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Research Article
Induction of Potato Resistance Against Bacterial Wilt Disease Using *Saccharomyces cerevisiae*

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Abstract

Background and Objective: Potato, the fourth important commercial crop in the world, is infected by brown rot disease, which is one of the most important bacterial plant disease worldwide. The present investigation aimed to study the influence of *Saccharomyces cerevisiae* on *Ralstonia solanacearum* and potato plant resistance to control bacterial wilt disease of potato. **Methodology:** The ability of *S. cerevisiae* to inhibit the growth of *R. solanacearum in vitro* was investigated. In pots experiment, potato slices were soaked in the three different treatments of *S. cerevisiae* (whole culture, cell-free extract and yeast cells), then cultivated in soil infested with *R. solanacearum*. The disease rating was recorded. Different systemic induced resistance parameters were estimated. The increase of the weight of tubers and the decrease of the percentage of infected tubers were calculated. The total count of bacteria, fungi and yeast of soil microflora of potato plant rhizosphere were enumerated. The one-way randomized blocks design was applied in pots experiment. Data were analyzed with the statistical analysis software packages CoStat. **Results:** The *S. cerevisiae* did not inhibit *R. solanacearum in vitro.* It was found to produce indole acetic acid, gibberellic acid and cytokinin in the amounts of 81.57, 464.56 and 4.37 μg mL^{-1}, respectively. In pots experiment, the three studied treatments of *S. cerevisiae* significantly increased ($p \leq 0.05$) the plant height and decreased the disease rating. The total phenols, flavonoids, polyphenol oxidase, peroxidase, ABTS, DPPH, reducing power and gibberellic acid were significantly increased ($p \leq 0.05$) as a response to the three studied treatments after 40 and 70 days of planting. Moreover, the antioxidant capacity, starch content were significantly increased ($p \leq 0.05$). While sugar contents of potato tubers were significantly decreased ($p \leq 0.05$). The rhizosphere microflora showed various responses. **Conclusion:** It was concluded that the cell-free extract was the best treatment that could be recommended as a safe and economic bio-agent for the management of bacterial wilt disease of potato.

Key words: Induced resistance, antioxidant, enzymes, *Ralstonia solanacearum*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The potato (*Solanum tuberosum* L.), the fourth most important commercial crop in the world, can be affected by many biotic and abiotic factors, including pathogens and environmental stresses[1]. Bacterial wilt disease, brown rot disease, caused by *Ralstonia solanacearum*, is the most important potato disease in tropical and sub-tropical regions of the world[2] . Globally, this disease affects about 1.7 million hectares of potatoes in approximately 80 countries[3]. The host range of this bacterial pathogen is extensively wide. This pathogen infects more than 200 plant species from more than 50 families and it is distributed worldwide and leads to a damaging economic loss[4]. Bacterial wilt disease is widely distributed in tropical, subtropical and warm temperate climates of the world and it occurs in about 45 countries in the southern hemisphere. In Africa, it is found in Angola, Congo, Ethiopia, Kenya, Nigeria, South Africa, etc[5]. Moreover, *R. solanacearum* is one of the most destructive pathogens, where it induces rapid and fatal wilting symptoms in host plants. Direct yield losses by *R. solanacearum* varies widely according to the host, cultivar, climate, soil type, cropping pattern and strain. For example, yield losses vary from zero to 91% in the tomato, 33-90% in the potato, 10-30% in tobacco, 80-100% in the banana and up to 20% in the groundnut[6]. However, there are difficulties associated with the management of this pathogen due to its abilities to grow endophytically, survive in soil, especially in the deeper layers and travel along the water and its relationship with weeds[7,8]. The management of bacterial wilt with physical, chemical, biological and cultural methods has been investigated for decades.

There is a growing fear about the bad influences of chemical pesticides all over the world. Microbial biocontrol agents have a number of important advantages over traditional chemical pesticides that make their commercial view particularly favorable and can be commercially developed with relative simplicity to control plant diseases. Biological control of different plant diseases was focused primarily using bacteria or filamentous fungi and so, the application of yeasts as biocontrol agents acts as a new trend against different pathogens[9]. Yeasts were used for management of fungal plant pathogens. *Saccharomyces cerevisiae* is one of the yeasts that is mostly used for this purpose. *S. cerevisiae* used for control of different fungal plant diseases. It used for control of fungi causing sour rot of table grapes[10], brown rot of sweet cherries[11], *Colletotrichum acutatum* during pre-harvest[12]. Furthermore, *S. cerevisiae* used for control of root-knot nematode[13].

Recently, the new trend of management of plant diseases depends upon induced systemic resistance in the plant such as generation of phenolic compounds. *S. cerevisiae* yeast extract was used for improving the health of butter lettuce (*Lactuca sativa* L.) where it increased the total phenolic compounds and chlorophyll content[14]. Such of these compounds, e.g., indole and auxin compounds which produced by numerous plant-associated bacteria and fungi. These phytohormones have biological effects on plant growth and several physiological processes[15-17]. These compounds possess two mechanisms, direct promotion of plant growth and indirect inhibition of plant pathogens. Here, this investigation focused on *in vitro*, the effect of *S. cerevisiae* on the growth of *R. solanacearum* and *in vivo*, estimation of some phytohormones associated with yeast growth and their effect on bacterial wilt disease of potato plants. Furthermore, the effect of *S. cerevisiae* on the microbial communities in soil was studied.

MATERIALS AND METHODS

Materials: A virulent bacterial isolate of *Ralstonia solanacearum* (race 3 biovar 2) was obtained from the culture collection of Bacterial Diseases Research Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

Saccharomyces cerevisiae was obtained from Microbial Activity Unit, Microbiology Department, Soils, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt.

Potato tubers cultivar (Spunta) was obtained from Nubaria district, Bahaira Government, Egypt.

All chemicals and solution are analytical grades.

Methods

Preparation of microbial inoculants for *in vitro* experiments: For the preparation of *R. solanacearum* suspension to be used *in vitro* experiments, the bacterium was seeded on Nutrient Glucose Agar (NGA) slants for 48 h. The pathogen was harvested and resuspended in sterilized saline solution (8.5 g NaCl L^{-1}). The bacterial culture was adjusted to 10^8 CFU mL^{-1}. Similarly, *S. cerevisiae* was inoculated on Yeast extract Peptone Dextrose Agar (YPDA) slants at 28°C for 48 h, harvested and resuspended in sterilized saline solution and adjusted to 10^8 CFU mL^{-1}.

Antagonistic activity of *S. cerevisiae* against *R. solanacearum*: The activity of *S. cerevisiae* to inhibit the growth of *R. solanacearum* was tested by using Agar plug

diffusion method[18] with some modification. *S. cerevisiae* was cultured by adding and spreading 1 mL of 10^8 CFU mL^{-1} culture on YPDA plates and incubated at 28°C for 2 days. Plugs of *S. cerevisiae* solid culture of diameter 10 mm were prepared by sterilized cork borer. These plugs were appropriately placed onto the center of solidified NGA plates freshly seeded with *R. solanacearum* (1 mL of a bacterial suspension of 10^8 CFU mL^{-1}). Three replicates were prepared. Plates were kept at 4°C for 2 h to permit the diffusion of the bioactive molecules produced by *S. cerevisiae*. The plates were incubated at 28°C for 48 h. The inhibition zones were measured (mm).

Estimation of growth regulator hormones of *S. cerevisiae*: *S. cerevisiae* was inoculated in Yeast extract–Peptone-Dextrose-Broth (YPDB) and incubated at 28°C for 48 h. The culture was adjusted to 10^8 CFU mL^{-1}. This yeast culture was centrifuged (10000 rpm/min for 20 min). The supernatant was sterilized by millipore filters (0.45 µm).

Determination of Indole Acetic Acid (IAA) was carried out according to the procedure of Mohite[19], gibberellic acid (GA_3) by the procedure of Berrios *et al.*[20] and cytokines by the procedure reported by Hoyerova *et al.*[21].

Preparation of bacterial pathogen for pots experiment: Bacterial pathogen (*R. solanacearum*) was inoculated in Nutrient Glucose Broth (NGB) and incubated in an incubator shaker (28°C and 120 RPM) for 48 h. Bacteria were harvested by centrifugation (10000 rpm/min for 20 min). The precipitate was re-suspended in sterilized tap water. The bacterial suspension was adjusted to 10^8 CFU mL^{-1} concentration.

Preparation of *S. cerevisiae* treatments for pots experiment: *S. cerevisiae* was inoculated in yeast extract peptone dextrose broth (YPDB) and incubated in an incubator shaker (28°C at 120 rpm) for 48 h. *S. cerevisiae* culture was adjusted to 10^8 CFU mL^{-1}. Three treatments of *S. cerevisiae* were prepared. The first treatment (whole culture) was obtained from the aforementioned *S. cerevisiae* culture. The second treatment was prepared by centrifugation (10000 rpm/min for 20 min). The supernatant was obtained and sterilized by filtrations through fritted glass and it was used as (cell-free extract treatment). While the third treatment was the *S. cerevisiae* precipitated cells, which were re-suspended in sterilized tap water for washing and re-centrifuged. Then, *S. cerevisiae* precipitated cells were re-suspended and adjusted to 10^8 CFU mL^{-1} (yeast cells only).

Pots experiment: This experiment was carried out at the Farm of Faculty of Agriculture, Mansoura University, Egypt during the period of 22nd November 2015 to 16th March 2016 at an open field. The plastic sacks of 35 cm diameter were filled with 20 kg non-sterilized soil. This soil was composed of a mixture of clay and sand 1:1 (w: w). The soil of each sack (pot) was irrigated by water and left to dry for 72 h, then infested by *R. solanacearum* (10^8 CFU mL^{-1}), 100 mL for each sack. The pots were left to dry for about 48 h before sowing.

The potato tuber slices of equal size were soaked in the three aforementioned treatments of *S. cerevisiae* (for 30 min) before planting. Two potato tuber slices were planted in each pot. After the emergency, one plant from each pot was harvested after 40 days for biochemical investigation (as indicated later). Another plant of each pot was harvested at the end of the experiment. After 70 days of planting, leaves from the same locations in the stem of potato plants were taken for biochemical investigation. Furthermore, after 70 of planting, plant length was measured. In addition, disease rating was determined. Five replicates were prepared for each treatment. The experiment was arranged complete randomized block design.

Disease rating: Wilt symptoms severity were recorded daily according to the scale of Kempe and Sequeira[22] where, (0 = no symptoms, 1= up to 25% wilt, 2 = 26-50% wilt, 3 = 51-75% wilt, 4 = 76-99% of the foliage wilted and 5 = dead plants. Each replicate was examined and disease rating was recorded. The mean value for each treatment was calculated.

Yield: Number and weight of tubers were estimated at harvesting time. In addition, the increase in potato tubers weight percentage was calculated according to Ezzat and Moussa[23] as the following:

$$\text{Increase in potato tubers weight percentage} = \frac{(\text{Tubers weight of treatment} - \text{Tubers weight of control})}{\text{Tubers weight of control}} \times 100$$

Infected tubers percentage: Tubers were stored for 6 weeks at room temperature to encourage the development of latent infection and facilitate symptoms detection[24]. After storage period, potato tubers were cut to determine the number of infected tubers and percentage of infected tubers was calculated. Furthermore, the reduction of the percentage of infected tubers was calculated according to Ezzat and Moussa[23] as the following:

$$\text{Decrease in Infected tubers (\%)} = \frac{\text{Infected tubers of control (\%)} - \text{Infected tubers of treatment (\%)}}{\text{Infected tubers of control (\%)}} \times 100$$

Biochemical evaluations: After 40 and 70 days of planting and after harvesting biochemical evaluations were conducted as the following.

Total phenols content of investigated leaves: Total phenolic contents of both fresh leaves and tubers were determined by using Folin-Ciocalteu reagent method according to Blainski *et al.*[25]. For quantitatively, determination a standard curve of gallic acid was prepared in the same manner. Total phenol contents were expressed as milligram Gallic Acid Equivalent (GAE).

Total flavonoids content of investigated leaves: Total flavonoids content of both fresh leaves and tubers were determined colorimetrically using aluminum chloride as described by Li *et al.*[26]. The concentration of total flavonoids contents was expressed as milligram Quercetin Equivalent (QE).

Reducing power of plant extracts: Reducing power of leaves extracts was determined according to the method of Li *et al.*[26]. The absorbance of the resultant color was measured spectrophotometrically at 700 nm. Higher absorbance of the reaction mixture indicated greater reducing power.

Determination of $ABTS^+$ cation radical: ABTS (2, 2'-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) assay was based on the method of Christodouleas *et al.*[27] with slight modifications. The absorbance of the resulting greenish-blue solution was recorded at wavelength 734 nm, the decrease in absorbance is expressed as a percentage of inhibition, which was calculated.

Determination of antioxidant activity by radical scavenging method: The free radical scavenging activity was determined using different concentrations of 2, 2 Diphenyl-1-picrylhydrazyl (DPPH) and measuring the bleaching of the purple color of DPPH[26].

The absorbance was measured at 517 nm. The percentage of inhibition was calculated.

Polyphenol oxidase and peroxidase activities: Extraction and activity of both enzymes were determined using a spectrophotometric method based on an initial rate of increase in absorbance at 410 nm were carried out at 4°C, according to Seleim *et al.*[28].

Chlorophylls and carotene content: Total chlorophyll, chlorophyll a, chlorophyll b and carotene in potato leaves were determined, according to Mackinney[29].

Determination of gibberellic acid in potato leaves: Gibberellic acid was determined, according to Nimir *et al.*[30]. A calibration graph was then obtained by using standard gibberellic acid solutions prepared by dissolving 0.04 g pure gibberellic acid in absolute alcohol and diluted to 100 mL in a volumetric flask with absolute alcohol.

Determination of sugars and starch contents: Total sugars content was determined with a slightly modified phenol-sulphuric acid method, according to Abidi *et al.*[31]. The reducing sugars content was determined by the modified method of Miller[32]. Non-reducing sugars content was calculated by subtraction. The starch content was determined by anthrone reagent, according to Ghavimi *et al.*[33].

Microbial count

Soil sampling: One soil sample was collected from rhizosphere soil from each pot. The soil samples from five replicates of each treatment were mixed well and left to be air dried then grounded after plant debris were removed.

Soil microbial populations: General microbial populations were determined as described by Larkin[34]. For each composite soil sample, 10 g of soil was weighed and added to 90 mL sterile saline and keep in shaker overnight for extract soil microorganisms from soil samples. The serial dilution method was performed. For total bacterial counts nutrient agar plates was used. For total yeast count, YPDA plates were used. For fungal count, Potato Dextrose Agar (PDA) medium was used. For total bacterial and yeast count plates incubated at 28°C for 3 days. While plates of the total fungal count were incubated at 25°C for 7 days prior to enumeration of viable colonies. Colonies were counted and log CFU mL^{-1} were calculated.

Statistical analysis: Data were analyzed with the statistical analysis software packages CoStat (version 6.4, CoHort Software, U.S.A). The one-way randomized blocks design was applied in pots experiment. Duncan's multiple range test was used to compare the means at probability ($p \leq 0.05$).

RESULTS AND DISCUSSION

The natural resistance of plants to diseases does not only depend on performed defenses, but also on induced mechanisms. Induced immunity is one of these mechanisms that were associated with local changes at the site of pathogen infection, such as hypersensitive response, which is one of the most efficient forms of plant defenses[35]. The hypersensitive response leads to an increase in the activity of peroxidase and polyphenol oxidase enzymes that involved

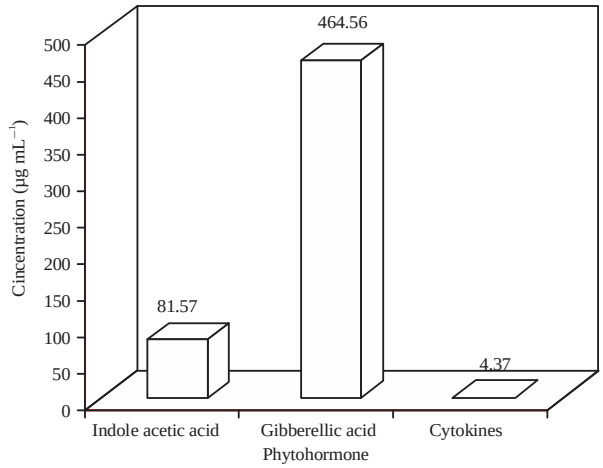

Fig. 1: Some phytohormones secreted by *S. cerevisiae*

in the defense mechanisms and finally, accumulation of phenolic compounds that play as an antimicrobial agent[36,37]. Additionally, plant growth-promoting microorganisms are known to enhance immune response and plant growth during production of phytohormones or auxins like indole-3-acetic acid, gibberellins and cytokinin[38]. Herein, a growing body of this study is an attempt to determine the impact of *S. cerevisiae* against bacterial wilt disease of potato caused by *R. solanacearum*. As well as, following the extended impact on the yield quantity, quality and the tolerance of the potato plants to biotic stress.

Initially, the antagonistic activity of *S. cerevisiae* against *R. solanacearum* in Petri dishes showed that *S. cerevisiae* had no antagonistic effect against *R. solanacearum*. These data are in contrast with that obtained by Nally *et al.*[10], who demonstrated that different yeast species (*Saccharomyces* and non-*Saccharomyces*) strains inhibited the growth of eight fungi causing sour rot of table grapes. Similarly, Lopes *et al.*[12] found that different isolates of *S. cerevisiae* antagonized *Colletotrichum acutatum*, the causal agent of post-bloom fruit drop.

Herein, *S. cerevisiae* cannot be used as a direct antagonistic factor for management of bacterial disease of potato. Therefore, this study was directed to investigate the ability of *S. cerevisiae* to produce some plant-growth hormones and use of *S. cerevisiae* to improve induced systemic resistance of potato against the studied pathogen.

Auxins secreted by *S. cerevisiae*: *S. cerevisiae* was cultured in YPDB under optimal conditions and the supernatant of the culture was chemically analyzed to investigate the auxins associated with the yeast growth. Obtained results showed that the efficiency of yeast in the secretion of auxins, i.e., indole acetic acid, gibberellic acid and cytokines, being

Table 1: Plant height, branches number and disease rating

	Characters		
Treatments	*Plant height (cm)	Branches number	**Disease rating
Control	19.60^{c}	2.0^{a}	4.2^{a}
Whole culture	25.60^{b}	2.8^{a}	1.0^{b}
Cell-free extract	29.60^{a}	2.8^{a}	0.8^{b}
Yeast cells	24.00^{b}	2.8^{a}	1.0^{b}

All recorded numbers are means of five replicates, means followed by the different letter (s) within each column are significantly different using Duncan's Multiple Range Test at $p \leq 0.05$. *Plant height was measured after 70 days of planting. **Disease rating scale: 0 = No symptoms, 1= up to 25% wilt, 2 = 26-50% wilt, 3 = 51-75% wilt, 4 = 76-99% of the foliage wilted and 5 = Dead plants

81.57, 464.56 and 4.37 µg mL^{-1}, respectively (Fig. 1). In which, these auxins play many different roles in plant growth and development[16]. Additionally, auxins are also involved in the regulation of changes in different growth processes associated with pathogens[17]. As well as, they may play an important role in plant defense against plant pathogens[15]. Consequently, *in vivo*, the response of bacterial wilt disease caused by *R. solanacearum* in potato plants towards the yeast understudy and/or its production of auxins has been investigated. These results are coinciding with the findings of Ezzat and Moussa[23] who found that *Bacillus subtillus*, *B. polymyxa* and *Pseudomonas fluorescens* increased the induced resistance of potato plant against *R. solanacearum*.

Pots experiment: The vital role of both yeast cell, cell-free extract and the whole culture of yeast on plant height and disease rating compared to control (Table 1). The three studied treatments led to significantly increase ($p \leq 0.05$) in plant height than the control treatment. Where the plant height in control treatment was 19.60 cm and the longest plants were observed with cell-free extract treatment (29.6 cm). On the other hand, there was no significant difference in plant height of the other two treatments, where the mean values of plant height of plants treated with the whole culture of *S. cerevisiae* and *S. cerevisiae* cells were 25.60 and 24.00 cm, respectively. In addition, there was no significant difference between the three tested treatments and control in branches number. Furthermore, the three tested treatments led to significant decrease ($p \leq 0.05$) in disease rating in comparing with control treatment. The mean value of disease rating of control treatment was 4.2. Whereas, there were no significant differences between the recorded mean values of the three tested treatments where the disease rating of the whole culture, yeast cells and cell-free extract were 1, 1 and 0.8, respectively.

These data may be due to phytohormones or auxins associated with the growth of the yeast that reflected on the

induced systematic resistance in plants. These may be due to the cytokinin of yeast that plays a beneficial role in cell division and cell enlargement[39]. However, other studies pointed out that there are several auxin mutants, which showed defects in the cell elongation, such defects interfere with the infection processes of pathogens that interact with the cell wall and colonize the extracellular spaces[40]. Another hypothesis suggested that a high level of auxin would confer cell expansion and cells with a less rigid cell better entry points for pathogens[17]. So, the opposing the auxin levels results in resistance of the host plant. In addition, the shortage of activity of auxin may be due to auxin homeostasis that defined as the binding of free auxin to small or large molecules as inactive hormones[41]. Further, yeast acting as natural stimulator which characterized by its content of protein, lipids, carbohydrates, nucleic acids, thiamine, minerals and other growth regulating hormones[42]. Moreover, a wide variety of yeasts has been investigated as biological control of some diseases of vegetables and fruits[43]. Endophytes fungi possess a synergistic effect of promoting plant growth and improving biotic and abiotic stress resistance as well[44]. Furthermore, they induce host plant defense against phytopathogenic organisms through regulating plant physiological responses[45]. Another investigated study pointed out that bio-agents, i.e., *B. polymyxa*, *P. fluorescens* and *B. subtillus* were decreased significantly ($p \leq 0.05$) the rating of bacterial wilt of potato, in addition to significant increase ($p \leq 0.05$) in plant height[23].

On the other side, the profile of some microbial counts in the rhizosphere of potato plants, i.e., *R. solanacearum*, the total bacteria, the total fungi and the total yeasts were affected by yeast treatments compared to control (Table 2). Interestingly, the number (log CFU g^{-1} soil) of *R. solanacearum* significantly decreased ($p \leq 0.05$) in response to the three yeast treatments during the intervals of 40 and 70 days compared to control, but no significant difference after 114 days from sowing, except in the case of *S. cerevisiae* cell treatment. In addition, the numbers of the total bacterial count of the three tested treatments after 40 and 114 days significantly decreased ($p \leq 0.05$) than the corresponding value of control treatment. However, after 70 days only the yeast cells treatment significantly decreased ($p \leq 0.05$) the number of the total bacterial count. Generally, the count of *R. solanacearum* and total microbial count decreased with the increase of period of study. Furthermore, log number of total fungi of the three investigated treatments significantly increased ($p \leq 0.05$) after 114 days. No significant response, except the yeast cell treatment, after 70 days. After 40 days, the whole culture and cell-free extract significantly increased ($p \leq 0.05$) the count of the total fungi. Concerning the total yeast count, the three tested treatments significantly increased ($p \leq 0.05$) the total yeast count after 70 days compared to control, but there was no significant difference among them after the 114 days. While, after 40 days the cell-free extract and yeast cells led to significant increments in total yeast count.

Several reasons may lead to the variations of the microbial count. The secreted hormones by *S. cerevisiae* affected the physiology of potato plant[16] that led to change in root exudates. Root exudates play an important role in plant defense and in the change in microflora[46]. The root exudates of treated plants may lead to inhibition of *R. solanacearum* and the decrease of its count. Furthermore, these root exudates might play a role in the decrease of log number of total bacterial count. Contrarily, these root exudates of treated plants might encourage fungi and yeast growth.

Table 2: Some microbial count in potato plant rhizosphere in pots experiment

		Microbial Count (Log CFU g^{-1} soil)			
Time (days)	Treatments	*R. solanacearum*	Total bacteria	Total fungi	Total yeast
40	Control	6.51^{a}	6.44^{a}	3.99^{bc}	4.53^{e}
	Whole culture	5.70^{b}	6.11^{b}	4.37^{a}	4.54^{e}
	Cell-free extract	5.85^{b}	6.10^{b}	4.54^{a}	4.63^{d}
	Yeast cells	5.73^{b}	6.04^{c}	3.80^{cd}	4.80^{c}
70	Control	6.43^{a}	5.97^{d}	3.78^{cd}	4.10^{g}
	Whole culture	5.24^{c}	5.95^{d}	3.70^{d}	5.69^{b}
	Cell-free extract	5.30^{c}	5.93^{d}	3.76^{cd}	5.88^{a}
	Yeast cells	5.03^{d}	5.67^{e}	4.10^{b}	5.73^{b}
114	Control	4.75^{e}	5.67^{e}	3.42^{e}	4.40^{f}
	Whole culture	4.69^{e}	5.58^{f}	3.69^{d}	4.38^{f}
	Cell-free extract	4.74^{e}	5.53^{f}	3.67^{d}	4.34^{f}
	Yeast cells	4.26^{f}	5.41^{g}	3.73^{d}	4.40^{f}

All recorded numbers are means of five replicates, means followed by the different letter(s) within each column are significantly different using Duncan's Multiple Range Test at $p \leq 0.05$

The filtrate of *S. cerevisiae* in the two treatments, i.e., whole culture and cell-free extract, may lead to change in rhizospheric microflora. This filtrate may encourage the growth of yeasts. This change in microflora might encourage microbial antagonistic of *R. solanacearum* that present in the rhizosphere[47]. Therefore, the filtrate of *S. cerevisiae* decreased log number (CFU g^{-1} soil) of studied bacterial pathogen.

The *S. cerevisiae* cells are competitors in soil microflora for other microorganisms. They are competitors for nutrient, iron and space[48]. Hence, these cells act on the change in rhizosphere microflora.

The *S. cerevisiae* cells might act as Plant Growth Promoting Bacteria (PGPB) in colonization on rot specific site that inhabited invading bacterial pathogen the plant[48,49].

Physiological characters: All studied treatments of *S. cerevisiae* led to significant increase ($p \leq 0.05$) of total polyphenols, flavonoids, polyphenol oxidase and peroxidase of potato plant leaves in compared with control treatment when they were estimated After 40 and 70 days from sowing (Table 3). The results indicated that the cell-free extract was superior to other two treatments in the aforementioned estimated factors. The leaves of potato plants treated with cell-free extract had total polyphenols 28.17 and 19.65 (mgGAE g^{-1} FW) after 40 and 70 days from sowing, respectively. Moreover, the leaves of the same treatment had total flavonoids 3.160 and 2.107 (mgQE g^{-1} FW) after 40 and 70 days. In addition, the same treatment recorded the highest significant increase ($p \leq 0.05$) in polyphenol oxidase and peroxidase. The activity of polyphenol oxidase and peroxidase enzymes were significantly increased ($p \leq 0.05$) compared to control where these enzymes have the ability to breakdown and scavenging free radical.

Furthermore, in the same manner, all tested treatments of *S. cerevisiae* caused significant increase ($p \leq 0.05$) in ABTS% inhibition, DPPH% inhibition and reducing power after 40 and 70 days compared with the control treatment (Table 4). The most effective treatment was the cell-free extract treatment that led to the highest significant increase ($p \leq 0.05$) in these factors. These factors were used as the indicators of the vitality of yeast treatments in increasing the efficacy of potato plants in scavenging free radicals that associated with *R. solanacearum* infection and strengthen the immune response of potato. Data indicated that cell-free extract enhancing the scavenging capacity of leaves of potato, which the ABT ranging between 14.30 and 5.36% during 40 and 70 days from sowing. While, the DPPH ranging between 3.42 and 1.87% inhibition from 40-70 days, respectively. The reducing power also showed to be 1.898 and 1.769 after 40 and 70 days. These results are matching with previous studies that showed the role of the biotic inducers in increasing the plant resistance against pathogens and their extended effect on the generation of antioxidant and anti-inflammatory agents[23,50-52]. As well as, the accumulation of secondary metabolites such as phenolics, saponins and terpenes are widely distributed in the higher plants as the

Table 3: Estimation of phenols and flavonoids in fresh weight basis after 40, 70 days

Time (days)	Treatments	Total phenols (mgGAE g^{-1} FW)	Total flavonoids (mgQE g^{-1} FW)	Polyphenol oxidase (Unit/min g FW)	Peroxidase (Unit/min g FW)
40	Control	11.67^{e}	0.074^{g}	8.54^{cd}	13.4^{f}
	Whole culture	22.66^{b}	1.911^{c}	11.87^{ab}	32.3^{c}
	Cell-free extract	28.17^{a}	3.160^{a}	13.54^{a}	47.5^{a}
	Yeast cells	22.17^{b}	0.886^{e}	11.72^{b}	19.8^{e}
70	Control	8.55^{f}	0.358^{f}	7.85^{d}	10.1^{g}
	Whole culture	13.05^{e}	1.118^{d}	9.35^{cd}	25^{d}
	Cell-free extract	19.65^{c}	2.107^{b}	11.55^{b}	39.1^{b}
	Yeast cells	15.20^{d}	0.356^{f}	10.07^{bc}	14.1^{f}

All recorded numbers are means of three replicates, means followed by the different letter(s) within each column are significantly different using Duncan's Multiple Range Test at $p \leq 0.05$. mgGAE/g FW: Milligram Gallic Acid equivalent (GAE)/gram Fresh weight. mgQE/g FW: Milligram quercetin equivalent (QE)/gram Fresh Weight

Table 4: Determination of ABTS, DPPH and reducing power after 40, 70 days

Time (days)	Treatments	ABTS inhibition (%)	DPPH inhibition (%)	Reducing power optical density at 700 nm
40	Control	6.43^{d}	1.05^{f}	0.770^{e}
	Whole culture	12.72^{b}	2.90^{b}	1.175^{d}
	Cell-free extract	14.30^{a}	3.42^{a}	1.898^{a}
	Yeast cells	10.56^{c}	2.25^{c}	1.368^{c}
70	Control	2.41^{g}	0.80^{g}	0.725^{e}
	Whole culture	4.77^{ef}	1.35^{e}	1.663^{b}
	Cell-free extract	5.36^{de}	1.87^{d}	1.769^{b}
	Yeast cells	3.96^{f}	1.30^{e}	1.341^{c}

All recorded numbers are means of three replicates, means followed by the different letter(s) within each column are significantly different using Duncan's Multiple Range Test at $p \leq 0.05$

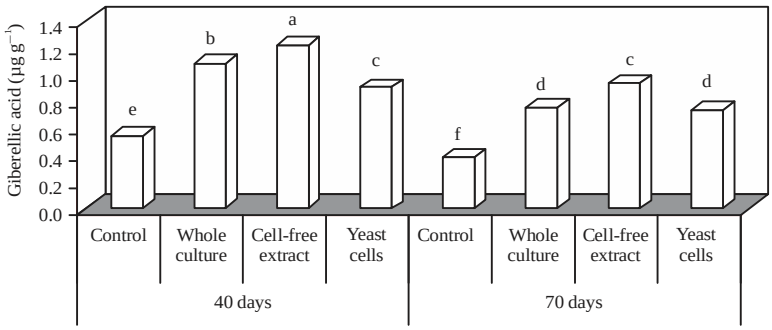

Fig. 2: Estimation of gibberellic acid in leaves on fresh weight basis after 40 and 70 days

All recorded numbers are means of three replicates, means followed by the different letter are significantly different using Duncan's Multiple Range Test at $p \leq 0.05$

Table 5: Estimation of chlorophyll and carotene contents (mg g^{-1} fresh weight) after 40 and 70 days

Time	Treatment	Total Chlorophyll	Chlorophyll a	Chlorophyll b	Carotene
40 days	Control	0.927[g]	0.673[g]	0.254[g]	0.291[h]
	Whole culture	1.379[f]	1.021[ef]	0.357[f]	0.332[f]
	Cell-free extract	1.635[e]	1.130[e]	0.505[e]	0.387[e]
	Yeast cells	1.309[f]	0.910[f]	0.399[f]	0.308[g]
70 days	Control	2.150[d]	1.561[d]	0.589[d]	0.674[d]
	Whole culture	3.199[b]	2.369[b]	0.829[c]	0.771[b]
	Cell-free extract	3.793[a]	2.621[a]	1.172[a]	0.898[a]
	Yeast cells	3.037[c]	2.112[c]	0.925[b]	0.714[c]

All recorded numbers are means of three replicates, means followed by the different letter(s) within each column are significantly different using Duncan's Multiple Range Test at $p \leq 0.05$

pathogen injury and they can serve either as physical barriers between the stressed cells and their environment or as a defense against pathogens and herbivores[53-55]. The phenolic compounds represent a large group of minor chemical constituents in potatoes, which play an important role in determining their organoleptic properties and recognized as the most abundant antioxidants in the human diet[56]. Further, the phenolic compounds have a wide-array of health providing characteristics[57]. Therefore, they have a potential use as a functional food for improving human health. The phenolic content of potatoes was reported to be high and ranged[58,59] from 530-1770 µg g^{-1}.

Additionally, the total chlorophyll, chlorophyll a and b were determined (Table 5), which are known as good parameters reflecting the health status of the plants. Data showed the superiority of yeast-free extract in enhancing these parameters in plants after 40 and 70 days, however, the yeast cells treatment was lower in this respect. As well as, the carotene contents showed to be significantly increased ($p \leq 0.05$) in all treatments compared to control. The content of potato leaves of gibberellic acid has been determined (Fig. 2). Data illustrated that positive response in the values of gibberellic acid has been occurred with the yeast treatments but in different ratios. Gibberellins produced by fungi and bacteria are secondary metabolites that elicit signals to establish symbiotic interaction with host plants[60,61] and have been shown to play a role in plant growth promotion[62]. The cell- free extract was also superior to other treatments, in which the values of gibberellic acids being 1.23 and 0.94 g L^{-1} after 40 and 70 days, respectively. The positive response of chlorophyll may be due to the vital role of cytokinines associated with the yeast strain that plays a role in the synthesis and maintenance of chlorophyll and influence on chloroplast development and metabolism. As well as, cytokinines play impact role on plant nutrients translocation by converting source tissues into active sinks[63]. Further, recent investigations provide support for the role of cytokinins as key regulators of plant defense response against non-cytokine producing plant-pathogenic bacteria such as *Pseudomonas syringae* by suppressing bacterially induced hypersensitive response symptoms and by increasing antioxidative enzyme levels[63,64].

Yield and infected tubers: There was no significant difference in the tubers number of potato among the treatments (Table 6). However, the tubers weight significantly increased ($p \leq 0.05$) in all treatments compared to control. The cell-free extract caused the higher increase in weight percentage (66.16%), while, yeast cells treatment showed lowest

Table 6: Tubers number, tubers weight and infected tuber percentage after harvesting

	Characters				
Treatments	Tubers number	Tubers weight (g)	Increase of tuber weight (%)	Infected tuber (%)	Decrease in Infected tubers (%)
Control	3.8 a	104.6[d]	-	85.00[a]	-
Whole culture	3.6 a	130.8[b]	25.05	38.95[bc]	54.18
Cell-free extract	4.0 a	173.8[a]	66.16	27.20[c]	68.00
Yeast cells	3.6 a	117.8[c]	12.62	52.22[b]	38.56

All recorded numbers are means of three replicates, means followed by the different letter(s) within each column are significantly different using Duncan's Multiple Range Test at $p \leq 0.05$

Table 7: Estimation of antioxidant capacity in tubers at harvesting time

Treatments	Total phenols (mgGAE/100 g FW)	Total flavonoids (mgQE/100 g FW)	ABTS inhibition (%)	DPPH inhibition (%)	Reducing power Optical density at 700 nm	Polyphenol oxidase (Unit/min g FW)	Peroxidase (Unit/min g FW)
Control	17.25[d]	0.993[d]	15.99[d]	1.89[d]	0.96[d]	0.483[d]	0.118[d]
Whole culture	19.16[b]	3.401[b]	24.40[c]	5.22[b]	1.90[b]	0.954[b]	0.405[b]
Cell-free extract	19.89[a]	6.523[a]	36.75[a]	6.15[a]	2.13[a]	1.172[a]	0.777[a]
Yeast cells	18.30[c]	2.053[c]	28.42[b]	3.24[c]	1.25[c]	0.792[c]	0.244[c]

All recorded numbers are means of three replicates, means followed by the different letter(s) within each column are significantly different using Duncan's Multiple Range Test at $p \leq 0.05$. mgGAE/100 g FW: Milligram Gallic Acid equivalent (GAE)/100 gram Fresh Weight. mgQE/100 g FW: Milligram quercetin equivalent (QE)/100 gram Fresh Weight

Table 8: Estimation of carbohydrates in tubers at harvesting time

Treatment	Starch (%)	Total sugars (mg g^{-1})	Reducing sugars (mg g^{-1})	Non-reducing sugars (mg g^{-1})
Control	12.71[c]	4.022[a]	0.400[a]	3.744[a]
Whole culture	14.15[b]	3.785[b]	0.388[a]	3.397[b]
Cell-free extract	15.33[a]	3.154[c]	0.278[c]	2.754[c]
Yeast cells	13.89[b]	3.188[c]	0.364[b]	2.824[c]

All recorded numbers are means of three replicates, means followed by the different letter(s) within each column are significantly different using Duncan's Multiple Range Test at $p \leq 0.05$

corresponding value (12.6%). In addition, infected tubers percentages of the three treatments were significantly decreased ($p \leq 0.05$) from the corresponding value of control treatment. The cell-free extract treatment led to the highest decrease in infected tubers (68.00%) whereas, yeast cells treatment revealed lowest corresponding value (38.56%). Hence, this increase of tubers weight and this decrease of infected tubers were due to the enhancement of systemic induce resistance and plant growth that resulted from the use of the three treatments of *S. cerevisiae.*

Tubers content: The total polyphenols and flavonoids of tubers had been determined (Table 7). The significant increases ($p \leq 0.05$) in their content compared to control. The cell-free extract treatment showed to be superior to other ones, in which the values of polyphenols and flavonoid being 19.89 mgGAE/100 g FW and 6.523 mgQE/100 g FW, respectively. As well as, the values of ABTS%, DPPH, reducing power, polyphenol oxidase and peroxidase were significantly increased ($p \leq 0.05$) compared to control. Interestingly, the cell-free extract treatment was the most potent treatment compared to the other treatments. Polyphenol oxidase enzymes (PPOs) are widely distributed within the plant and act in the defense responses[65]. Other investigations pointed out that the overexpression of PPO in tomato leads to a significant increase ($p \leq 0.05$) in resistance to *Pseudomonas syringae* pv. tomato in harmonious interactions[66]. The efficacy of enzyme in disease resistance due to the hydroxylating monophenols to o-diphenols and oxidizing these compounds to quinones, which are toxic to the microorganisms than original phenolic compounds[67]. Likewise, peroxidase enzymes are contributed in plant resistance to pathogens, where strengthening the cell wall[36].

Moreover, the content of starch, total sugars, reducing sugars and non-reducing sugars have been also investigated (Table 8). Starch contents were significantly increased ($p \leq 0.05$) in the three treatments from the control. The cell-free extract led to the higher value of starch content (15.33%) while yeast cell treatment had the lowest starch content (13.89%). Contrary, reducing sugars, non-reducing sugars and total sugars values of the three treatments were significantly decreased ($p \leq 0.05$) compared to control. Finally, yeast extract is one of the most common natural elicitors used for the enhancement of secondary metabolites *in vitro* plant culture[68], because of their natural origin, these extracts are safe products that can be used in agricultural applications for stimulating the plant defense mechanism and improving the nutraceutical quality of some edible plants[69]. The previous

investigation pointed out that elicitation with yeast extract caused an increase in the total phenolic compounds and chlorophyll content in lettuce plants[14].

This study can be applied for management of bacterial wilt disease of potato as a safe ecofriendly alternative tool of pesticide. The preparation and application of this bio-agent is simply and economically.

CONCLUSION

S. cerevisiae did not inhibit *R. solanacearum in vitro. S. cerevisiae* produced some auxin-like substances that were estimated in its filtrate. Three different treatments of *S. cerevisiae* (whole culture, cell-free extract, yeast cells) were used to control bacterial wilt disease of potato in pots experiment. These three treatments increased the systemic induced resistance, leading to decrease in disease rating and percentage of infected tubers and increased the productivity of potato plant. The cell-free extract was the superior treatment. The three tested treatments caused changes in the microflora of rhizosphere.

SIGNIFICANCE STATEMENTS

This study revealed that different treatments of *S. cerevisiae* (whole culture, cell-free extract and cells) can be used for management of bacterial wilt disease of potato. Therefore, this study encourages the use of these treatments in the management of other plant diseases. This investigation is a trial in the new re of biological management of plant diseases by yeasts that were used less than bacteria and fungi. This investigation confirmed that the effective bio-control agents may be not antagonized plant pathogen *in vitro* and they inhibit this plant pathogen in an indirect way by increasing systemic induced resistance.

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