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Management of Bacterial Wilt of Tomato using Spent Mushroom Substrate

Ali Waqas[1], Musharaf Ahmad[1], Shah Fahad[2]*, Muhammad Aftab Alam[1], Murad Ali khan[3], Muhammad Qasim Jan[3] and Mahmood Hussain[4]

[1]*Department of Plant Pathology, The University of Agriculture, Pakistan*

[2]*Department of Agriculture, The University of Swabi, Pakistan*

[3]*Department of Horticulture, The University of Agriculture, Pakistan*

[4]*Department of Weed Science, The University of Agriculture, Pakistan*

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***Corresponding author:** Shah Fahad, Department of Agriculture, The University of Swabi, Khyber Pakhtunkhwa, Pakistan

Abstract

The anti-bacterial effect of spent mushroom substrate, a waste product of mushroom industry, opposed to tomato bacterial wilt (BW). The study was performed at mushroom house of the Plant Pathology Department, Agriculture University (UAP), Peshawar, Pakistan during 2021. In both the in vitro and in vivo experiments, the ideal dosage, application technique, and time of deterioration were identified. According to the in-vitro studies, all doses produced noticeably greater inhibition zones (ZIs) than the control treatment. The 15g concentration produced 19.67 mm ZIs followed by 10g (16.13 mm ZIs). The 5g concentration produced 13.00 mm (ZI). Similarly, the greater the deterioration time i.e., Higher doses of 14 DBI, i.e., 30g powder preparation, led to significantly improved results than control treatments., enlarge root length, stem length and fresh biomass by 65.8%, 100% and 111%, respectively as compared to control treatment (0 g and 0 autoclaving) the greater dose that is 30g preparation of powder (per kg soil), autoclaved and combined with soil in pots 7 days before inoculation (DBI) attain remarkable results. The enhanced plant development parameters such as mean root lengths, stem lengths, and fresh biomasses for this treatment (SMS) were 18.62 cm, 43.75 cm and 37.50 cm. Our results indicate that 30g of dried powder, autoclaved and combined with infested soil 14 DBI might be utilized as a productive IDM component to fight tomato BW.

Keywords: Spent mushroom substrate; Bacterial wilt; *Ralstonia Solanacearum*

Introduction

Tomato (*Lycopersicon esculentum* Mill) is a commercial vegetable and one of the most used vegetables all over the world. As compared to other countries of the world, Pakistan annually produces a very small amount of tomatoes (0.562 million metric tons) including the small share (0.1618 million metric tons) annually produced by Khyber Pakhtunkhwa province of the country. Likewise, the average tomato yield of Pakistan (9.5 tons/hectare) is much lower than the world's average (38 tons/hectare) FAOSTAT [1]which needs immediate attention in terms of crop protection, crop production and crop marketing and management. Tomato diseases play a significant role in lowering the average yield/ha in the country. Among plant diseases, bacterial wilt, induced by *Ralstonia solanacearum*, is economically one of the most important diseases of tomato crop in Pakistan.

To control bacterial wilt of tomatoes and other vegetables, several management measures have been tried. Sanitary and cultural practices are the most frequent strategies for minimizing infection [2]. To control tomato wilt, chemicals are generally not suggested [3]. Bio-control tactics such as the use of antagonists that strongly affect pathogen populations, along with antibiosis, nutrient competition, and the generation of plant systemic resistance are some of the good options [4]. Alternative, sustainable, easily obtainable, and environment-friendly disease control approaches are offered for the successful treatment of plant diseases such as tomato wilt. One of these eco-friendly approaches is the use of spent mushroom substrate (SMS) to control plant infections [5].

Spent mushroom substrates (SMS) are organic substrates left over after mushroom crop is harvested. About 5 kg of substrate is required to grow 1 kilogram of mushrooms. Because of the exponential increase in the consumption of mushrooms world-wide, a husge amount of SMS (as big as 17 million tons) is generated

annually Finney, et al. [6] which must be properly disposed of. Although mushroom production has not become commercialized in Pakistan yet, it is gaining momentum especially at the resource-poor farmer's field level where the cheaply available agricultural raw materials are used as ingredients of SMS to reduce cost of production. The production of white button mushrooms i.e., Agaricus spp. Tahir and Hassan [7] and oyster mushrooms, *Pleurotus ostreatus* [8], which are locally popular, is on the rise, generating a sizable amount of SMS annually.

One way of sustainable waste management of this SMS is to use it for plant disease control. Fresh SMS could be dried, turned into a fine powder and used as a soil amendment by thorough mixing of finely ground powder with soil or mulching of the powder on top of soil. SMS water extract could also be used as a foliar spray for pathogens that attack above-ground parts of plants [9]. SMS can also be composted to get SMC (spent mushroom compost) which in turn could be used as soil amendment and its aqueous extract could be used for as foliar spray to control foliar plant diseases.

Besides being rich in nutrients and anti-biotic-producing pathogen-suppressing bacteria [10,11], SMS contain plenty of mycelia of mushrooms leftover after the harvest of the crop. Carbohydrate and protein elicitors are released from fungal mycelia [12]. These elicitors, when recognized by host plants, trigger resistance in many plants against many pathogens leading to systemic acquired resistance or SAR [13,14]. Hence, the application of SMS to plants may be useful in controlling plant diseases through inducing SAR in host plants.

The goal of this study was to explore the tomato wilt disease-control efficiency of SMS, a byproduct of the mushroom house at the University of Agriculture's department of plant pathology. The specific objectives of the study were: To test the effect of SMS water extract is at inhibiting in-vitro growth of *Ralstonia solanacearum*. To test different doses of SMS powder, activate SAR in tomato plants when applied (mixed with soil) 0 days before inoculation (0 DBI), seven days before inoculation (7 DBI) and 14 days before inoculation (14 DBI). To test the effects of various doses of autoclaved and non-autoclaved SMS powder mixed with soil 7 DBI.

Materials and methods

Source of spent mushroom substrate (SMS) and preparation of water extract

SMSs of oyster mushroom were obtained from the mushroom house of the Plant Pathology Department, Agriculture University (UAP), Peshawar, Pakistan. To make fine powder (2 mm mesh or finer) the SMS were sun dried and ground with the help of an electric grinder. The powders were kept in plastic containers, and stored at 4^0C until used. To make water extract, 5g, 10g and 15g SMS powders were separately mixed with 100 ml sterilized distilled water (SDW), soaked for 24 hours [15,16], passed through two layers of cheese-cloth and then filtered through Whatman filter paper (20 µm). The filtrate was used immediately or stored briefly at 4^0C until used.

In-vitro bacterial growth inhibition by SMS water extract

Figure 1: Effect of various aqueous extract concentrations of SMS powder on *R. solanacearum* in vitro growth inhibition.

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From the plant bacterial culture bank of the Department of Plant Pathology, UAP, Pakistan, a pre-identified and pure culture of *R. solanacearum* was acquired and streaked on TZCNA (tetrazolium chloride nutrient agar). The resulting pathogenic culture (white fluidal colonies with pink centers) was mass-cultured on nutrient agar (NA) medium (beef extract = 3 g; peptone = 5 g; agar = 15 g per one-liter medium) and 48 hours were spent incubating the plates at 28 °C. By pouring sterilized distilled water on them and scraping with sterilized cotton swabs, the ensuing bacterial growth was eliminated from the surfaces of NA plates. To dissolve bacterial clumps, magnetic stirrers were used, and uniform bacterial suspension was prepared [17]. Up to (OD600 = 0.3) and 10^8 cfu/ml the suspension was diluted Lin et al. [18] using spectrophotometer. This solution was used to inoculate tomato plants and conduct in-vitro testing to see how well it inhibited bacterial development (Figure 1).

Efficacy of SMS-water extracts of oyster and button mushrooms was tested by agar well diffusion method using sterilized corn borer. To prepare bacterial lawns, Each NA plate received 100 ul of the bacterial solution (10^8 cfu/ml), which was expertly dispersed above the surface [19]. The required number of wells were made into the medium using a template. About 100 ul of SMS extract was loaded per well. Well having 100 ul streptomycin sulphate (200 ppm) and SDW was served as positive and negative controls, respectively. The plates were incubated for 48h at 30 °C. Zones of inhibition of bacterial growth throughout the discs were measured in millimetres using a transparent plastic ruler. The experiment was repeated twice using CRD with six replications.

Experiment 1: SMS powder doses and inoculation times.

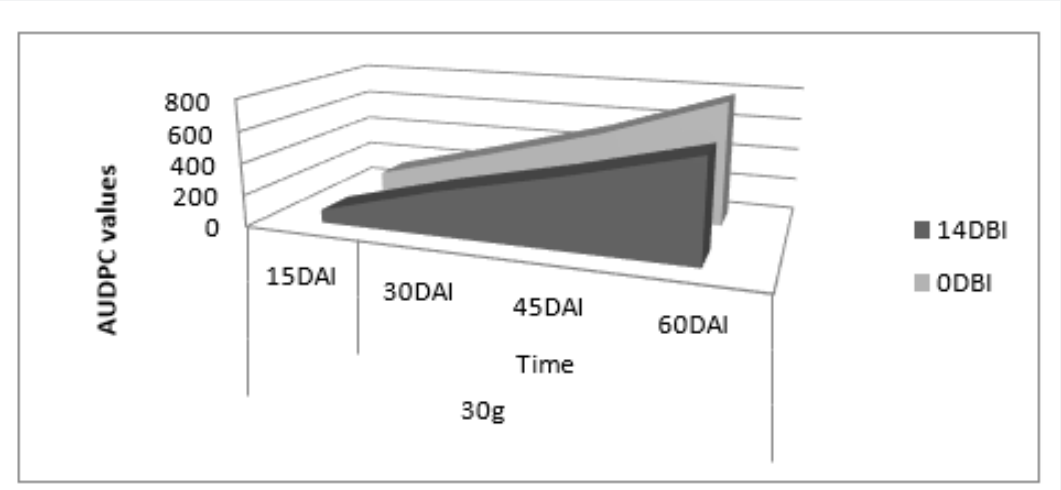

Figure 2: Effect of pre-inoculation exposure times of tomato plants to different doses of SMS powder preparation on values AUDPC of plants (tomatoes) inoculated with R. salonaceraum.

Tomato nursery plants (Rio Grande) were raised in large earthen pans each having pasteurized combination of clay and sand (2:1 w/w). Thirty-day old tomato plants were transplanted (1 plant per pot) to earthen pots each having 1 kg of field soil. There were 3x3 = 9 treatments, and each treatment was replicated 8 times (so, 9x8 = 72 pots). The pots were divided into 3 main groups, each having 24 pots. In the first main group, each pot received 0g SMS powder. In the second main group, each pot received 15g SMS powder. In the third main group, each pot received 30g SMS powder. Each main group had 3 sub-groups of 8 pots each. The first batch of each main group (total of 24 pots) was inoculated by pouring 20 ml/pot of the above-mentioned bacterial suspension right at the time of transplantation, i.e., there was no waiting period between powder application and inoculation (i.e., 0 DBI). The second batch of each main group (total of 24 pots) was inoculated by pouring 20 ml/pot of the above-mentioned bacterial suspension 7 days after transplantation, i.e., there was a waiting period of 7 days between plant's exposure to SMS powder and inoculation (i.e., plants were exposed to SMS powder for 7 days before inoculation or 7 DBI). The third batch of each main group (total of 24 pots) was inoculated by pouring 20 ml/pot of the above-mentioned bacterial suspension 14 days after transplantation, i.e., there was a waiting period of 14 days between plant's exposure to SMS powder and inoculation (i.e., plants were exposed to SMS powder for 14 days before inoculation or 14 DBI). Before inoculation, the soil was made pre-moist. The study design used was 3^2 factorial experiment in CRD where we have two factors each with three levels in this design. As per horticultural recommendations, the plants were fertilized and watered for 60 days' post-transplant, the experiment was runned (Figure 2).

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Experiment 2: SMS powder doses and effect of autoclaving SMS

In this experiment, the same three SMS powder doses were used. However, one-half of the pots received autoclaved and the other half received non-autoclaved powder preparations. There were 3x2 = 6 treatments, and each treatment was replicated 8 times (so, 6x8 = 48 pots). The pots were divided into 2 groups, each group having 24 pots. One group received autoclaved SMS powder doses and the other group non-autoclaved SMS powder doses. In case of autoclaved SMS group, 8 pots received 0g SMS powder, 8 received 15 g SMS powder per pot and another 8 pots received 30 g SMS powder per pot. In case of non-autoclaved SMS group also, 8 pots received 0g SMS powder, 8 received 15 g SMS powder per pot and 8 pots received 30g SMS powder per pot. Powder mixing and transplantation (1 plant/pot) was done the same day, but inoculation was done 7 days after the exposure of plants to SMS powder or 7 DBI. All the remaining details of the experiment were the same as mentioned above (Figure 3).

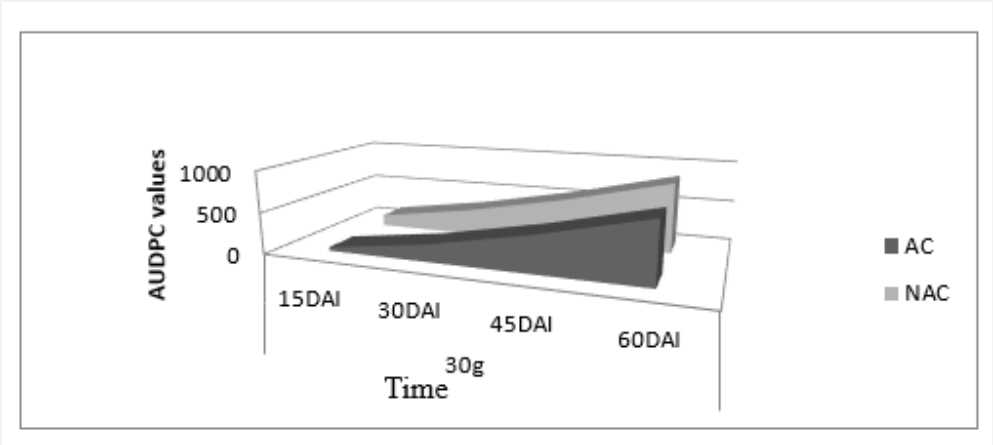

Figure 3: Effect of various doses and autoclaving of SMS powder preparation on values of AUDPC of plants (tomatoes) treated with R. salonaceraum.

Disease severity assessment

Using a 5-category disease-rating scale, at 15 day intervals, data of disease severity were gathered. (that is at 15, 30, 45 and 60 days post-transplanting). The different categories of the scale are: 0=No visible wilt, 1 = 1-25% wilt, 2 = 26-50% wilt, 3= 51-75% wilt, 4 = 76-100% wilt (plant dead). To determine %DS (i.e., disease severity) values, Bdliya and Dahiru formula, (2006), was used:

$$\%DS = \frac{\Sigma n}{4N} x100$$

Σ_n = [0 (A) +1 (B)+2(C)+3(D)+4(E)] where the alphabets A, B, C, D and E indicate the plants number showing wilting/ category; N= sample size (the total examined number of plants), and 4= peak category of disease (wilt) scale. The formula below was used to determine the AUDPC or disease progress curve. (Madden et al., 2007):

$$AUPDC = \Sigma_{i=0}^{n} \left(\frac{x_i + 1 + x_i}{2} \right) \left(T_{i+1} - T_i \right)$$

n: Total number of assessments, T_i: Time at the ith assessments, T_{i+1} = Time at the consecutive higher assessment, X_i: The % DS at the ith assessment, X_{i+1} = The % DS at the consecutive higher assessment

Data on plant growth characters

Data were also recorded on plant height (cm), fresh biomass (g), fresh root-length (cm), and number of fruits per plant.

Determination cfu/g soil over-time

The beginning pathogen's cfu/g soil was detected soon at the end of inoculation. A sterilized corn borer with a 10 mm diameter and 12 cm length was utilized to collect soil cores throughout the experiment in order to measure the cfu/g of soil at any moment. From each pot containing tomatoes, two soil cores randomly selected. To construct a composite sample, every soil core from each treatment was properly combined [20,21]. Corrosive elements like stones and other waste were taken out of each composite sample. Ten-fold dilution series (up to 10^{-8}) were created from each composite sample. To create the required ten-fold serial dilution, from every composite sample of every treatment a 5g sample was separately mixed with 45 ml of sterilized distilled water in a tiny beaker. This soil suspension was noted as 10-1 dilution. Next, a 5 ml suspension was taken from this 10^{-1} dilution and added to another small beaker having 45 ml sterilized distilled water. This was notes as 10^{-2} dilution. This way, the series was extended to10-8dilution. About 100 µl of each treatment's 10^{-7} and 10^{-8} dilution

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were placed on TZCNA plates. To make the semi-selective medium for BW pathogen, 1 ml of tetrazolium chloride (0.5% solution) in100 ml of Nutrient agar was added to the sterilized medium just before being placed into Petri plates. The plates were incubated for 24 h at 27°C, to obtain the individual colonies for counting purpose. Every creamy colony with a pink centre was counted as a BW pathogen to determine the cfu/g soil. At the completion of the experiment, the identical steps were taken once more. (i.e., 60 days' post-transplant). Bacterial counts were changed into $\log_{10}$. To determine the treatment-based reduction (i.e., cfu/g soil) of the bacterial population in soil, last $\log_{10}$ values were subtracted from starting $\log_{10}$ values.

Statistics Analysis

The recorded data including root length, shoot length/ plant heights, cfu/g soil, inhibition zones, disease severity and plant fresh biomass were taken as dependent variables whereas the doses and treatments were taken as independent variables. Using statistix 8.1, the data were analyzed statistically. In case of any significant differences, the least significant difference (LSD) test was used to separate the means.

Results

Aqueous extract of SMS inhibiting in-vitro growth of BW pathogen

The results (in-vitro growth inhibition of BW pathogen) obtained for each of the 5%, 10%, and 15% concentrations differed from each other high significantly ($p < 0.001$) as shown in Table 1. The biggest (19.67 mm) zone of inhibition (ZI) was achieved by 15% aqueous extract of SMS powder, followed by 10% extract (16.13 mm) and 5% (13.00 mm) aqueous extract.

Table 1: Effect of various aqueous extract concentrations of SMS powder on *R. solanacearum in vitro* growth inhibition.

Treatments	Inhibition zone (mm)	% of antibiotic
5% aqueous extract of SMS	13.00 d	59.97
10% aqueous extract of SMS	16.13 c	79.94
15% aqueous extract of SMS	19.67 b	92.47
Antibiotic	26.68 a	-
SDW	0.00 e	0

Treatments LSD = 3.46; A six-replicate mean is used to calculate each treatment value.

Values with the same alphabets do not differ from one another significantly ($p>0.05$).

Optimum doses and pre-inoculation exposure time of plants to SMS powder

The results of doses and pre-inoculation exposure times of tomato plants to the SMS powder preparation are showed in Tables 2-4. The treatments were determined to have a highly significant ($p \leq 0.001$) difference from one another in terms of how they affected the variables which are dependent, namely length of root, stem, and fresh biomass. In general, all plant yield parameters were improved by increasing the powder dosages and the plants' pre-inoculation exposure to SMS powder preparation. Length of root, stem and fresh biomass treated with 30g/kg soil of the plants applied 14 days before inoculation were all increased by 65.8%, 100%, and 111%, respectively, in contrast to control plants.

Optimum doses and exposure of tomato plants to autoclaved and non-autoclaved (0 autoclaving) SMS powder

The results of autoclaved and non-autoclaved doses (0g, 15g, and 30g Kg^{-1} potted soil; applied 7 DBI) of SMS powder preparation are presented in Tables 5-7. A significant difference between the treatments was observed ($p \leq 0.05$). In terms of preventing bacterial wilt and improving plant growth parameters, autoclaved powder preparation was shown to be better to non-autoclaved. As compared to control treatment (0 g and 0 autoclaved), mean root lengths, stem lengths, and fresh biomasses were 18.62 cm, 43.75 cm (62.04%), and 37.50 cm (84.09%) when 1 kg potted soil combined with 30g powder preparation.

Cfu/g of soil as impressed by different doses of autoclaved and non-autoclaved SMS powder preparation

The results (Tables 8) showed that increasing powder dosages and powder degradation time significantly decrease the number of bacterial count g^{-1} soil. In 30g(14DBI) the population decrease recorded was high. The number of pathogens was decreased from $\log_{10} = 8.34$ to $\log_{10} = 7.71$ (a drop of 0.63 units), after which in 30g (7 DBI), the number of bacteria per g of soil decreased from beginning $\log_{10} = 8.33$ to final $\log_{10} = 7.81$. (a decrease of 0.52 units).

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Table 2: Effect of various SMS powder dosages and application times on the root length (cm) of tomato plants treated with *R. solanacearum*.

Application times →	0DBI	7DBI	14DBI	Mean
Doses↓				
0g	15.00 EF	14.62 EF	13.87 F	14.50 C
15g	16.37 E	20.12 CD	22.25 B	19.58 B
30g	18.87 D	21.87 BC	24.87 A (65.8%)	21.87 A
Mean	16.75 C	18.87 B	20.33 A	

DBI = Days before inoculation; LSD of application times X Doses = 2.12

A four-replicate mean is used to calculate each value. Values with the same alphabets do not differ from one another significantly ($p > 0.05$). Percent increase over un-amended control values are mentioned in brackets [{(24.87-15) ÷15}x100 = 65.8%.]

Table 3: Effect of various SMS powder dosages and application times on the stem length (cm) of tomato plants treated with *R. solanacearum*.

Application times →	0DBI	7DBI	14DBI	Mean
Doses↓				
0g	25.50 G	26.62 G	25.37 G	25.83 C
15g	32.75 F	38.50 E	43.12 C	38.12 B
30g	41.25 D	45.12 B	51.00 A **(100%)**	45.79 A
Mean	33.16 C	36.75 B	39.83 A	

DBI = Days before inoculation

LSD value for interaction of application times and Doses = 1.77

A four-replicate mean is used to calculate each value. Values with the same alphabets do not differ from one another significantly ($p > 0.05$). Percent increase over un-amended control values are mentioned in brackets [{(51.0-25.5) ÷25.5}x100 = 100%.]

Table 4: Effect of various SMS powder dosages and application times on the fresh biomass (cm) of tomato plants treated with *R. solanacearum*.

Application times →	0DBI	7DBI	14DBI	Mean
Doses↓				
0g	21.50 E	20.75 E	20.12 E	20.79 C
15g	27.87 D	35.25 C	39.12 B	34.08 B
30g	35.00 C	40.00 B	45.37 A **(111%)**	40.12 A
Mean	28.12 C	32.00 B	34.87 A	

DBI = Days before inoculation

LSD value for interaction of application times and Doses = 2.04

A four-replicate mean is used to calculate each value. Values with the same alphabets do not differ from one another significantly ($p > 0.05$). Percent increase over un-amended control values are mentioned in brackets [{(45.37-21.5) ÷21.5}x100 = 111%.]

Table 5: Effect of SMS powder dosages and autoclaving on the length of root in (cm) of plants (tomatoes) treated with *R. salonaceraum*.

Methods↓	Doses			
	0g	15g	30g	Mean
Autoclaved	12.62	16.87	18.62	16.04 A
Non autoclaved	12.37	14.25	16.75	14.45 B
Mean	12.50 C	15.56 B	17.68 A	

Methods LSD = 1.02; Doses LSD = 1.25;

Each table's value is the mean of four replicates. Values with the same alphabets do not

differ from one another significantly ($p > 0.05$). Interactions were non-significant.

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Table 6: Effect of SMS powder dosages and autoclaving on the length of stem in (cm) of plants (tomatoes) treated with *R. salonaceraum*.

Methods↓	Doses			
	0g	15g	30g	Mean
Autoclaved	25.25 D	38.75 B	43.75 A -62.04%	35.91 A
Non autoclaved	27.00 D	33.87 C	39.25 B	33.37 B
Mean	26.12 C	36.31 B	41.50 A	

LSD of methods and Doses = 1.82

Each table's value is the mean of four replicates. Values with the same alphabets do not differ from one another significantly ($p > 0.05$). Percentage increase over un-amended control values are mentioned in brackets [(43.75-27.00) ÷27.00}x100 = 62.04%.]

Table 7: Effect of SMS powder dosages and autoclaving on the biomass in fresh in (cm) of plants (tomatoes) treated with *R. salonaceraum*.

Methods↓	Doses			
	0g	15g	30g	Mean
Autoclaved	19.75 C	33.87 B	37.50 A -84.09%	30.37 A
Non autoclaved	20.37 C	32.25 B	33.50 B	28.70 B
Mean	20.06 C	33.06 B	35.50 A	

LSD of methods and Doses= 2.24

Each table's value is the mean of four replicates. Values with the same alphabets do not differ from one another significantly ($p > 0.05$). Percentage increase over un-amended control values are mentioned in brackets [{(37.50-20.37) ÷20.37}x100 = 84.09%.].

Table 8: Effect of doses of SMS powder and application timing on reduction in soil BW pathogen population density.

Application times →	0DBI	7DBI	14DBI	Mean
Doses↓				
0g	0.09 F	0.10 F	0.09 F	0.09 C
15g	0.25 E	0.30 D	0.37 C	0.31 B
30g	0.51 B	0.51 B	0.63 A	0.55 A
Mean	0.28 C	0.31 B	0.36 A	

DBI and Doses LSD = 0.02

Each table's value is the mean of four replicates. Values with the same alphabets do not differ from one another significantly ($p > 0.05$).

Original log values

Log values for 0g and 0DBI,7DBI and 14DBI are (8.334-8.235), (8.348-8.247), (8.34-8.243)

Log values for 15g and 0DBI,7DBI and 14DBI are (8.332-8.079), (8.336-8.036), (8.342-7.965)

Log values for 30g and 0DBI,7DBI and 14DBI are (8.342-7.831), (8.334-7.823), (8.34-7.713)

Cfu/g of soil as impressed by doses and autoclaving of SMS powder preparation

The results of doses and autoclaving of SMS powder preparation are given in Tables 9. The results of 30 g autoclaved SMS powder mixed per kg potted soil were found to be better than non-autoclaved as they reducing bacterial numbers g^{-1} soil. Bacterial counts dropped from a $\log_{10}$ beginning value of 8.35 to a $\log_{10}$ end value of 7.79. The same powder preparation technique, that is 30g/kg soil autoclaved, decreased the number of bacteria from beginning $\log_{10}$ = 8.33 to end $\log_{10}$ = 7.81. The little reduction in population of bacteria was observed when 15g powder mixture (autoclaved) was mixed with 1 kg soil in pot.

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Values of AUDPC as impressed by doses and time of powder application

Results on the impact of varied dosages and deterioration times for the SMS powder preparation on AUDPC values are shown in Table 10. There was significant difference ($p \leq 0.05$) between the treatments. The treatment with the greater dosage of (30g/kg soil applied) 14 DBI gave the lowest AUDPC value.; a reduction of 27.54% against the control treatment (that is 0g/kg soil and 0 DBI). AUDPC having great values observed for 15g Kg^{-1} soil applied 0DBI.

Table 9: Effect of and autoclaving of SMS powder mixed to potted inoculated soil and different doses on pathogen population.

Methods↓	Doses			
	0g	15g	30g	Mean
Autoclaved	0.09 E	0.33 D	0.54 A	0.32 B
Non autoclaved	0.09 E	0.48 C	0.51 B	0.36 A
Mean	0.09 C	0.41 B	0.52 A	

Doses and Methods LSD = 0.0163

Each table's value is the mean of four replicates. Values with the same alphabets do not differ from one another significantly ($p > 0.05$).

Original log values

Log values for autoclaved 0g, 15g and 30g SMS are (8.334-8.244), (8.348-8.014), (8.352-7.799)

Log values for non-autoclaved 0g, 15g and 30g SMS are (8.344-8.255), (8.34-7.845), (8.334-7.812)

Table 10: Effect of dosages of powder preparation of SMS and application times on the values of AUDPC plants (tomatoes) treated with *R. salonaceraum*.

Application times →	0DBI	7DBI	14DBI	Mean
Doses↓				
0g	433.00 A	421.17 A	428.00 A	427.39 A
15g	343.50 C	349.33 C	374.50 B	355.78 B
30g	283.00 E	313.00 D	339.50 C	311.83 C
			-27.54%	
Mean	353.17 B	361.17 B	380.67 A	

LSD of DBI and Doses = 17.561

Each table's value is the mean of four replicates. Values with the same alphabets do not differ from one another significantly ($p > 0.05$). Percentage increase over un-amended control values are mentioned in brackets [{(433-339.5) ÷339.50}x100 = 27.54%.].

Table 11: Effect of various doses and autoclaving of SMS powder preparation on values of AUDPC of plants (tomatoes) treated with *R. salonaceraum*.

Methods↓	Doses			
	0g	15g	30g	Mean
Non Autoclaved	493.13	422.97	387	434.37 A
Autoclaved	406.83	338	308.67	351.17 B
Mean	449.98 A	380.48 B	347.83 C	

Methods LSD = 15.02

Doses LSD = 18.40

Each table's value is the mean of four replicates. Values with the same alphabets do not differ from one another significantly ($p > 0.05$). Interactions were non-significant.

AUDPC values as afflicted by different doses and autoclaving of SMS powder preparation

Table 11 provides information on how varied dosages and autoclaving of the SMS powder formulation affect the decline in AUDPC values. There was significant difference ($p \leq 0.05$) between the treatments. In terms of lowering AUDPC values, autoclaved SMS powder preparation combined with potted soil was shown to be more successful than non-autoclaved. Autoclaving the SMS

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powder gave better results than non-autoclaving, reducing the AUDPC value to 351.17. Likewise, the higher dose of 30g SMS powder was found to be better than the smaller doses, reducing the AUDPC value to 347.83.

Discussion

Numerous researchers have been able to successfully control different plant diseases using SMSs of different edible mushrooms as soil amendments or their water extracts as sprays and soil drenches against various pathogens. For example, spent mushroom substrate/composts of shiitake and oyster mushrooms have been reported to control bacterial wilt of potato and tomato [22,23]. Likewise, several serious fungal diseases were successfully controlled by SMSs: these diseases include stem rot of onions [24]; damping off (Phytophthora drechsleri) in cucumber [25]; damping off (Rhizoctonia solani) of tomatoes [26]; and late blight (Phytophthora capsici) in peppers [27]. Khan, et al. [28] successfully managed collar rot (Sclerotium rolfsii) of chick pea using SMS of button mushroom. Using SMSs of shiitake and oyster mushrooms, Istifadah and Herawati [26] successfully controlled early blight in tomatoes. SMS of shiitake edible mushroom was found to suppress bacterial wilt of tomato [28] and water extracts of hatakeshimeji mushroom successfully controlled cucumber anthracnose [28]. Control of plant diseases as well as soil health improvement as a result of 8 added SMSs, in turn, result in increases of crop yields. This fact has been reported by multiple researchers. Adding SMSs of various edible mushroom species to soil, several researchers have reported increases in the yield of many crop plants. Jonathan, et al. [29] added SMS of Pleurotus pulmonarius to depleted garden soil and found significant increases in the plant growth parameters and yield of four different vegetables. Idowu and Kadiri [30] obtained significantly higher yields of okra by adding SMS/compost of *Pleurotus ostreatus* to soil.

Depending upon the composition, SMS fertilizes soil by providing a lot of mineral nutrients to it which are then taken up by plants, resulting in the enhancement of crop yield. The various nutrients and their amounts reportedly Jasinska [31] provided by SMSs to soil include N (1-4.2%), P (0.1-0.4%), K (0.5-1.8%), Mg (0.2-0.4%), and good amounts of trace elements such as Fe, Cu, Zn, Mn, Mo, and B. Besides enriching soil with nutrients, the use of SMSs also enhances soil organic matter Zhu, et al. [32] which improves soil structure, maintains soil pH around neutral (6-7) which is ideal for the growth of many crops and release many plant-needed Phyto-hormones [33]. The release of micro- and macro-nutrients by SMSs added to soil also increase the activities of plant-friendly soil microflora which, in turn, helps in the enhancement of crop yield [34].

Several mechanisms have been hypothesized by different researchers for the SMSs-mediated control of plant diseases. Some of these include: (i) the release of anti-microbial phenolic compounds by SMSs Aslam and Saifullah [35]; Ishihara, et al. [36]; (ii) the presence of systemic acquired resistance or SAR-activating bacterial and fungal isolates in the SMSs of various edible mushrooms Istifadah and Herawati [25]; (iii) the presence of PAMPs (pathogen-associated molecular patterns) in SMSs which act as elicitors for the activation of host plant defences Ishihara, et al. [36]; (iv) the generation of microbe-mediated antifungal volatile compounds during incubation of SMSs in wet soil Fujita, et al. [38]; (v) enhancement of plant-friendly soil microflora in the rhizosphere of plants; and (vi) enrichment of soil with nutrients as well as the development in the physical structure of soil as a result of addition of SMSs to soil. SMSs contain remains of fungal mycelia after the harvest of mushroom crop. Chitins and glucans are important skeletal polysaccharides of cell walls of both pathogenic as well as non-pathogenic fungi. So, they act as PAMPs or MAMPs (microbe associated molecular patterns). PAMPs/MAMPs and SMSs-released chemicals, both of which act as elicitors of host defences, are important as they activate SAR which can last and protect plants against many pathogens for the whole season [12,13].

Our results exhibited that aqueous extracts prepared from higher doses of SMS were more effective in inhibiting the in-vitro growth of the BW pathogen. These results can be explained on the basis of the release of higher amounts of anti-bacterial compounds by higher doses of SMS. Many researchers Aslam and Saifullah [35]; Ishihara, et al. [36] found that SMS release phenolic compounds which directly kill bacteria. It is obvious that bigger amounts of SMS will release higher amounts of phenolic compounds resulting in higher anti-bacterial activity. We also found that higher dose (30g SMS powder/kg potted soil) and pre-inoculation of tomato plants to SMS powder for longer times (14 DBI) were more effective than lower doses and shorter exposure times in terms of reducing AUDPC values and pathogen counts (i.e., cfu/g soil) and enhancing plant growth perimeters such as root length, plant fresh biomass and shoot length. Higher doses of SMS, when applied to soil, will release more phenolic compounds Aslam and Saifullah [35]; Ishihara, et al. [36] resulting in greater decline of pathogen population in soil and reduction in AUDPC values. The reduction in AUDPC values is also caused by improvement in the host resistance because of SAR activation due the presence of PAMPs (pathogen-associated molecular patterns) as well as some fungal and bacterial isolates in SMS powder Ishihara et al. [37]; Istifadah and Herawati [26]. The enhancement in plant growth characters can be explained on the basis of reduction in the amount of disease, provision of a lot of nutrients to soil by the added SMS and the SMS-mediated enhancement of plant-friendly soil microflora and improvement in the physical structure of soil [39-41].

Our results that higher dose (30g SMS powder/kg potted soil) when autoclaved performed better than the same amount of non-autoclaved SMS powder in terms of enhancing plant growth characters and reducing AUDPC values/pathogen population in

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soil is most probably due to the autoclaving-mediated release of SAR-activating chemicals as well as degradation products of chitins and glucans, present in the fungal hyphae (still present in SMS after the harvest of mushrooms) as skeletal polysaccharides. These chemicals and cell wall degradation products act as elicitors of host defences, and activate SAR in host plants against many plant diseases. SAR can last and protect plants against many pathogens for the whole season [12,13, 42-51].

Conclusion and Recommendations

In conclusion, the use of SMS water extract at a higher concentration of 15% resulted in the largest zone of inhibition (ZI) at 19.67 mm, followed by the 10% concentration with a ZI of 16.13 mm. Furthermore, exposing tomato plants to SMS powder for an extended period (14 days before infestation) and at a higher dosage of 30g per preparation led to significant increases in root length (65.8%), stem length (100%), and fresh biomass (111%). Additionally, the application of a higher dose of SMS powder (30g/kg soil), autoclaved and mixed with potted soil 7 days before infestation, produced notable improvements in plant growth parameters, including an 18.62 cm increase in root length, a 43.75 cm (62.04%) increase in stem length, and a 37.50 cm (84.09%) increase in fresh biomass compared to the control. As recommendations, it is advised to utilize the higher SMS powder dose (30g/kg soil) that has been autoclaved and mixed with infested soil 14 days before infestation as an effective component of integrated disease management for combatting tomato bacterial wilt. This treatment is particularly recommended for establishing disease-free nurseries and cultivating disease-free tomato crops in greenhouses and small fields.

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