plant growth directly by the production of plant growth regulators or by stimulating nutrient uptake by producing siderophores or antibiotics to protect plants from soil borne pathogens. Further significant increase in seedling vigour would have occurred by better synthesis of hormones like auxins, cytokinins, etc. This finding is consistent with the result obtained by Passari et al., (2019), were demonstrated the four bacterial isolates (BPSAC77, BPSAC101, BPSAC121, and BPSAC147) mediated enhancement in the germination of tomato seeds. Inoculation of BPSAC147 increased the germination percentage to 100% compared to 90% in the untreated, control seeds. Similarly, Lasudee et al. (2018) who stated that *S. thermocarboxydus* S3 increased the germination percentage of mung bean seeds (95–98%) which was statistically higher than the control. Kanini et al. (2013) reported the emergence of the tomato plants, the in vivo experiments were the tomato seeds treated with *S. rochei* ACTA1551 germinated at a higher rate into *F. oxysporum* infected soil compared to untreated seeds (Kanini et al. 2013; Lasudee et al. 2018; Passari et al. 2019).

Green house experiments on tomato plants: Efficacy of formulations on fusarium wilt disease of tomato:

Fusarium oxysporum is a soil-borne pathogen and can spread through root system and it would be better to protect the infection sites rather than alter the entire soil microbial community. Biocontrol agents can protect the infection site by producing specific nutrients and other compounds against pathogen (Passari et al. 2019). Therefore, biocontrol agent must be introduced to root systems well in advance of *Fusarium oxysporum f. sp. lycopersici* infestation (Lu et al., 2004; Kanini et al. 2013). Additionally, this procedure is much easier to implement and more applicable for large scale cultivation, compared to the classic one which

includes enrichment of the soil with the biocontrol agents which is time consuming one and difficult in real farming conditions (Whipps 2001; Nerey et al. 2010). Actinobacteria are identified to be existing in the rhizosphere of plants, and *Streptomyces* sp. are well known as endophytic in root tissues, where they can be able to function as probable biocontrol agents and furthermore modulate plant growth (Rey and Dumas 2017; Jones et al. 2019).

Figure 1: Cross sections of tomato stems (×100).



In the present study, lowest disease severity of 19.1% in treatment of self fusant (SFSg 5) *S. griseus* suspension (root dipping - 9×10^8 cfu / mL) followed by 19.5% in treatment

of chitin amended *S. griseus* (root dipping - 9×10^8 cfu/mL) was recorded (Table 2).

Isolate *S. griseus* exhibited biocontrol activity against *F. oxysporum* in tomato plants which may be due secondary metabolites production are in agreement with previous studies on the potential of *Streptomyces* sp., isolates to be used as biocontrol agents was documented in the previous studies (Passari et al. 2017). The finding is correlated with the earlier findings, were tomato seedlings treated with application of biocontrol agents mainly belongs to *S. enissocaesilis* IC10 and *S. rochei* Y28; *S. flavofuscus* CPP-53; *S. griseorubens* E44G from Saudi Arabia; *S. rochei*

ACTA1551; *S. miharaensis* KPE62302H; *S. psammoticus* KP1404 (Kim et al. 2011) from Korea and *S. plicatus* from Egypt against *Fusarium* wilt disease of tomato incited *F. oxysporum* f. sp. *lycopersici* showed efficient reduction in wilt disease severity and were reported as successful biocontrol agents to control tomato *Fusarium* wilt (Abd-Allah 2001; Kim 2012; Kanini et al. 2013; Rashad et al. 2017; Abbasi et al. 2019). In the same line, application of non-pathogenic *Fusarium oxysporum* and *Trichoderma harzianum* also showed efficient reduction in wilt incidence (Silva and Bettio 2005; Shishido et al. 2005; Yigit and Dikilitas 2007; Verma et al. 2017; Kamal and Sharma 2019).

Table 2. Efficacy of biocontrol agents on disease severity and yield of tomato under greenhouse conditions

Treatments	Disease severity (%)	Yield (g/plant)
T ₁ – <i>S. griseus</i> (Seed treatment – 10 g / kg)	26.0 ^a	485.0
T ₂ – Chitin amended <i>S. griseus</i> (Seed treatment – 10 g/ kg)	23.1 ^a	455.0
T ₃ – Chitin amended <i>S. griseus</i> suspension (Root dipping – 4×10^8 cfu/mL)	19.5 ^a	520.0
T ₄ – self fusant (SFSg 5) <i>S. griseus</i> suspension (Root dipping - 4×10^8 cfu/mL)	19.1 ^a	550.0
T ₅ – foliar spray using crude chitinase enzyme (1L) of <i>S. griseus</i> with Apsa 80 (113.3 IU/mL) after planting	28.9 ^{ab}	416.0
T ₆ – carbendazim treated seeds (2 g/kg of seeds)	31.5 ^{ab}	425.0
T ₇ – Healthy Control	49.3 ^{de}	425.0
T ₈ – Inoculated Control	61.1 ^h	120.0

Values are the mean of three replications. In a column, means followed by common letters are not significantly different at 5% level by DMRT.

Efficacy of *S. griseus* on growth attributes of tomato:

Better growth may be responsible for better synthesis of auxin and cytokinin which would have helped in better partitioning efficiency which later resulted in increased economic yield. PGPR synthesize phytohormones that promote plant growth at various stages (Passari et al. 2017). In the present study, compared with control, highest shoot length of 70.60 cm, fresh weight of 23.60 g, dry weight of 6.65 g and root length of 55 cm, fresh 15.55 g, dry weight dry weight 3.08 g was observed in the treatment of self fusant (SFSg 5) *S. griseus* suspension followed by nearby values were reached in treatment of chitin amended *S. griseus* (root dipping) was recorded (shoot and root length - 67.50 cm, 52.65 cm; Fresh & dry weight of shoot - 24.7 g; 15.75 g; Fresh & dry weight of root - 6.8 g, 2.85 g). Correspondingly, the chemical treatments had less effect compared with these formulations nevertheless showed moderate increase over control (Table 3). The possible reason might be associated with the initial increase in root growth by the application of PGRP strains, which would have helped in promoting better absorption of essential nutrients that are responsible for highly active photosynthesis as well as protein synthesis (Passari et al. 2017; Jones et al. 2019).

Our study, however, is the first report on the ability of endophytic *S. griseus* to exhibit PGP and biocontrol

activity in tomato plants. Similar results were reported in the previous studies who evidenced that endophytic *S. thermocarboxyodus* BPSAC147; *Streptomyces* sp. isolate PM4 and PM5; *Streptomyces* sp. isolate SNL2 and *S. caeruleatus* isolate ZL2 significantly increased the growth against *F. oxysporum* in tomato plants, relatively non-inoculated control plants (Zamoum et al. 2015; Goudjal et al., 2016; Dias et al. 2017; Passari et al. 2019). Furthermore, Goudjal et al. (2014) and Kanini et al. (2013) also reported that *Streptomyces* sp. CA-2 and AA-2 and *Streptomyces rochei* ACTA1551 significantly increased the shoot and root length of tomato plants, relative to control plants. In earlier studies, inoculation of *Trichoderma* sp., *G. intraradices* showed significant increase in growth and yield of tomato plant (Ilham et al. 2003; Akkopru and Demir 2006; Martínez-Medina et al. 2014; Ghazalibiglar et al. 2016; Lee et al. 2016; Jones et al. 2019).

Microscopic sections and histological studies: After assessing the disease severity plant tissues of specific treatment and the corresponding controls also were studied histochemically. Control plant showed normal cambium layers, xylem vessels per bundle (Plate. 1c). The cambium layers, xylem vessels per bundle, and the vessel diameter decreased in plants infected with *F. oxysporum* and not treated with *S. griseus* (Plate. 1a). Cambium is in charge of

secondary growth and regression or its total differentiation has a negative effect on vascular development with inefficient water transport in the plant and subsequent

stunting (Cal et al. 2000). Several authors have described the reduction in the number of xylem vessels as an effect of vascular pathogens including *F. oxysporum f. sp. lycopersici* (Cal 1997; Cal 2000; Abbasi et al. 2019).

Table 3. Efficacy of formulations on growth attributes of tomato under greenhouse condition.

Treatments	Shoot			Root		
	Length (cm)	Fresh weight (g)	Dry weight (g)	Length (cm)	Fresh weight (g)	Dry weight (g)
T ₁ – <i>S. griseus</i> (Seed treatment – 10 g / kg)	63.98 ^{cf}	22.75 ^e	6.30 ^{fg}	51.55 ^{gh}	14.5 ^f	2.48 ^d
T ₂ - Chitin amended <i>S. griseus</i> (Seed treatment – 10 g/ kg)	65.68 ^{fg}	25.8 ^h	6.5 ^{gh}	52.05 ^{gh}	14.75 ^e	2.55 ^{dc}
T ₃ – <i>S. griseus</i> suspension (root dipping - 9 x 10 ⁸ cfu/mL)	67.50 ^e	24.7 ^g	6.8 ^{gh}	52.65 ^{gh}	15.75 ^h	2.85 ^{fg}
T ₄ - self fusant (SFSg 5) <i>S. griseus</i> suspension (root dipping - 9 x 10 ⁸ cfu / mL)	70.60 ^h	23.60 ^f	6.65 ^h	53.0 ^h	15.55 ^b	3.08 ^{gh}
T ₅ - Crude chitinase enzyme of <i>S. griseus</i> suspension (root dipping - 113.3 IU / mL)	58.35 ^d	21.75 ^{dc}	5.8 ^{dc}	50.6 ^{gh}	13.85 ^{cf}	2.35 ^{cd}
T ₆ - Partially purified chitinase enzyme of <i>S. griseus</i> suspension (root dipping – 1000 IU / mL)	60.35 ^{dc}	20.8 ^{cf}	5.05 ^b	49.85 ^e	13.85 ^e	2.015 ^{ab}
T ₇ - carbendazim (seed treatment - 2 g/ kg)	54.7 ^{bc}	19.85 ^e	4.5 ^a	22.65 ^a	13.35 ^b	1.91 ^a
T ₈ - Inoculated control	46.85 ^a	15.85 ^a	5.08 ^b	39.35 ^{dc}	9.8 ^a	1.75 ^a

Values are the mean of three replications. In a column, means followed by a common letter are not significantly different at 5% level by DMRT.
(g / kg – gram/kilo gram; cfu – colony forming units; IU / mL – International Units/Milli Liter; Cm – Centimeter; g – Gram)

The mode of application of *S. griseus* showed marked difference in the suppression of morphological effects caused by *F. oxysporum f. sp. lycopersici* in tomato plants. Changes in cambium layers, xylem vessels per bundle and the vessel diameter under microscopy were observed (Plate. 1b) and these changes were most apparent in plants of treated with *S. griseus* (treatment 3). Morphological changes observed in plants treated with *S. griseus* in seeding before transplanting might have a role in resistance to *Fusarium* tomato wilt, and they remain systemic in the tomato plants (Passari et al. 2019).

The changes could be related to the growth regulators production causing cell growth, division and differentiation also the phytohormones, the major factor which controlling the plant vascular differentiation. This result was supported by the previous studies that showed tomato (*Lycopersicon esculentum*) plants of 'Lorena' were induced with a conidial suspension (107conidia/mL) of *Penicillium oxalicum* before inoculation with *F. oxysporum f. sp. lycopersici*, the wilt pathogen. In non-induced plants, the pathogen produced almost a complete loss of cambium (75 to 100% reduction) whereas plants induced with *P. oxalicum* showed less

disease, did not lose the cambium, had a lower number of bundles, and had less vascular colonization by *F. oxysporum f. sp. lycopersici* (35 to 99%). Further the suppression of disease caused by *F. oxysporum f. sp. lycopersici* due to application of *P. oxalicum* was demonstrated previously to be related to mechanisms of induced resistance in tomato plants (Cal et al. 1995; Cal et al. 1997; Cal et al. 2000; Passari et al. 2019; Abbasi et al. 2019).

Different patterns of root colonization between cultivars and *F. oxysporum f. sp.* pathosystems have been reported previously, such as *F. oxysporum f. sp. lentis*; purple passionfruit – *F. oxysporum* and *F. solani*; chickpea - *F. oxysporum f. sp. ciceris*; melon - *Fusarium oxysporum* race 1.2; bean - *F. oxysporum f. sp. phaseoli* (Zvirin et al., 2010; Garcia-Sanchez et al. 2010; Jimenez-Fernandez et al. 2013; Ortiz et al. 2014; Pouralibaba et al. 2016; (Abbasi et al. 2019).

CONCLUSION

The findings of the present study focused on the screening of *Streptomyces griseus* to control the phytopathogenic

fungi *Fusarium oxysporum* f. sp. lycopersici. The best antifungal producer, *Streptomyces griseus* from the prawn cultivating soil proved to be capable of protecting tomato plants from *Fusarium* wilting under greenhouse conditions while confirmation that it promoted the plants growth was derived. Resistance process may functional through secondary metabolites by restricting the pathogen invasion and histological changes could be related to the growth regulators and phytohormones production in *S. griseus* treated plants. Hence plant growth promoting *S. griseus* can be used as biocontrol agent and better crop protection. Thus, the finding of present investigation holds a good promise in tomato wilt management.

Conflict of Interests: Authors declare no conflict of interests to disclose.

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