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Efficacy of Streptomyces Formulation for the Induction of Defense Response in Rice Challenged with Bacterial Leaf Blight Pathogen *Xanthomonas oryzae* pv. *oryzae*

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Abstract

The present study was conducted during the *kharif* season (June–September, 2019) at the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India (11.01°N, 76.93°E) to evaluate the efficacy of a *Streptomyces*-based formulation in managing bacterial leaf blight (BLB) of rice. Biological control has been gaining increasing importance in recent years for the management of plant diseases. A water-soluble starch-based formulation was developed using dried spores and mycelia of *Streptomyces* sp. TC1, a strain exhibiting inhibitory activity against *Xanthomonas oryzae* pv. *oryzae*, the causal organism of BLB. The TC1 formulation effectively reduced seed-borne infection and enhanced the germination percentage of paddy seeds by 78.7%. Foliar application of the formulation at varying concentrations significantly induced systemic resistance in rice plants, as evidenced by increased activity of defense-related enzymes such as peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), superoxide dismutase (SOD), and elevated total phenol content. Among the tested concentrations, the 0.4% TC1 formulation was identified as optimal, resulting in a 56.6% reduction in disease incidence under pot culture conditions compared to the untreated control. Furthermore, application of the TC1 formulation up to 1% concentration did not exhibit any phytotoxic effects on rice plants. The findings suggest that the TC1 formulation could serve as a promising biocontrol agent for the eco-friendly and sustainable management of bacterial leaf blight in rice cultivation.

Keywords: Rice, seed germination, defense enzymes, *Streptopmyces* sp., *Xoo*

1. Introduction

The use of microbial based products for the management of plant diseases is gaining more importance nowadays. The inadvertent use of chemical pesticides leads to soil pollution and also results in resistance development among the target pathogens. *Streptomyces* genus is known for production of several secondary metabolites and widely used for the management of certain plant diseases (Al-Quwaie, 2024; Khan et al., 2023; Dow et al., 2023; Le et al., 2022). A promising *Streptomyces* sp. TC1 (GenBank KC954629) isolate exhibited antimicrobial activity against bacterial leaf blight pathogen *Xanthomonas oryzae* pv. *oryzae* under *in vitro* conditions (Jaivel et al., 2014).

Formulation of the TC1 isolate and subsequent use as a biocontrol agent for the management of bacterial leaf blight disease in rice was investigated. Formulation is a crucial aspect for producing inoculants containing an effective microbial strain and can determine the success or failure of a biological agent (Aristide et al., 2022). Formulation typically consists of establishing the active ingredient (i.e., microorganism) in a suitable carrier together with additives that aid in the stabilization and protection of the microbial cells during storage and transport, and at the target site (Bashan, 1998).

The spore-peat moss-sand formulation of *Streptomyces lydicus* WYEC108 applied to *P. ultimum*-infested sterile or nonsterile soil planted with pea and cotton seeds, showed significant increases in average plant stand, plant length, and plant weight in both cases compared with untreated control plants grown in similar soils (Yuan and Crawford, 1995).

Induced Systemic Resistance is a plant defense mechanism that is activated in response to certain stimuli, particularly beneficial microbes like *rhizobacteria*. ISR enhances the

plant's ability to defend itself against a broad spectrum of pathogens and pests without directly attacking them. During ISR, several enzymes play key roles in strengthening the plant's defense including peroxidase (PO), polyphenoloxidase (PPO), phenylalanine ammonia-lyase (PAL), catalase, superoxide dismutase (SOD). Induced systemic resistance activates the multiple defense mechanisms which include increased pathogenesis related (PR) proteins (peroxidase, chitinase, etc) (Xue et al., 1998). Induction of resistance in rice with *A. vasica* was evident from increased accumulation of PR proteins and other related compounds. Biological active compounds which are present in plants act as elicitors to induce resistance in host plants resulting in a reduction of disease development (Vidhyasekaran, 1992).

The idea that biocontrol agents might induce resistance in the host was first suggested on the basis of experiments showing that bacterial treatments protected potato tubers from subsequent infection by *P. solanacearum* (Kempe and Sequeira, 1983). More recently, it has been shown that the biocontrol agent *P. fluorescens* strain CHAO (Maurhofer et al., 1994) induces SAR-associated proteins, confers systemic resistance to a vira1 pathogen, and induces accumulation of salicylic acid, which plays a role in signal transduction in SAR (Gaffney et al., 1993; Ryals et al., 1996).

Streptomyces can inhibit Xoo through antagonistic mechanism and are also capable of producing growth regulators (Kawicha et al., 2023; Suarez-Moreno et al., 2019; Bhai et al., 2016). Another mechanism by which disease suppression occurs in foliage by rhizosphere bacteria is induced systemic resistance or ISR (Le et al., 2021; Dias et al., 2017; Choudhary et al., 2007). Based on this background, a starch-based formulation containing Streptomyces sp. was prepared and evaluated for its effectiveness in managing bacterial leaf blight disease in rice. Additionally, the formulation was tested for its ability to control seed-borne infections and to induce systemic resistance in rice plants.

2. Materials and Methods

2.1. Development of Streptomyces sp. TC1 formulation

The experiment was conducted during *kharif* (June–September, 2019) at Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India (located at an altitude of 426.7 m above MSL, 11.01°N latitude and 76.93°E longitude) to study the efficacy of *Streptomyces* based formulation on the management of bacterial leaf blight disease in rice. The TC1 strain was developed into water dispersible powder formulation according to the procedure described by Jones and Burges (1998). The spore and mycelia of the TC1 strain were collected from the fermentation broth by means of centrifugation and were added to soluble starch at 3% level. The developed TC1 formulation contained 10^8 cfu g^{-1} of the product at the time of preparation.

2.2. Effect of TC1 formulation on seed infection, seed germination and seedling vigour

2.2.1. Disease incidence (Blotter method)

The Taichung Native 1 **(**TN1) variety paddy seeds were used to find out the efficacy of various concentrations (0.2, 0.4, 0.6, 0.8 and 1.0%) of *Streptomyces* sp. TC1 formulation. The seeds (100 seeds) were soaked in each treatment with formulation for 2 h and replicated four times. A control was maintained by soaking the seeds in distilled water. Twenty five seeds of each treatment were placed on moist blotters (Anonymous, 1993) in petriplate and incubated (20±2°C) for 12 h at alternated natural UV light and 12 h darkness. The seeds were examined for growth of seed borne pathogens on eighth day of treatment and seed infection was expressed in percentage.

2.2.2. Seed germination and vigour index

The TN1 variety paddy seeds were soaked in *Streptomyces* sp. TC1 formulation for 18 h and then dried under shade. Three replicates of 25 seeds were uniformly placed on standard germination paper roll-towel medium and kept in germination room maintained at 25±2°C and 90±2% relative humidity. After the test period of 14 days, the seedlings were evaluated as total number of normal seedlings and germination as percentage. Similarly different concentration of formulation was used for evaluating the percent germination. The fresh weight and dry weight of the seedlings were also evaluated.

The vigour index of fourteen days old rice seedlings was calculated by measuring the root and shoot length by following the procedure of Abdul-Baki and Anderson (1973).

2.3. Evaluation of TC1 formulation against bacterial blight

A pot culture experiment was conducted to evaluate the efficacy of different concentrations of TC1 formulation against bacterial blight disease of rice. Twenty five days older TN1 variety paddy seedlings were transplanted and the plants were maintained in pots. Cell suspension of *Xoo* was prepared by mixing 48 h old culture grown on NA medium with 10 mL sterile distilled water. The cell suspension containing 10^9 cfu mL^{-1} was used for inoculating rice leaves by leaf clip method (Kauffman et al., 1973).

The TC1 formulation was sprayed three times at 15 days interval from 45 days after sowing. Each treatment was replicated thrice in factorial completely randomized design (CRD). The symptoms were recorded as % diseased leaf area following the Standard Evaluation System (SES) for Rice (Anonymous, 2002) and the Percent Disease Index (PDI) was calculated using the formulae of McKinney (1923).

2.4. Induction of biochemical defense mechanisms by TC1 formulation in rice

The effect of TC1 formulation on induction of biochemical defense mechanisms in TN 1 rice variety plants were carried out with different concentrations viz., 0.2, 0.4, 0.6, 0.8

and 1.0% of TC1 formulation. Control plants were sprayed with distilled water. After 48 h of formulation spray, *Xoo* was inoculated by leaf-clip method and leaf samples were collected at 0, 1, 2, 3, 4 and 5 days after inoculation (DAI) of the pathogen. The enzyme was extracted from leaves at ice-cold condition (4°C) and used as a source for assaying the change in the activity of defense related enzymes viz., peroxidase (PO), polyphenoloxidase (PPO), phenylalanine ammonia-lyase (PAL), catalase, superoxide dismutase (SOD) and phenol. The defense enzymes were analyzed using the standard protocols for peroxidase (Puttur, 1974), polyphenoloxidase (Mayer et al., 1965), phenylalanine ammonia-lyase (Dickerson et al., 1984), catalase (Chaparro Giraldo et al., 2000), superoxide dismutase (Mishra and Fridovich, 1972) and phenol (Spies, 1955) respectively.

3. Results and Discussion

3.1. Preparation of TC1 formulation

This study focuses on developing a starch-based formulation of *Streptomyces* sp. TC1 to evaluate its effectiveness in pot culture experiments for managing bacterial leaf blight in rice. The formulation was prepared using the dried spores and mycelium of *Streptomyces* sp. TC1. The effect of different concentration of TC1 formulation on the seed germination, seed infection and seedling vigour of TN1 variety paddy seeds was also evaluated. The TC1 formulation was prepared with starch as a carrier material and silica as an adjuvant. Addition of silica fume enhances the flowability of product by reducing the clump and cake formation during formulation process of *B. thuringiensis* subsp. *aizawai* (Teera-Arunsiri et al., 2003).

3.2. Effect of TC1 formulation on seed infection, germination and vigour of rice seedlings

The rhizosphere bacteria when applied as a seed treatment greatly reduced the foliage disease incidence. For instance, seed treatment of tomato with *Streptomyces* reduces the damping off symptoms caused by *Rhizoctonia solani* to the extent of 53.3% (Patil et al., 2010). In this present study, the treatment of TN1 variety paddy seeds with various concentrations of TC1 formulation showed reduced seed infection compared to control. The seeds treated with 1% TC1 formulation produced 81.8% reduced seed infection over control. The seed germination of TN1 variety paddy seeds treated with 1% TC1 formulation was found to be increased by 28.3% more than the control (Table 1). The increase in seed germination and biomass production of seedling was observed due to the inhibition of seed borne pathogens as well as the production of growth promoting substances by *Streptomyces* sp. TC1. The seeds treated with TC1 formulation at 0.6% and above showed increased seedling biomass than the positive controls. The effect of various concentrations of TC1 formulation on vigour of TN1 variety rice seedlings was presented in Table 2. A maximum vigour index of 1748.0 was observed with T_6 treatment which was 69.2% increase over control. The treatments which received Streptocycline and Kocide101 recorded 28.3 and 22.3% increased vigour index over control.

3.3. Effect of TC1 formulation on bacterial leaf blight control in rice

Any formulation prior to the field study has to be tested on a small scale. Hence pot culture studies were carried out to evaluate the biocontrol potential of TC1 formulation for BLB control in rice. Various microbial antagonists have been investigated as potential biocontrol agents of plant diseases. Many species of actinomycetes, especially those belonging to the genus *Streptomyces*, are well known as biocontrol agents that inhibit several soil-borne and airborne plant pathogens (Sonowal et al., 2024; Silva Sousa et al., 2008, Augustine et al., 2005). The biocontrol potential of Streptomyces was reported in many cereal crops (Bestha et al., 2025; Ashwini et al., 2023; Newitt et al., 2019).

The pot culture experiment was conducted using developed TC1 formulation in rice plants challenge inoculated with *Xoo* cells. Several inoculation techniques have been used to inoculate *Xoo* in rice leaves for developing pathogenicity including needle-pricking method, spraying method and

Table 1: Effect of TC1 formulation on seed infection and germination

Treatment	Seed infection percentage	% reduction over control	Germination (%)	% increase over control	Biomass	
					Fresh weight	Dry weight
T_1: Control	58.7		61.3		0.060±(0.01)	0.015±(0.02)
T_2: 0.2% TC1 formulation	30.7	47.7	64.0	4.4	0.066±(0.02)	0.021±(0.01)
T_3: 0.4% TC1 formulation	20.0	65.9	69.3	13.0	0.068±(0.01)	0.023±(0.01)
T_4: 0.6% TC1 formulation	13.3	77.3	74.7	21.7	0.073±(0.01)	0.027±(0.03)
T_5: 0.8% TC1 formulation	12.0	79.6	77.3	26.1	0.079±(0.02)	0.031±(0.02)
T_6: 1.0% TC1 formulation	10.7	81.8	78.7	28.3	0.084±(0.01)	0.034±(0.01)
T_7: Streptocycline (100 ppm)	9.3	84.1	72.0	17.4	0.067±(0.01)	0.022±(0.01)
T_8: Kocide101 (0.2%)	12.0	79.6	70.7	15.2	0.069±(0.03)	0.023±(0.01)

Values are mean±SD

Table 2: Effect of TC1 formulation on vigour of TN1 variety paddy seedling

Treatment	Root length (in cm)	% increase over control	Shoot length (in cm)	% increase over control	Vigour index	% increase over control
T_1: Control	10.5±(0.40)		6.3±(0.26)		1032.9	
T_2: 0.2% TC1 formulation	13.7±(0.90)	30.8	6.8±(0.25)	6.8	1312.0	27.0
T_3: 0.4% TC1 formulation	14.3±(0.71)	35.7	7.0±(0.38)	10.9	1475.4	42.8
T_4: 0.6% TC1 formulation	15.1±(0.50)	43.3	7.4±(0.44)	17.5	1679.3	62.6
T_5: 0.8% TC1 formulation	14.9±(0.63)	39.8	7.5±(0.41)	19.1	1719.1	66.4
T_6: 1.0% TC1 formulation	14.6±(0.72)	37.7	7.7±(0.28)	22.6	1748.0	69.2
T_7: Streptocycline (100 ppm)	11.6±(0.58)	10.3	6.8±(0.33)	7.7	1324.8	28.3
T_8: Kocide101 (0.2%)	11.3±(0.79)	7.9	6.6±(0.27)	3.5	1263.6	22.3

Values are mean±SD

dipping method (Zaragosa and Mew, 1979). Most of these inoculation techniques require wounding of the host tissues in order to introduce the inoculum to the infection site. In this present study, the *Xoo* culture was inoculated into rice leaves by leaf clipping method. According to Hoque and Mansfield (2005), the clipping method of inoculation was found to be better in all the cases irrespective of genotype and incubation period.

Three sprays of TC1 formulation were given at 45th, 60th and 75th days after sowing after challenge inoculation with *Xoo*. The application of TC1 formulation as curative spray resulted in BLB disease reduction to considerable level compared to control. Observations were taken on the tenth day after each spray. The maximum reduction in BLB severity was observed after tenth day of second spray. The plants which received 0.4% TC1 formulation showed on par results with increased concentrations of TC1 formulation.

The Percent Disease Index (PDI) was calculated using the Standard Evaluation System for rice. The PDI of TC1 formulation treated plants was significantly lesser when compared to the control. The lowest PDI of 20.82, 12.64 and 15.75 were recorded in the treatment with Streptocycline (100 ppm) at the 10th day after first, second and third spray respectively. This was followed by the Kocide101 treatment which showed a reduced disease intensity of 25.76, 16.37 and 21.63 PDI after 10th day of each spray respectively. Among the TC1 formulation the plants which received 0.4% to 1.0% level showed on par results in disease suppression. The plants which received 0.4% formulation showed reduction in BLB disease incidence about 39.28, 25.91 and 33.12 PDI after 10th day of each spray respectively (Table 3). The treatment T_3 which received 0.4% TC1 formulation recorded 56.6% reduced PDI over the control (T_1) after 10th day of second spray and showed on par results with the plants that received TC1 formulation at 1% level (62.6%). Hence, 0.4% TC1 formulation was considered as optimum concentration for BLB control in rice. The disease suppression efficacy of Streptocylcine was found to be more compared to all other treatments followed by Kocide101. This might be due to the instant inhibitory action of positive control on the reduction of BLB pathogen.

Table 3: Effect of TC1 formulation on bacterial leaf blight of rice

Treatment	Percent disease index (PDI)			% Reduction over control		
	55 DAS	70 DAS	85 DAS	55 DAS	70 DAS	85 DAS
T_1: Control	47.43±(2.10)	59.71±(1.09)	66.13±(3.75)	-	-	-
T_2: 0.2% TC1 formulation	42.67±(2.22)	29.95±(2.98)	35.54±(2.24)	10.0	49.8	46.3
T_3: 0.4% TC1 formulation	39.28±(3.17)	25.91±(3.78)	33.12±(2.75)	17.2	56.6	49.9
T_4: 0.6% TC1 formulation	38.45±(3.37)	24.49±(3.48)	31.38±(3.43)	18.9	58.9	52.6
T_5: 0.8% TC1 formulation	37.67±(1.00)	23.93±(2.05)	29.67±(2.85)	20.6	59.9	55.1
T_6: 1.0% TC1 formulation	36.93±(2.58)	22.36±(3.48)	28.19±(3.83)	22.1	62.6	57.4
T_7: Streptocycline (100 ppm)	20.82±(2.15)	12.64±(2.84)	15.75±(1.86)	56.1	78.8	76.2
T_8: Kocide101 (0.2%)	25.76±(2.99)	16.37±(2.69)	21.63±(2.50)	45.7	72.6	67.3
SEd	1.013	1.315	1.413			
CD (p=0.05)	2.173	2.821	3.031			

Values are mean±SD

3.4. Induction of systemic resistance by TC1 formulation

The biocontrol agents suppress the plant pathogens not only by means of antimicrobial metabolites, but it also having influence in developing the induced systemic resistance by synthesis of plant defence chemicals. Plants respond to a variety of chemical stimuli produced by soil and plant associated microbes. Such stimuli can either induce or condition plant host defenses through biochemical changes that enhance resistance against subsequent infection by a variety of pathogens (Hata et al., 2021; Leeman et al., 1995). The fluorescent pseudomonads known to produce the antibiotic 2,4-diacetylphloroglucinol (DAPG) may also induce host defenses (Iavicoli et al., 2003). Hence, the plants treated with TC1 formulation under pot culture experiments were also analyzed for plant defence enzymes at different time intervals. There will be increasing trend in accumulation of biochemical defense mechanisms in rice plants observed after spraying with TC1 formulations. Most of the defense enzymes were synthesized maximum upto 72 h after inoculation with *Xoo* after which tends to decline. The increase in catalse activity was found to be low compared to other defense enzymes like PO, PAL and PPO.

The enzyme activities were constantly measured in rice plants on 0, 24, 48, 72, 96 and 120 h after challenge inoculation with *Xoo*. The enzyme activities were found to be higher in treatments which received various concentrations of TC1 formulation compared to Kocide101 (0.2%), Streptocycline (100 ppm) and inoculated control. Increased peroxidase activity was observed in rice plants sprayed with TC1 formulation followed by challenge inoculation of *Xoo*. Significant induction was detected at 24 h after pathogen inoculation and the trend increased upto 72 h. Plants sprayed with Kocide101 and Streptocycline also showed increased peroxidase activity compared to inoculated control plants (Table 4). Similar kinds of results were obtained for PPO activity which increased upto 72 h and gradually reduced there after (Table 5). Maximum PPO activity was observed in plants which received 1% TC1 formulation. The untreated control recorded the lowest PPO activity compared to all other treatments.

Induction of systemic resistance as evident from the increased accumulation of pathogenesis related (PR) proteins and other defense related compounds was observed in rice plants following application of leaf extract of *Datura metel* and challenge inoculation with either *R. solani* or *Xoo* (Kagale et al., 2004). In resistant cultivars, the defense response is characterized by an increase in peroxidase activities, the deposition of lignin into the plant cell wall, host cell death, and limitation of bacterial multiplication (Reimers et al., 1992).

The induction of PAL activity was higher in the rice plants sprayed with TC1 formulation followed by challenge inoculation of *Xoo*, when compared to the plants sprayed with Kocide101 and Streptocycline. It was observed that application of TC1 formulation enhanced the PAL activity and reached the maximum at 72 h. The initial PAL activity of 16.27 increased to 34.46 for the treatment T_6 which received 1% spray of TC1 formulation (Table 6).

The increased levels of catalase activity was observed at 24 h after treatment and the maximum activity at 72 h (Table 7). Whereas increased levels of SOD activity was observed upto 48 h. Further the enzyme activity was declined but remained at higher level when compared to control plants (Table 8).

The level of phenolic content remained higher in the rice plants sprayed with TC1 formulation and the maximum accumulation of phenols was observed at 96 h after treatment. The phenol content of 2.015 mg of catechol g^{-1} was comparatively higher in 1.0% TC1 formulation than the control (Table 9). The

Table 4: Changes in the peroxidase activity of rice leaves in response to treatment with TC1 formulation

Treatment	Change in absorbance min^{-1} g^{-1} of fresh tissue at 420 nm					
	Time interval (h)					
	0	24	48	72	96	120
T_1: Control	2.157±(0.30)	2.316±(0.23)	2.472±(0.17)	2.796±(0.27)	2.534±(0.13)	2.341±(0.14)
T_2: 0.2% TC1 formulation	2.063±(0.14)	2.425±(0.35)	2.860±(0.07)	3.106±(0.24)	2.871±(0.31)	2.540±(0.36)
T_3: 0.4% TC1 formulation	2.175±(0.24)	2.584±(0.26)	2.927±(0.41)	3.241±(0.14)	3.021±(0.24)	2.876±(0.19)
T_4: 0.6% TC1 formulation	2.087±(0.29)	2.617±(0.24)	2.984±(0.20)	3.276±(0.19)	3.125±(0.51)	2.907±(0.29)
T_5: 0.8% TC1 formulation	2.158±(0.09)	2.745±(0.10)	3.181±(0.32)	3.436±(0.21)	3.276±(0.11)	2.983±(0.44)
T_6: 1.0% TC1 formulation	2.115±(0.20)	2.781±(0.18)	3.240±(0.22)	3.574±(0.23)	3.408±(0.25)	3.126±(0.51)
T_7: Streptocycline (100 ppm)	2.038±(0.28)	2.497±(0.36)	2.914±(0.08)	3.265±(0.40)	2.911±(0.18)	2.827±(0.28)
T_8: Kocide101 (0.2%)	2.136±(0.19)	2.427±(0.29)	2.892±(0.31)	3.153±(0.21)	2.886±(0.43)	2.644±(0.22)
SEd	0.082	0.146	0.117	0.087	0.131	0.119
CD (*p*=0.05)	0.176	0.312	0.252	0.188	0.281	0.255

Values are mean±SD

Table 5: Changes in the polyphenol oxidase activity of rice leaves

Treatment	Change in absorbance min^{-1} g^{-1} of fresh tissue at 495 nm					
	Time interval (h)					
	0	24	48	72	96	120
T_1: Control	1.197±(0.24)	1.382±(0.37)	1.476±(0.36)	1.591±(0.25)	1.372±(0.41)	1.184±(0.13)
T_2: 0.2% TC1 formulation	1.248±(0.13)	1.427±(0.15)	1.623±(0.28)	1.855±(0.17)	1.632±(0.27)	1.414±(0.42)
T_3: 0.4% TC1 formulation	1.138±(0.35)	1.398±(0.23)	1.684±(0.09)	1.918±(0.10)	1.706±(0.18)	1.447±(0.21)
T_4: 0.6% TC1 formulation	1.265±(0.26)	1.546±(0.27)	1.729±(0.13)	1.982±(0.28)	1.823±(0.43)	1.615±(0.08)
T_5: 0.8% TC1 formulation	1.208±(0.08)	1.571±(0.09)	1.786±(0.23)	2.048±(0.29)	1.883±(0.12)	1.637±(0.28)
T_6: 1.0% TC1 formulation	1.146±(0.11)	1.492±(0.19)	1.806±(0.19)	2.131±(0.18)	1.947±(0.22)	1.685±(0.18)
T_7: Streptocycline (100 ppm)	1.256±(0.50)	1.512±(0.21)	1.638±(0.08)	1.892±(0.27)	1.726±(0.27)	1.513±(0.19)
T_8: Kocide101 (0.2%)	1.142±(0.29)	1.473±(0.15)	1.595±(0.22)	1.817±(0.36)	1.649±(0.18)	1.432±(0.11)
SEd	0.134	0.109	0.068	0.129	0.126	0.098
CD (*p*=0.05)	0.287	0.235	0.147	0.277	0.270	0.211

Values are mean±SD

Table 6: Changes in the phenylalanine ammonia lyase activity of rice leaves

Treatment	µmol of trans-cinnamic acid min^{-1} g^{-1} leaf tissue at 290 nm					
	Time interval (h)					
	0	24	48	72	96	120
T_1: Control	16.12±(0.11)	19.25±(0.15)	22.23±(0.57)	25.43±(0.19)	24.54±(0.20)	21.16±(0.21)
T_2: 0.2% TC1 formulation	16.04±(0.38)	20.17±(0.37)	26.25±(0.31)	32.14±(0.26)	31.71±(0.11)	28.47±(0.36)
T_3: 0.4% TC1 formulation	16.18±(0.15)	20.95±(0.13)	26.87±(0.11)	33.30±(0.06)	32.12±(0.08)	29.74±(0.20)
T_4: 0.6% TC1 formulation	16.10±(0.28)	21.96±(0.10)	26.46±(0.15)	33.71±(0.28)	32.68±(0.35)	31.12±(0.44)
T_5: 0.8% TC1 formulation	16.19±(0.48)	22.41±(0.36)	26.95±(0.40)	34.14±(0.37)	33.21±(0.38)	31.48±(0.61)
T_6: 1.0% TC1 formulation	16.27±(0.34)	22.14±(0.24)	27.05±(0.55)	34.46±(0.49)	33.85±(0.23)	31.91±(0.37)
T_7: Streptocycline (100 ppm)	16.14±(0.29)	20.03±(0.20)	24.44±(0.38)	30.27±(0.23)	28.64±(0.33)	25.02±(0.57)
T_8: Kocide101 (0.2%)	16.08±(0.51)	21.11±(0.29)	23.94±(0.46)	29.24±(0.12)	26.44±(0.18)	24.92±(0.29)
SEd	0.125	0.128	0.185	0.144	0.104	0.167
CD (*p*=0.05)	0.268	0.275	0.397	0.310	0.224	0.359

Values are mean±SD

Table 7: Changes in the catalase activity of rice leaves

Treatment	µmol of H_2O_2 consumed min^{-1} g^{-1} of fresh tissue					
	Time interval (h)					
	0	24	48	72	96	120
T_1: Control	0.78±(0.07)	0.96±(0.04)	1.29±(0.05)	1.67±(0.04)	1.61±(0.05)	1.53±(0.02)
T_2: 0.2% TC1 formulation	0.69±(0.02)	1.10±(0.09)	1.58±(0.04)	1.84±(0.01)	1.77±(0.03)	1.68±(0.09)
T_3: 0.4% TC1 formulation	0.71±(0.02)	1.22±(0.11)	1.80±(0.02)	2.04±(0.08)	1.95±(0.11)	1.81±(0.06)
T_4: 0.6% TC1 formulation	0.70±(0.06)	1.27±(0.04)	1.91±(0.05)	2.33±(0.06)	2.21±(0.08)	2.06±(0.06)
T_5: 0.8% TC1 formulation	0.81±(0.04)	1.33±(0.05)	2.05±(0.01)	2.48±(0.03)	2.39±(0.01)	2.21±(0.02)
T_6: 1.0% TC1 formulation	0.82±(0.07)	1.45±(0.04)	2.18±(0.04)	2.56±(0.04)	2.41±(0.05)	2.28±(0.04)

Table 7: Continue...

Treatment	µmol of H_2O_2 consumed min^{-1} g^{-1} of fresh tissue					
	Time interval (h)					
	0	24	48	72	96	120
T_7: Streptocycline (100 ppm)	0.74±(0.06)	1.26±(0.08)	1.84±(0.08)	2.13±(0.01)	2.07±(0.03)	1.85±(0.01)
T_8: Kocide101 (0.2%)	0.76±(0.04)	1.18±(0.02)	1.73±(0.05)	1.98±(0.03)	1.90±(0.02)	1.79±(0.05)
SEd	0.021	0.035	0.024	0.023	0.030	0.024
CD (*p*=0.05)	0.046	0.076	0.051	0.050	0.065	0.052

Values are mean±SD

Table 8: Changes in the superoxide dismutase activity of rice leaves

Treatment	units min^{-1} g^{-1} of fresh tissue					
	Time interval (h)					
	0	24	48	72	96	120
T_1: Control	2.03±(0.02)	4.93±(0.03)	8.25±(0.05)	7.15±(0.07)	6.80±(0.04)	6.17±(0.02)
T_2: 0.2% TC1 formulation	2.17±(0.02)	5.86±(0.05)	9.14±(0.01)	8.52±(0.03)	7.15±(0.08)	6.42±(0.07)
T_3: 0.4% TC1 formulation	2.32±(0.03)	5.47±(0.03)	10.29±(0.03)	9.25±(0.02)	7.82±(0.05)	6.78±(0.04)
T_4: 0.6% TC1 formulation	2.21±(0.01)	5.22±(0.02)	11.28±(0.04)	9.03±(0.03)	8.23±(0.01)	6.95±(0.11)
T_5: 0.8% TC1 formulation	2.08±(0.01)	5.78±(0.02)	10.62±(0.07)	10.38±(0.05)	8.47±(0.03)	7.36±(0.06)
T_6: 1.0% TC1 formulation	2.13±(0.04)	6.17±(0.04)	11.77±(0.04)	10.68±(0.09)	8.98±(0.02)	7.42±(0.04)
T_7: Streptocycline (100 ppm)	2.25±(0.01)	4.61±(0.01)	9.81±(0.10)	8.82±(0.01)	7.53±(0.03)	6.84±(0.03)
T_8: Kocide101 (0.2%)	2.34±(0.03)	5.10±(0.04)	9.44±(0.04)	8.11±(0.02)	7.39±(0.04)	6.58±(0.02)
SEd	0.012	0.013	0.031	0.028	0.020	0.029
CD (*p*=0.05)	0.027	0.029	0.068	0.061	0.043	0.063

Values are mean±SD

Table 9: Changes in the phenolic content of rice leaves

Treatment	mg of catechol g^{-1} fresh tissue at 650 nm					
	Time interval (h)					
	0	24	48	72	96	120
T_1: Control	0.623±(0.37)	0.744±(0.37)	0.878±(0.36)	1.036±(0.22)	1.384±(0.27)	1.275±(0.38)
T_2: 0.2% TC1 formulation	0.644±(0.16)	0.857±(0.14)	1.074±(0.51)	1.362±(0.11)	1.615±(0.39)	1.508±(0.54)
T_3: 0.4% TC1 formulation	0.642±(0.21)	0.923±(0.28)	1.128±(0.28)	1.446±(0.31)	1.781±(0.10)	1.627±(0.68)
T_4: 0.6% TC1 formulation	0.708±(0.28)	0.978±(0.20)	1.257±(0.13)	1.523±(0.61)	1.832±(0.26)	1.708±(0.32)
T_5: 0.8% TC1 formulation	0.658±(0.30)	0.934±(0.08)	1.289±(0.35)	1.603±(0.29)	1.917±(0.08)	1.751±(0.59)
T_6: 1.0% TC1 formulation	0.691±(0.17)	0.969±(0.37)	1.343±(0.67)	1.772±(0.77)	2.015±(0.42)	1.832±(0.34)
T_7: Streptocycline (100 ppm)	0.632±(0.43)	0.942±(0.17)	1.191±(0.28)	1.506±(0.18)	1.783±(0.38)	1.692±(0.20)
T_8: Kocide101 (0.2%)	0.679±(0.29)	0.898±(0.28)	1.187±(0.26)	1.478±(0.26)	1.775±(0.25)	1.623±(0.45)
SEd	0.133	0.103	0.165	0.215	0.129	0.182
CD (*p*=0.05)	0.286	0.221	0.354	0.461	0.278	0.390

Values are mean±SD

Streptocycline (100 ppm) and Kocide101 (0.2%) treated plants recorded the phenol content of 1.783 mg of catechol g^{-1} and 1.775 mg of catechol g^{-1} on 96 h after treatment.

Physiological observation of *Adathoda vasica* treated rice plants indicated that restriction of *Xoo* colonization was correlated with the pronounced increase of peroxidase, PAL,

β-1, 3-glucanase, polyphenol oxidase and phenol activity after challenge inoculation with the target pathogen. The enzyme activities were found to be increase upto 72 hrs after that the production tends to decline (Govindappa et al., 2011)

4. Conclusion

The water-soluble starch-based formulation using *Streptomyces sp. TC1* spores and mycelia shows potential as a biocontrol agent against *Xanthomonas oryzae* pv. *oryzae*. It reduces seed infection and enhances germination by 78.7%. Spraying the formulation boosts plant defense chemicals, including PO, PPO, PAL, SOD, and phenols, strengthening rice resistance. The 0.4% concentration is most effective, reducing disease incidence by 56.6%. These results highlight *Streptomyces sp.* TC1 as a sustainable, eco-friendly solution for managing bacterial leaf blight in rice.

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