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UNREVEALING THE BIOCONTROL EFFICACY OF SPENT MUSHROOM COMPOST AGAINST BACTERIAL WILT DISEASE IN TOMATO CAUSED BY *Ralstonia solanacearum*

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ABSTRACT

Bacterial wilt of tomato (*Solanum lycopersicum*), caused by *Ralstonia solanacearum*, is a major constraint to tomato production worldwide. This study evaluated spent mushroom compost (SMC) from *Agaricus bisporus* and *Lentinula edodes* as an eco-friendly approach for bacterial wilt management. The pathogen was isolated from diseased tomato plants and identified through morphological characterization. Button mushroom SMC was slightly alkaline and contained higher macro- and micronutrient levels than shiitake SMC. *In vitro* assays showed that weathered SMC, particularly from *A. bisporus*, exhibited the greatest antagonistic activity against *R. solanacearum*, with inhibition increasing as compost concentration increased. Pot culture experiments further demonstrated that weathered *A. bisporus* SMC significantly enhanced tomato growth and yield while reducing wilt incidence. These findings indicate that weathered SMC, especially from *A. bisporus*, is a promising, sustainable, and value-added organic amendment for the integrated management of bacterial wilt in tomato.

Key words: *Ralstonia solanacearum*, Bacterial wilt, Spent mushroom compost and Biocontrol agent

Introduction

Tomato (*Solanum lycopersicum*), a member of the family Solanaceae, is one of the most widely cultivated vegetable crops worldwide owing to its high nutritional and economic value. With an annual production exceeding 160 million tonnes, it contributes nearly 15% of global vegetable production (Pritesh *et al.*, 2011). However, tomato cultivation is severely affected by several diseases, among which bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi *et al.*, (1995) is one of the most destructive. The pathogen infects more than 450 plant species belonging to 54 botanical families (Allen *et al.*, 2005). In India, bacterial wilt has become a major constraint to tomato production, particularly in Himachal Pradesh, where it was first reported in Kangra Valley in 1981 and later spread to the mid-hill and sub-humid regions, including Mandi, Kangra, and Solan districts (Sood and Singh 1993; Gupta *et al.*, 1998). Among the different races and biovars of *R. solanacearum*, race 1 biovar III

predominates in Himachal Pradesh (Kalha and Sood 1994; Chauhan 2020).

Conventional management of bacterial wilt relies largely on chemical pesticides, which often result in environmental pollution and the development of resistant pathogen populations. Consequently, there is increasing interest in sustainable and eco-friendly disease management strategies based on beneficial microorganisms and organic amendments.

Spent mushroom compost (SMC), a nutrient-rich byproduct of mushroom cultivation, contains residual mycelium, straw, and diverse beneficial microorganisms that improve soil fertility and suppress soil-borne pathogens (Aslam and Saifullah 2013; Koberl *et al.*, 2013). In addition to serving as an organic manure and soil conditioner, SMC supplies essential nutrients such as nitrogen, phosphorus, and potassium and promotes beneficial microbial communities, including *Bacillus*, *Pseudomonas*, and *Lysobacter* spp. (Kim *et al.*, 2011;

Wang *et al.*, 2020). SMC derived from edible mushrooms such as *Agaricus bisporus* and *Lentinula edodes* has also been reported to possess antimicrobial and biocontrol properties, making it a promising resource for sustainable crop protection (Susana *et al.*, 2009; Altaf and Inam-ul-Haq 2020).

Therefore, the present study was undertaken to evaluate the physicochemical properties and antagonistic potential of spent mushroom compost from *Agaricus bisporus* and *Lentinula edodes* against *R. solanacearum*, and to assess its effectiveness in reducing bacterial wilt and improving tomato growth under pot conditions.

Materials and Methods

Collection of spent mushroom compost

Fresh and six-month-old spent mushroom compost (SMC) of button mushroom (*Agaricus bisporus*) and shiitake mushroom (*Lentinula edodes*) were collected during 2024–25 from the Centre for Mushroom Research and Training, Department of Plant Pathology, CSKHPKV, Palampur.

Estimation of pH and nutrient content of spent mushroom compost

Six-month-old SMC samples of *A. bisporus* and *L. edodes* were analyzed for pH and nutrient content in the Department of Soil Science, CSKHPKV, Palampur.

pH estimation: The pH of powdered SMC was determined using a 1:10 (w/v) compost-to water suspension following the method of Jackson (1973).

Total nitrogen estimation: Total nitrogen content was determined by digesting ground SMC samples with concentrated sulphuric acid and a digestion mixture (K_2SO_4 , $CuSO_4$, and selenium powder). The digested samples were distilled with sodium hydroxide using a nitrogen analyzer (Pelican KEL Plus–Classic DX), and the liberated ammonia was trapped in boric acid and titrated against standard sulphuric acid (Jackson, 1973).

Sample digestion for nutrient analysis: SMC samples were digested with a diacid mixture (HNO_3 : $HClO_4$) until a white residue was obtained. The residue was dissolved in 6 N HCl and the volume was made up to 50 mL with distilled water for subsequent nutrient analysis.

Estimation of total phosphorus, total potassium and Sodium content

Phosphorus content in digested spent mushroom compost (SMC) samples was determined by the vanado-molybdate phosphoric acid yellow colour method using a

spectrophotometer at 420 nm, while potassium was estimated using a flame photometer after appropriate dilution of the digested samples (Jackson, 1973).

Estimation of micronutrients:

Magnesium was estimated by Atomic Absorption Spectrophotometry (AAS) following the method of Jackson (1973). DTPA-extractable iron (Fe), zinc (Zn) and copper (Cu) were determined by AAS according to Lindsay and Norvell (1978).

Antibacterial assay against *Ralstonia solanacearum*

Tomato plants exhibiting typical bacterial wilt symptoms, including vascular discoloration, were collected and brought to the Bacteriology Research Laboratory, Department of Plant Pathology. Stem tissues were washed thoroughly and subjected to a bacterial ooze test by suspending small vascular tissue pieces in sterile water. The appearance of bacterial streaming confirmed the presence of *R. solanacearum*. Surface-sterilized tissue pieces were then macerated in sterile distilled water, and the bacterial suspension was streaked onto Kelman's triphenyl tetrazolium chloride (TZC) agar medium. Plates were incubated at $28 \pm 1^\circ C$ for 24–36 h, and characteristic virulent colonies (fluidal, dull white with pink centres) were purified by repeated streaking on TZC medium. A single colony was grown in nutrient broth, and the bacterial suspension was standardized to approximately 10^8 CFU mL^{-1} .

Fresh and six-month-old spent mushroom compost (SMC) of *Agaricus bisporus* and *Lentinula edodes* were shade-dried, ground into powder, and aqueous extracts were prepared at 10, 20, 30, 40 and 50% (w/v). The extracts were sterilized by tyndallization. Sterile Whatman No. 42 filter paper discs (5 mm diameter) were soaked in each extract and placed on nutrient agar plates seeded with *R. solanacearum*. Discs soaked in sterile distilled water served as the negative control. Following incubation at $28 \pm 1^\circ C$ for 72 h, antibacterial activity was determined by measuring the diameter of the inhibition zone (mm).

Evaluation under pot conditions

A pot experiment was conducted to evaluate the effect of spent mushroom compost (SMC) on tomato growth, yield, and bacterial wilt incidence. Pots (30 cm diameter) containing sterilized sandy loam soil and farmyard manure (3:1) were artificially infested with *Ralstonia solanacearum* (10^8 CFU mL^{-1}) one week before transplanting. Two-month-old seedlings of a susceptible tomato cultivar were transplanted (one plant per pot) with five replications per treatment.

Treatments included fresh SMC of *Agaricus bisporus* (T₁), fresh SMC of *Lentinula edodes* (T₂), weathered (six-month-old) SMC of *A. bisporus* (T₃), weathered SMC of *L. edodes* (T₄), and a 1:1 mixture of weathered SMC from both mushrooms (T₅), each incorporated at 50% (w/w) of the potting mixture. Healthy and inoculated controls were maintained. Pots were arranged in a completely randomized design (CRD). At maturity, shoot length, root length, number of fruits per plant, fruit yield per plant and wilt incidence were recorded. Wilt incidence was calculated using the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

$$\text{Disease control (\%)} = \frac{\text{Incidence in control} - \text{Incidence in treatment}}{\text{Incidence in control}} \times 100$$

Data on growth and yield parameters were subjected to square root transformation, while wilt incidence was angular transformed prior to statistical analysis.

Results and Discussion

Identification of pathogen

Virulent colonies of *R. solanacearum* developed on Triphenyl Tetrazolium Chloride (TZC) medium within 24–48 h and appeared fluidal, opaque, with pink centres and white margins (Fig. 1). Typical virulent colonies were purified by repeated subculturing on TZC medium. Pure cultures were preserved in sterile distilled water at 4°C and periodically revived every three months to maintain virulence.

Morphological Characteristics: The pathogen was initially identified based on its cultural and morphological characteristics. On TZC medium, the bacterial isolate produced typical colonies of *Ralstonia solanacearum*, appearing dull white, fluidal, irregular, with a light pink centre and whitish margins, indicating a virulent strain and these observations consistent with the descriptions of El-Ariqi *et al.*, (2005), Seleim *et al.*, (2011) and Sharma (2022).



Fig. 1: Virulent colonies of *Ralstonia solanacearum* on TZC.

Table 1: Nutrient Profiling of Button and Shiitake spent mushroom compost.

Nutrient	Button mushroom SMC	Shiitake mushroom SMC
Macronutrients (%)		
Nitrogen (N)	1.43	1.25
Phosphorus (P)	0.48	0.04
Potassium (K)	1.20	0.18
Micronutrients (mg/kg)		
Iron (Fe)	156.78	21.25
Magnesium (Mg)	0.72	0.47
Zinc (Zn)	193.30	56.00
Copper (Cu)	64.22	24.89
Sodium (Na)	1158.00	1121.00

Simple staining: The bacterial cells appeared as small, rod-shaped, purple-stained cells under the microscope, consistent with the morphology of *R. solanacearum* described by Kelman (1954).

Gram staining: The cells were small, rod-shaped, and pink in colour, confirming the Gram-negative nature of the bacterium. These findings agree with those of Schaad *et al.*, (2001), who described *R. solanacearum* as reddish-pink, rod-shaped cells following Gram staining. Similar observations have also been reported by Umesha *et al.*, (2005), Zhang *et al.*, (2011), Narasimha and Srinivas (2012) and Zulperi and Sijam (2014). Suslow *et al.*, (1982) described the KOH test as a rapid and reliable alternative to Gram staining for differentiating Gram-negative bacteria. Similar results have been reported by Shekhawat *et al.*, (1988), Sambasivam and Girija (2006), and Rahman *et al.*, (2010).

KOH solubility test: A positive KOH test was observed by the formation of a slimy mucoid thread when

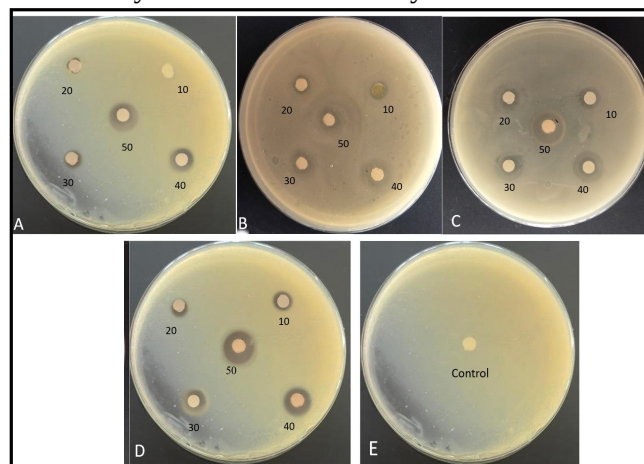


Fig. 2: *In vitro* evaluation of spent mushroom compost of shiitake and button mushroom at different concentrations by using paper disc method. Shiitake SMC A: Fresh, B: Weathered, Button SMC C: fresh, D: Weathered, E: Control.

mushroom compost (SMC) from button (*Agaricus bisporus*) and shiitake (*Lentinula edodes*) was evaluated against *R. solanacearum* at concentrations of 10–50% under laboratory conditions (Table 2).

At 10% concentration, no inhibition was observed with fresh or weathered shiitake SMC, whereas fresh and weathered button SMC produced inhibition zones of 1.67 and 1.83 mm, respectively. At 20%, shiitake SMC showed moderate inhibition (1.17 mm in fresh and 1.67 mm in weathered compost), while button SMC exhibited significantly higher inhibition zones of 2.33 mm (fresh) and 3.00 mm (weathered). Increasing the concentration to 30% further enhanced inhibition, with fresh and weathered shiitake SMC producing zones of 2.33 and 3.67 mm, respectively, compared with 9.17 and 9.67 mm for fresh and weathered button SMC (Fig. 2).

At 40%, fresh and weathered shiitake SMC recorded inhibition zones of 6.83 and 7.00 mm, respectively, whereas button SMC showed 9.17 mm (fresh) and 10.00 mm (weathered). The highest inhibition was observed at 50%, where weathered shiitake SMC (10.67 mm) was significantly more effective than fresh shiitake SMC (9.67 mm). Fresh button SMC produced a 14.83 mm inhibition zone, while weathered button SMC recorded the maximum inhibition (15.33 mm), significantly outperforming all other treatments. No inhibition was observed in the control, confirming that pathogen suppression was due to the bioactive constituents and antagonistic microorganisms present in the SMC. These findings agree with Meunchang *et al.*, (2005) and Soh *et al.*, (2014), who reported that SMC harbours beneficial microbial communities and bioactive metabolites capable of suppressing soil-borne pathogens such as *Fusarium* spp., *Pythium* spp. and *R. solanacearum*. The superior performance of weathered SMC may be attributed to enhanced microbial succession and the establishment of stable antagonistic microbial populations during compost maturation, as suggested by Ahlawat and Gaur (2010).

Efficacy of spent mushroom compost on growth parameters, yield and wilt incidence in tomato under pot conditions

The data presented in Table 3 demonstrated that spent mushroom compost (SMC) significantly improved tomato growth, yield, and bacterial wilt management under pot conditions.

The combined application of weathered *Agaricus* and *Shiitake* SMC (T_5) was the most effective treatment, recording the highest shoot length (63.00 cm), root length (29.80 cm), fruit number (7.60 plant⁻¹) and yield (142.20 g plant⁻¹), along with the lowest wilt incidence (30.92%) and highest disease control (64.68%). Weathered SMC

treatments (T_3 and T_4) consistently outperformed fresh SMC treatments (T_1 and T_2). In contrast, the untreated control exhibited the poorest growth and yield, with the highest wilt incidence (87.59%). Although the healthy check remained disease-free, it produced lower growth and yield than the weathered SMC treatments, indicating the additional growth-promoting benefits of SMC. Statistical analysis revealed significant differences among treatments, identifying the combined application of weathered SMC as the most effective strategy for enhancing tomato productivity and suppressing bacterial wilt.

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