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Antagonistic Yeast-based Biocontrol against *Penicillium* sp. Spoilage and Prolonging the Shelf Life of Tomatoes (*Solanum lycopersicum*)

R.A.O.P. Ranasinghe, S.N. Prematilake *

Department of Science and Technology, Uva Wellassa University, Badulla, Sri Lanka, 90000

*** Corresponding author email address**: saranga@uwu.ac.lk

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Abstract

The tomato (*Solanum lycopersicum*) is the second most extensively cultivated crop globally. However, an estimated 25% to 42% of the worldwide tomato yield is lost during the post-harvest stage, primarily due to various factors, including pathogen infections. Especially, *Penicillium* species are a significant cause of post-harvest losses, particularly under refrigerated storage conditions. Antagonistic yeasts have shown promising potential in controlling fungal pathogens. Therefore, this study focuses on isolating yeasts and evaluating their antagonism against *Penicillium* sp. to enhance the shelf life of tomatoes. Four yeast strains were isolated from soil and citrus leaves (YS004, YCL001, YCL002, YCL004),and *Penicillium* sp. was isolated from infected tomatoes. A dual culture assay was conducted to evaluate the antagonistic activities of each yeast strain against *Penicillium* sp. Three yeast strains had shown significant antagonistic activity against *Penicillium* sp. (one-way ANOVA, $p < 0.05$), yet only YS004 (68.27 ± 0.33) exceeded the 55% of percent inhibition of radial growth (PIRG). In the *in vivo* assay, tomatoes treated with YS004 (4.04×10^{-5} cells mL^{-1}) exhibited the lowest disease severity percentage of 45 ± 2.89 and the lowest disease incidence of 43.33 ± 5.77%. The tomatoes treated with YS004 achieved a shelf life of 19 days compared to 12 days for the control samples. Also, tomatoes treated with YS004 showed statistically significant retention of moisture content ($p = 0.0000$; $p < 0.05$) and reduced weight loss ($p = 0.0000$; $p < 0.05$). A sensory evaluation was done using 30 untrained panellists, and the results indicated that YS004 enhanced the overall acceptability of tomatoes. Therefore, applying the YS004 yeast solution is a promising and effective bio-control agent that can extend the shelf life of tomatoes by controlling the Penicillium pathogen in refrigerated conditions.

Keywords: Antagonistic yeast, *Penicillium* spp., Dual culture assay, Shelf life, *In vivo* assay

1. Introduction

Tomato (*Solanum lycopersicum*) is a member of the family Solanaceae, which is an edible berry-like fruit that is known for its rich nutritional profile, including antioxidants, carotenes, ascorbic acids, phenolic compounds, minerals, and fibre [1,2,3]. Tomatoes are widely recognized for their health benefits, such as anticancer properties, reduced cardiovascular disorders, and anti-inflammatory properties [4-8]. Furthermore, they significantly enhance a meal's nutritional and texture profile [4,5]. According to FAOSTAT (Food and Agriculture Organization of the United Nations), global tomato production reaches approximately 182.3 million tons annually, cultivated across 4.85 million hectares, and tomatoes are the second most extensively grown crop worldwide [1]. Asia contributes significantly to this output, accounting for 61.1% of global tomato production [4]. However, 25%-42% of global tomato yield is during post-harvesting [9].

Even in Sri Lanka, 20 - 40% of the total harvest of vegetables is lost during the post-harvest stage, with tomatoes being among the most vulnerable [10]. Freshly harvested delicate vegetables like tomatoes, which have high moisture content, are highly susceptible to mechanical damage, pathogen attacks, and chemical interactions [10]. As a result, these factors reduce the quality, shelf life, and consumer acceptance of fresh tomatoes, ultimately leading to a loss of economic gains [10,11]. According to previous studies, poor handling, technical issues, poor transportation, improper storage conditions, pest attacks, and pathogen infections are common post-harvest challenges [11-13].

Out of all the diseases affecting tomatoes, fungal diseases are the most prevalent, significantly compromising each commodity's quality, safety, and shelf life [12]. Among the fungal pathogens, *Penicillium* spp. are the most common causative agents of blue mould and green mould diseases in tomatoes [13]. Common *Penicillium* spp. such as *P. expansum*, *P. digitatum*, and *P. italicum* can be found in soil and plants, classifying them as phytopathogens [13-15]. *Penicillium* can contaminate each commodity through different pathways, including airborne spores, contact with contaminated surfaces (shelves, tools), cross-contamination, and infection through soil or organic matter [16]. Infected tomatoes exhibit several distinctive symptoms, such as powdery blue-green mould, softening of the tissues, wrinkling, discolouration and the presence of white mycelium [17,18]. *Penicillium* spp. require specific

environmental conditions to thrive in fruits. Usually, fungi prefer moderate temperatures (15 °C to 25 °C), yet some species can survive and proliferate in refrigerated conditions [19]. Moreover, they can persist on both moist & dry surfaces and require certain nutrients to survive. Tomatoes with high moisture content, rich nutrient profile, and delicate surfaces provide an ideal substrate for *Penicillium* spp. to thrive [21]. There are several strategies to combat these challenges, including physical, chemical, and biological methods [9,12].

Recently scientists have become more interested in biocontrol methods due to their demonstrated efficacy at laboratory and industrial scales. Bacteria (*Bacillus thuringiesnsis*, *Pseudomonas fluorescens*), fungi (*Trichoderma* spp., *Metarhizium anisopliae*), nematodes (*Heterohabditis* spp.), mites, and viruses are successfully used as biocontrol method in industrial scale [12,14,22]. However, yeasts have emerged its popularity as a biocontrol agent over bacteria and moulds due to their advantages [12,22]. They are more genetically stable, and they adapt to extreme external (temperature fluctuation, relative humidity fluctuation, wide pH range, low oxygen level) and internal (high sugar level, low pH) environments, making them effective in different food commodities [12,14,22].

Despite all the research done to manage different fungal diseases, no proper investigation has been conducted to find an antagonistic yeast against the *Penicillium* pathogen of tomatoes. Therefore, this study focuses on isolating antagonistic yeast as a biocontrol agent against *Penicillium* sp. to enhance the shelf life of tomatoes, evaluating the antagonism using the dual culture method, and determining the efficacy of the isolated yeast strains in real-time application.

2. Material and Methods

2.1 Isolation of antagonistic yeast

Twenty-five samples (ten soil samples, five tomato leaf samples, four coconut water samples, and six samples of leaves of citrus plants) were collected randomly from various areas in Badulla district in Sri Lanka (6.9934° N, 81.0550° E). Samples were taken using a sterile spatula and forceps, in sterile plastic bags and bottles. Sample suspensions were prepared by adding each sample (10.00 g) to distilled water (100.00 mL), mixed well, and vortexed. Each suspension was serially diluted from 10^{-1} to 10^{-6} [23]. Each diluted sample (100 µL) was pipetted onto plates with potato dextrose agar medium (PDA). The diluted samples were spread evenly on the agar surface using a sterile glass spreader [23]. The culture plates were incubated at 26 ± 2°C for 48 h - 72 h. Once colonies were visible, colonies with different morphologies were selected [24,25]. Each selected yeast colony was re-cultured on new PDA plates at 26 ± 2°C for 48 h - 72 h to obtain pure cultures [23].

2.2 Isolation of *Penicillium* from the infected tomato sample

Healthy mature tomatoes were collected from a local market in Badulla town in Sri Lanka (6.9934° N, 81.0550° E). Tomatoes were washed with running tap water to remove any visible dirt. For surface sterilization, tomatoes were dipped in 1% - 2% sodium hypochlorite for 30 seconds. The tomatoes were rinsed thrice with sterile water to remove any residue from the disinfectant [23]. Cleaned tomatoes were stored under refrigeration for a prolonged period until they spoiled. Small pieces from the infected areas of the tomato were cut using a sterile scalpel blade. The tomato pieces were placed onto the surface of a PDA plate [23]. The agar plates were incubated at 26 ± 2°C for 48 h – 72 h. After the fungi started to grow on the agar plates, under the sterile condition, a *Penicillium* sp. colony was transferred to a new agar plate to obtain a pure culture. The isolated *Penicillium* sp. was identified by their characteristics through morphological examination using the light microscope [18].

2.3 Dual plate assay

Preliminary *in vitro* screening was conducted for all the isolated yeasts to evaluate their antagonistic activities against isolated *Penicillium* sp., according to Hassan *et al*. (2021) and Al-Maawali *et al*. (2020), with minor modifications. A six-millimeter mycelial disc was taken from a seven-day-old isolated *Penicillium* sp. culture and placed at 1cm from the edge of the petri dish. Two-day-old yeast culture was streaked on the same media, 1cm from the plate edge (opposite end). A culture plate with *Penicillium* sp. but without the yeast served as the control, while another control was prepared with only yeast. and it was served as the control. Triplicates were done for each treatment [23,26]. The plates were incubated at room temperature (28 ± 2 °C) for 10 -12 days or until complete mycelial growth was achieved in the control plate. Percent inhibition of radial growth (PIRG) was calculated using the following formula;

$$PIRG = \frac{(R_1 - R_2)}{R_1} \times 100\% \quad (1)$$

Where R_1 = Radial growth of *Penicillium* sp. in the control plate; and R_2 = Radial growth of *Penicillium* sp. cultivated with potential antagonistic yeast. Only isolates with PIRG > 55% were selected for further experiments.

2.4 Determination of the yeast cell concentration for effective control of *Penicillium* sp.

The diluted cell suspension was prepared using a sterilized inoculation loop [27]. A small amount of yeast colonies was transferred aseptically from the PDA plate into a sterile saline solution (10 mL) and mixed well to make a homogenized solution [27,28]. The known volume of yeast suspension was loaded into one of the counting chamber's wells in the hemocytometer [29]. After that, the hemocytometer was placed on the microscope stage and focused on the grid lines under low magnification. The yeast cells were counted within a defined grid area [30]. The counting process was repeated in multiple areas to ensure the accuracy of the hemocytometer. The concentration of yeast cells was calculated using the following formula [30].

Concentration of the yeast cells =

$$\left(\frac{Average\ cell\ count \times Dilution\ factor}{Number\ of\ squares\ counted \times Volume}\right) \quad (2)$$

2.5 *In vivo* assay

This experiment was done according to Hassan *et al*., 2021 with minor modifications. Healthy mature tomatoes (0.035-0.055 kg) were collected from a local market in Badulla town in Sri Lanka. Tomatoes were washed with running tap water to remove any visible dirt. For the surface sterilization, tomatoes were dipped in 1% - 2% sodium hypochlorite for 30 seconds. The tomatoes were rinsed 3 times with sterile water to remove any residue from the disinfectant. Washed tomatoes were air-dried before being sterilized using 70 %(v/v) ethanol [23]. For each tomato, a wound was formed using a sterile glass rod. Antagonistic yeast suspension (10 mL), selected in section 2.4., was taken to a test tube as a reference. The reference solution was serially diluted to obtain a dilution series at 10^{-2} and 10^{-4}. Each suspension was applied to the wounds. *Penicillium* sp. was inoculated onto the same wound using a sterile inoculation loop. For the control treatment, only *Penicillium* sp. was inoculated. All the tomatoes were stored in separate sterile polythene bags at refrigeration conditions (4 ± 2 °C). After 15 days of incubation, the diameter of the lesions formed on the fruits was measured to assess disease development. Finally, the average lesion expansion (cm day^{-1}) was calculated using the following formula;

$$\frac{\left(\begin{matrix}Lesion\ diameter\\ in\ final\ day\end{matrix}\right)-\left(\begin{matrix}Lesion\ diameter\\ on\ day\ 0\end{matrix}\right)}{\sum storage\ days} \quad (3)$$

Disease reduction over control percentage (%) was calculated using the following formula;

$$\frac{\left(\begin{matrix}Lesion\ diameter\\ of\ control\end{matrix}\right)-\left(\begin{matrix}Lesion\ diameter\\ of\ treated\ tomato\end{matrix}\right)}{Lesion\ diamter\ of\ control} \quad (4)$$

Three replicates were conducted for each treatment [30].

2.6 Physical and chemical analysis

The yeast cell suspension was diluted to the selected concentration (section 2.5) using sterile water. The cell suspension was stirred for five minutes for a homogeneous solution [31]. The diluted yeast cell suspension was filtered through a sterile 0.22 µm filter to remove any potential microbial contaminants. The spray was transferred into a sterilized bottle.

2.6.1 Shelf life

Randomly selected healthy matured tomatoes (same size range, colour and shape) were used for the analysis. Ten tomatoes were treated with the yeast spray, and ten were used as the control. Subsequently, all the tomatoes were refrigerated at a controlled temperature of 4 ± 2 °C until signs of spoil manifested [32].

2.6.2 Decay%

This analysis was conducted according to Hassan *et al.* (2021). Randomly selected healthy matured tomatoes (same size range, colour and shape) were used for the analysis. Ten tomatoes were sprayed with the prepared yeast spray, and another ten were used as the control. Subsequently, all the tomatoes were refrigerated at a controlled temperature of 4 ± 2 °C until signs of spoil manifest. After 12 days of storage, fungal disease signs and their severity on the tomato surface were observed. Disease incidence was calculated using the following formula [23];

$$Disease\ incidence\ = \frac{(No.of\ infected\ tomatoes)}{Total\ no.of\ tomatoes} \times 100\% \quad (5)$$

Disease severity was calculated using the following formula, and a scale (0 - 4) was used as the index of the formula (0 = no symptoms of an infection; 1 = 11-20% of the surface of tomatoes were infected; 3 = 21- 30% of the surface of tomatoes were infected; 4 = 31% and above surface area of tomatoes were infected).

Disease Severity% =

$$\frac{\sum(Severity\ rating \times No.of\ fruit\ with\ that\ fruit\ rating)}{Total\ no.of\ tomatoes\ assesed \times highest\ scale} \times 100\% \quad (6)$$

2.6.3 Physiological weight loss

According to Dhami *et al.* (2023), randomly selected healthy mature tomatoes (Same size range, colour, and shape) were used for the analysis and weighed using an electronic balance. Ten tomatoes were sprayed with the selected yeast spray, and another ten were sprayed with sterile distilled water to refer to them as the control. All the tomatoes were kept in the refrigerator under 4 ± 2 °C. The weight of tomatoes in the refrigeration condition was measured consecutively within three days' intervals from the purchase day to 15 days [32]. Weight loss was calculated using the following formula;

$$\text{Weight Loss\%} = \frac{Initial\ Weight - Final\ Weight}{Initial\ Weight} \times 100\% \quad (7)$$

2.6.4 pH

The analysis was done according to Sualeh *et al.* (2016). Randomly selected healthy matured tomatoes (same size range, colour and shape) were used for the analysis. All the tomatoes were washed with tap water and air-dried before being used for further analysis. 30 tomatoes were sprayed with the yeast solution, while another 30 were used as the control (sprayed distilled water). They were stored in the refrigerator (4 ± 2 °C), and pH was measured consecutively within three days' intervals from the purchase day to 15 days

[33]. For that, tomatoes were blended using a food processor, and puree was filtered through a cotton cloth. A benchtop pH meter (TRANS, BP3001, Singapore) as used to measure the pH of each sample.

2.6.5 Titratable acidity (TA)

The analysis was done according to Sualeh *et al.* (2016). Randomly selected healthy matured tomatoes (same size range, colour and shape) were used for the analysis. All the tomatoes were washed with tap water and air-dried before being used for further analysis. 30 tomatoes were sprayed with the yeast solution, while another 30 were used as the control (sprayed distilled water). They were stored in the refrigerator (4 ± 2 °C), and TA was measured consecutively within three-day intervals from the purchase day to 15 days. Tomatoes were blended using a food processor, and puree was filtered through a cotton cloth [33]. As the indicator, three drops of Phenolphthalein were added to the 2 mL of the filtrate, and it was titrated with 0.1 N NaOH solution until a light pink colour was observed. The titratable acidity was calculated using the following formula.

$$\text{TA\%} = \frac{[Volume\ of\ NaoH(mL)] \times [0.1\ N\ NaOH] \times [0.0064]}{Volume\ of\ the\ sample(mL)} \times 100\% \quad (8)$$

2.6.6 Moisture content

This analysis was carried out following Dhami *et al.* (2023). Randomly selected healthy matured tomatoes (same size range, colour and shape) were used for the analysis. All the tomatoes were washed with tap water and air-dried before being used for further analysis. 30 tomatoes were sprayed with the yeast solution, while another 30 were used as the control (sprayed with distilled water). They were stored in the refrigerator (4 ± 2 °C), and moisture content was measured consecutively within three-day intervals from the purchase day to 15 days. After that, tomato samples (2.00 g) were dried at 105 °C for 24 hours and cooled in a desiccator. The weight was measured using an analytical balance (KERN & SOHN, ABJ 120 - 4NM, Germany). The moisture content (percentage of dry weight) was calculated using the following formula [32].

$$\frac{(Weight\ loss\ of\ the\ sample)}{Initial\ weight\ of\ the\ sample} \times 100\% \quad (9)$$

2.6.7 Total soluble solid (TSS)

The analysis was done according to Sualeh *et al.* (2016). Randomly selected healthy matured tomatoes (same size range, colour and shape) were used for the analysis. All the tomatoes were washed with tap water and air-dried before being used for further analysis. 30 tomatoes were sprayed with the yeast solution, while another 30 were used as the control (sprayed distilled water). They were stored in the refrigerator (4 ± 2 °C), and TSS was measured consecutively within three-day intervals from the purchase day to 15 days. For that, tomatoes were blended using a food processor, and puree was filtered through a cotton cloth [33]. The total soluble solid (brix value) was measured using a calibrated hand refractometer (Bellingham + Stanley, Opti refractometer, United Kingdom).

2.6.8 Sensory evaluation

A sensory evaluation was done using 30 random untrained individuals on the 7th day after treating the samples according to the research of Deltsidis, A.I. *et al.* (2018). The panel was given two types of samples including tomatoes treated with YS004 and control tomatoes that were sprayed with distilled water. They were stored in the refrigerator (4 ± 2 °C) for 7 days before serving. Each sample was labelled using a three-digit identification code (300,400). All the training panel was given pieces of diced tomatoes (from each type) and asked to evaluate using five descriptive attributes, including visual appearance, smell, flavour, texture, and overall acceptability. They were asked to rate using a Likert scale (5 = Excellent, 4 = Good, 3 = Acceptable, 2 = Poor, 1 = Very poor). The data were analyzed using the Friedman test with a significance level of 0.05 using the Minitab 17 version [34].

3. Results and Discussion

3.1 Isolation of antagonistic yeast

A total of 04 yeasts strains were isolated from a soil sample, three citrus leaves samples and they were named as YS004, YCL001, YCL002, and YCL004. Using the characteristic morphology yeast colonies were identified [25,35] (Fig. 1 & 2).

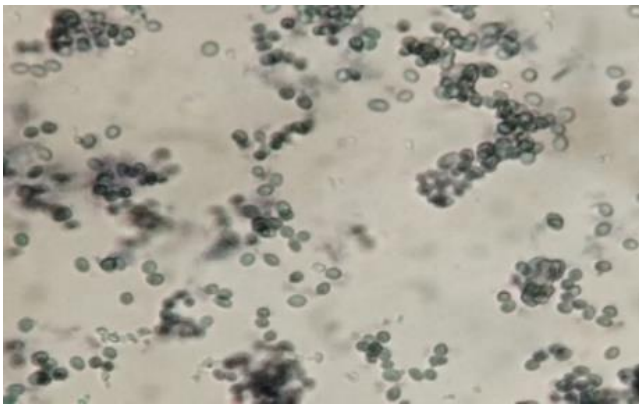


Fig. 1. Microscopic view of isolated YS004 yeast strain with the magnification × 40

Fig. 2. Isolated YS004 yeast strain cultures on PDA

3.2 Isolation of *Penicillium* from the tomato sample

Penicillium sp. was isolated from an infected tomato sample and identified by its characteristics through a morphological examination using a light microscope [18] (Fig. 3 & 4).

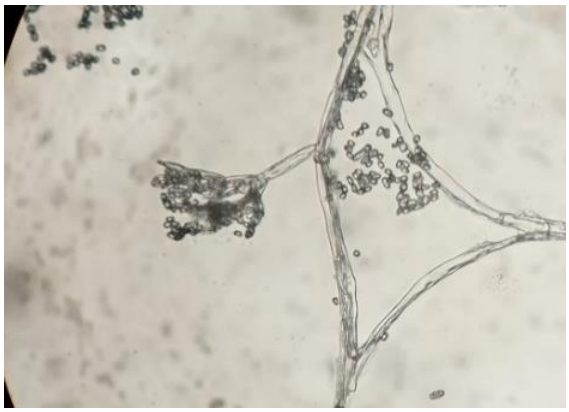


Fig. 3. Isolated *Penicillium* strain under the microscopic view with the magnification of × 40

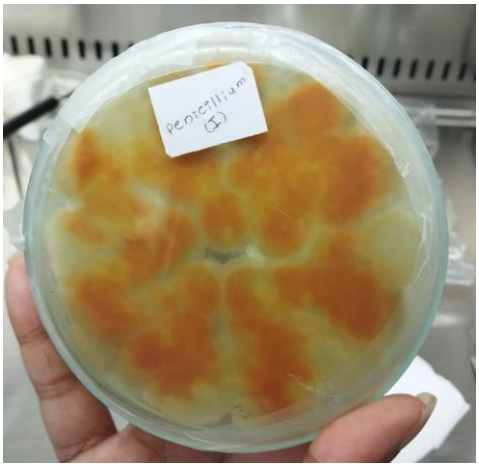


Fig. 4. Isolated *Penicillium* on inverted PDA plate

3.3 Dual plate assay

The dual culture screening method was employed to evaluate the antagonistic activities of isolated yeasts against *Penicillium* sp. pathogen [23,26]. Here ability of the yeasts to inhibit fungal growth was measured by co-culturing both strains in the same plate and calculating the PIRG values of each strain combination.

The results of One-Way ANOVA demonstrated a statistically significant difference in PRIG values across the tested yeast isolates ($p = 0.000$; $p < 0.05$). Out of the yeast strains tested, only three strains showed a positive inhibitory effect after 10 days of incubation (Fig.5). Yeast strain YS004 showed the highest antagonistic activity, achieving a PRIG value of 68.27 ± 0.33%, surpassing 55% of the PIRG value. In comparison, YCL001 and YCL004 showed PRIG values of 51.68 ± 0.37% and 25.10 ± 0.27%, respectively (Table 1). YCL002 showed no inhibitory activity against *Penicillium* sp. The inhibitory effects of these yeasts may be attributed to several antagonistic mechanisms, including competition for nutrients and space, production of inhibitory compounds (such as enzymes or volatile organic compounds), mycoparasitism, and more [36]. However, among the tested strains, YS004 showed the most potent inhibitory effect, indicating its suitability for further experimentation in controlling *Penicillium* sp. infections.

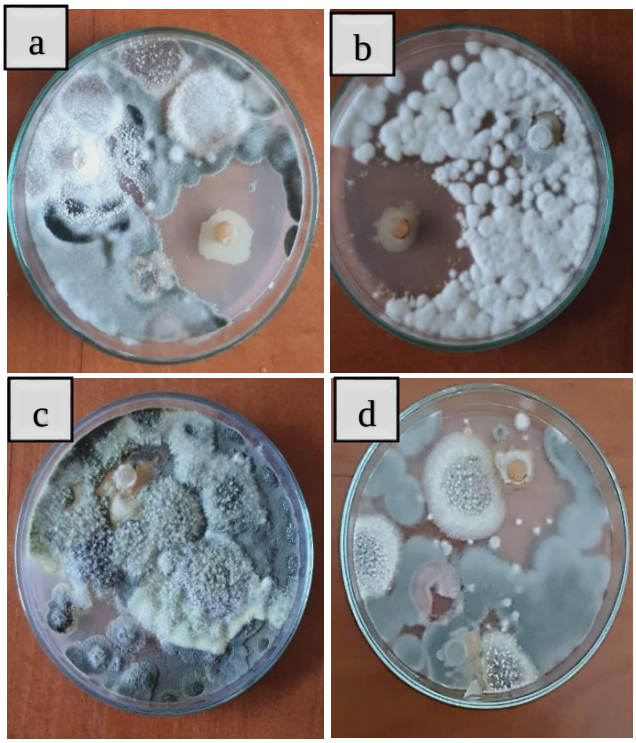


Fig. 5. *In vitro* inhibition of *Penicillium* sp. using (a) YS004, (b) YCL001, (c) YCL002, (d) YCL004

Table 1
Inhibitory effects of yeast isolates against *Penicillium* sp. using dual culture assay after ten days of incubation at 28 ± 2 °C.

Yeast isolates	Source	PIRG%
YS004	Soil	68.27 ± 0.33[a]
YCL001	Citrus leaves	51.68 ± 0.37[b]
YCL002	Citrus leaves	No inhibitory effect
YCL004	Citrus leaves	25.10 ± 0.27[d]

3.4 Determination of the optimal yeast cell concentration for effective control of *Penicillium* sp.

A 0.01 mL of YS004 yeast cell suspension was pipetted, and the slides were observed under the microscope with 10x magnification [27]. The concentration of the yeast cells was calculated as 4.04×10^5 cells $mL^{-1,}$ and this suspension was taken as the reference for the later part of the experiment.

3.5 In *vivo* assay

Healthy mature tomatoes in the same weight range, size, and colour were selected to minimize variability and bias. This study interprets that tomatoes treated with 4.04 × 105 cells mL^{-1} concentration of YS004 suspension had smaller fungal lesion diameters than the other samples. According to Table 2, isolate YS004 with 4.04×10^5 cells mL^{-1} concentration showed the highest fungal control efficacy with 0.65 ± 0.01 cm lesion diameter, 0.033 ± 0.0003 cm day^{-1} lesion expansion rate (%), and 43.97 ± 0.86 disease reduction rate (%) as compared to the others, proving it is the most effective concentration for pathogen inhibition in this case. Therefore, a homogenized yeast solution with 4.04×10^5 cells mL^{-1} was used for further analysis.

Table 2
Biocontrol effects of YS004 against *Penicillium* sp. using *in vivo* assay after 15 days of incubation at 4 ± 2 °C

Concentration of yeast suspension (cells mL^{-1})	Average lesion diameter (cm)	Lesion expansion rate %	Disease reduction rate (over control) %
4.04×10^1	1.07 ± 0.01[b]	0.054 ± 0.0003[b]	4.17 ± 1.03[a]
4.04×10^3	1.14 ± 0.02[a]	0.057 ± 0.0004[a]	2.01 ± 1.32[b]
4.04×10^5	0.65 ± 0.01[c]	0.033 ± 0.0003[c]	43.97 ± 0.86[c]
Control	1.16 ± 0.01[a]	0.058 ± 0.0002[a]	-

3.6 Physical and chemical analysis

3.6.1 Shelf life

Typically, tomatoes have a shelf life of around 7 – 12 days when stored in the refrigerator [11]. In this experiment, the shelf life of the tomatoes was considered as the period from the application of each treatment to the initiation of spoiling and became unacceptable for consumption [32]. The control samples showed the first signs of visual deterioration on the 12th day, characterized by softening and discolouration. In contrast, the tomatoes treated with YS004 showed visual deterioration only by the 19th day.

By the 20th day, the control samples were initiated spoiling by mould and reduced their consumer acceptability. The tomatoes sprayed with YS004 solution started spoiling by mould on the 35th day after spraying. At the onset of spoilage, there were clear signs of visual deterioration including softening the fruit, darkening to a brownish-dark red hue, and releasing an unpleasant odour. By the 40th day, all the tomatoes had completely rotten. The acceptable shelf life of tomatoes treated with yeast was 19 days compared to the 12 days for control samples. Therefore, it is evident that antagonistic yeast spray notably extended the overall shelf life of tomatoes, proving it is more effective.

3.6.2 Decay%

After 12 days, observations were recorded. The disease incidence of the treated tomatoes showed much difference compared to the control samples (Table 3). The analysis shows that yeast spray has a statistically significant effect on the disease incidence of tomatoes ($p = 0.000$; $p < 0.05$). Moreover, the disease severity of each group showed a statistically significant difference over the other as $p = 0.001$; $p < 0.05$. That means the disease severity percentage of tomatoes treated withYS004 yeast solution showed a lower rate of 45 ± 2.89 (%) than the control (75 ± 5.00 (%)). According to the result, it is evident that the YS004 solution not only prevented the disease incidence but also exhibited a significant reduction in the severity of the disease on tomatoes, proving it is more effective.

Table 3
Effects of treatments (sterile distilled water and YS004) on disease incidence and severity percentage of tomatoes stored at 4 ± 2 °C for 12 days.

Treatment	Disease incidence%	Disease severity%
Sterile distilled water	100 ± 0.00[a]	75 ± 5.00[a]
YS004 solution	43.33 ± 5.77[b]	45 ± 2.89[b]

3.6.3 Physiological weight loss

Over time, due to moisture loss, respiration, and ethylene production, the physiological weight of tomatoes tends to decrease [37]. This can lead to tissue shrinkage, softening and reducing overall acceptability. The analysis shows that the application of YS004 yeast suspension had a statistically significant effect on the weight loss of tomatoes ($p = 0.0000$;

p < 0.05). The control tomatoes sprayed with distilled water exhibited the highest average weight loss of 2.58 ± 0.02 %. The least weight loss of 1.73 ± 0.12% was observed from the tomatoes treated with YS004 yeast solution.

The trend in average weight loss was non-linear for both tomato samples. Yet, the rate of weight loss was faster in control samples than in the treated tomatoes. According to the results (Fig. 6), the average weight loss of both samples over the first six days was notably similar. However, after six days, the weight loss of control samples increased more rapidly while the treated samples continued to lose weight more gradually. Therefore, these results suggest that applying YS004 yeast solution effectively slows down the rate of weight loss in tomatoes, promising an effective bio-control agent to extend the shelf life of tomatoes.

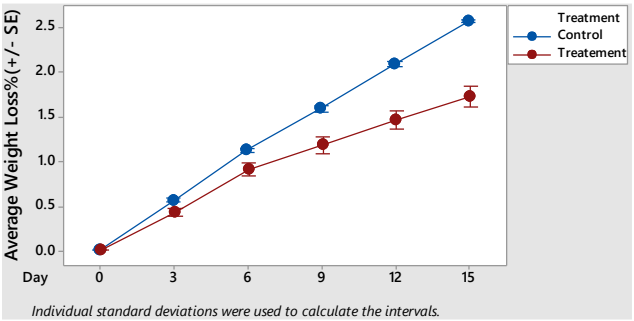


Fig. 6. Variation of the average weight loss of the tomatoes with storage days in the treated and control samples

3.6.4 pH

Previous studies have indicated that tomatoes increase their pH as they mature due to the consumption of organic acid for respiration [38]. However, the pH increase rate can vary depending on the treatment applied. From this analysis, it is evident that YS004 yeast spray had no significant effect on the pH of tomatoes (p = 0.102; p < 0.05; Friedman Test). In this case, the pH of the control and treatment samples were initially recorded as 4.10 and 4.12. The highest pH of the tomatoes was recorded as 4.37 and 4.20 for the control and treated samples, respectively. The trend of pH of tomatoes was not linear for both treated. However, the pH of the control samples increased more rapidly while the treated samples continued to increase the pH at a lower rate. Therefore, the yeast treatment did not markedly influence the pH of the tomatoes, as seen from the p-value of 0.102. However, it may still play a role in moderating the overall post-harvest quality of the tomatoes.

3.6.5 Titratable acidity

Titratable acidity (TA%) is a crucial parameter that decides the acceptability of tomatoes, as it directly influences their overall flavour and quality profile [38]. Previous studies indicate that the titratable acidity of the tomatoes decreases over time during the post-harvest period [38]. At the early stage of maturity, tomatoes have more organic acid, which is later consumed for respiration during maturity [39]. This leads to a decrease in the acidity of the tomatoes over time. This study measured titratable acidity

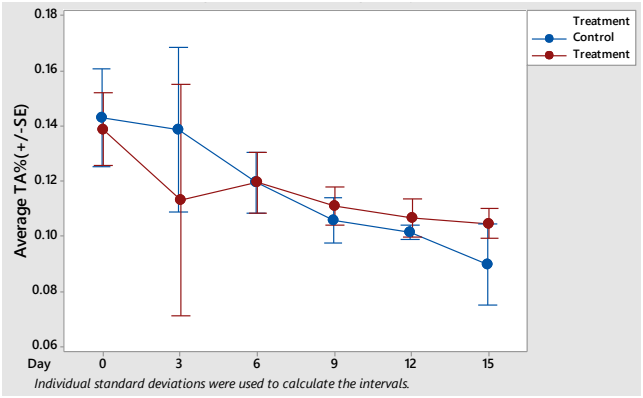
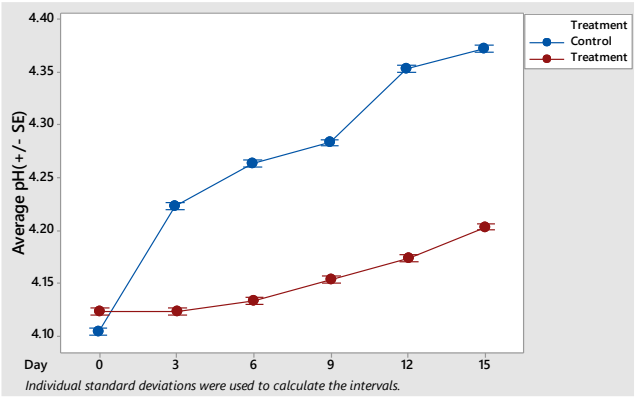


Fig. 7. Variation of average pH of the tomatoes with storage days in the treated and control samples

consecutively at three-day intervals after treating the YS004 yeast solution. This analysis shows that YS004 yeast spray had no significant effect on TA% (p = 0.860; p > 0.05).

The highest TA of 0.15 ± 0.02 was recorded for control tomatoes at the initial stage (day 3). The lowest TA of 0.09 ± 0.02 was recorded from the control samples, and at that time, treated tomatoes got 5.76% TA. The trend of the TA was not linear for both treated and control samples. However, the TA of the control samples decreased notably more rapidly while the treated samples continued to decrease the TA at a lower rate. The yeast treatment did not markedly influence the tomatoes' acidity, as seen from the p-value of 0.860, though it may still play a role in moderating the



overall post-harvest quality of the fruit.

Fig. 8. Variation of average TA of the tomatoes with storage days in the treated and control samples

3.6.6 Moisture content

Tomatoes usually have higher moisture content compared to other vegetables [32]. Therefore, it is important to have its moisture content in an acceptable range for human consumption. Previous studies indicated that the moisture content in tomatoes decreases over time, especially in refrigeration storage [32].

In this study, the moisture content of the tomatoes was measured consecutively at three-day intervals after treating with YS004 yeast solution. From this analysis, it is evident

that YS004 yeast spray has a significant effect on moisture content ($p = 0.000$; $p > 0.05$). As usual, the highest moisture content of 92.47 ± 0.06 % was recorded at the initial stage. The lowest moisture content of 90.10 ± 0.01 was recorded from the control samples after 15 days of storage. The lowest moisture content of YS004 yeast solution sprayed tomato was 90.85 ± 0.09 %. The trend of the moisture content of both samples was not linear. The moisture content (%) of tomatoes decreased over the storage period. Therefore, these results suggest that applying YS004 yeast solution effectively slows down the rate of TA% variability of tomatoes over time, promising an effective bio-control agent to extend the shelf life of tomatoes.

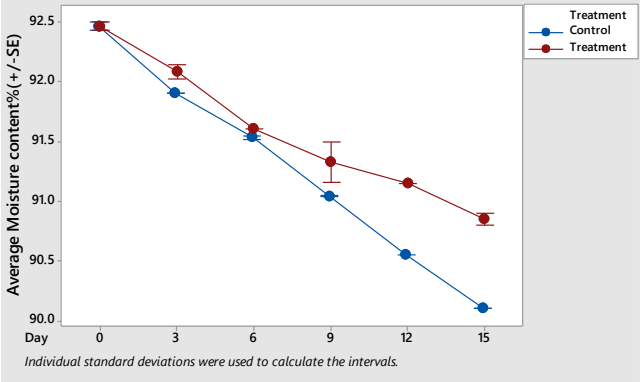


Fig. 9. Variation of average moisture content of the tomatoes with storage days in the treated and control samples

3.6.7 Total soluble solids (TSS)

The total soluble solids represent the concentration of dissolved solids like sugars in fruits, affecting their overall quality, including the sweetness/tartness of tomatoes [40]. In this study, the TSS of the tomatoes was measured consecutively at three-day intervals after applying YS004 yeast solution. This analysis shows that YS004 yeast spray had no significant effect on total soluble solids ($p = 0.742$; $p > 0.05$). On day 0, the highest brix value of 4.1 was observed, gradually decreasing in both samples. The lowest brix value was recorded as 3.7 from the control samples. The brix values were changed from 4.1 to 3.7 in control samples while 4.1 to 3.8 in treated samples within 15 days. The trend of the total soluble solid values of both samples were not linear. Therefore, this experiment showed that applying YS004 yeast spray did not significantly affect tomatoes' total soluble solid values over time.

3.6.8 Sensory evaluation

An important aspect of this study is the sensory evaluation, as the quality and consumer preference for fresh vegetables like tomatoes are critical factors [34]. The results showed a significant treatment effect on visual appearance, smell, flavour, texture, and overall acceptability ($p < 0.05$; Friedmann Test).

The diagram (Fig. 10) represents the sensory evaluation of tomatoes focusing on attributes like flavour, overall acceptability, appearance, texture, and smell. Each axis of the diagram corresponds to one of these attributes, and scores range from 1 (poor) to 5 (excellent). This comparison shows that the treated tomatoes outperform the control in all sensory attributes. The higher scores, especially in flavour and texture, suggest that the yeast-treated sample was more desirable. The larger area covered by the treated sample in the diagram indicates that it was the best option regarding overall sensory attributes.

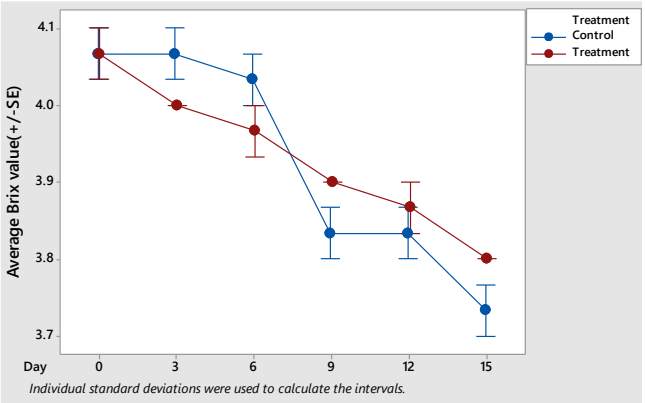


Fig. 10. Variation of average brix values of the tomatoes with storage days in the treated and control samples

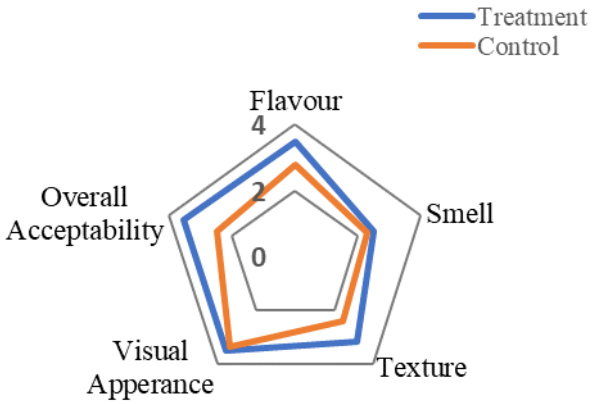


Fig. 11. Diagram indicating ratings of sensory parameters of tomatoes

4. Conclusion

Overall, the findings of this study suggest that applying YS004 yeast solution can be a promising effective bio-control method for extending the shelf life of tomato varieties in Sri Lanka by controlling *Penicillium* pathogen in refrigeration conditions. However, further study is needed to address the limitations of this experiment. Future studies should focus on the molecular biological identification of YS004 to confirm its taxonomy and assess its safety as a food-grade microorganism. Additionally, isolating and characterizing the bioactive compounds responsible for antagonistic activity could enhance the efficacy. This approach may help to refine the current findings and improve the application of YS004 as a promising solution for managing post-harvest tomato spoilage by *Penicillium* sp.

Conflicts of Interest

There are no conflicts to declare.

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