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THE SYNERGISTIC EFFECTIVENESS OF *SACCHAROMYCES CEREVISIAE* AND ALCOHOLIC EXTRACTS OF *MYRTUS COMMUNIS* AND *POPULUS EUPHRATICA* AGAINST *RHIZOCTONIA SOLANI* IN *IN VITRO* CONDITIONS

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Abstract

A laboratory study was conducted at a college of agriculture, university of Diyala during 2018 to assess the synergistic effectiveness of yeast *Saccharomyces cerevisiae* and plant extracts *Myrtus communis* and *Populus euphratica* in the inhibition of *Rhizoctonia solani* growth in vitro. Results showed that *S. cerevisiae* recorded increment in inhibition percentage of *R. solani* growth in the first and second methods 75.55 % and 46.66 % respectively as compared with control 0 %. The effective concentration (EC50) of *M. communis* extract in the yeast *S. cerevisiae* and *R. solani* reached 9120 ppm and 3311 ppm respectively, whereas the effective concentration (EC50) of *P. euphratica* extract in the yeast *S. cerevisiae* and *R. solani* reached 8709 ppm and 3019 ppm respectively. The inhibitory activity of *S. cerevisiae* with *M. communis* and *S. cerevisiae* with *P. euphratica* against *R. solani* growth reached 82.22% and 78.88% respectively compared with control 0%.

Keywords*: *Saccharomyces cerevisiae*, *Myrtus communis*, *Populus euphratica* and *Rhizoctonia solani

Introduction

Rhizoctonia solani is an endemic pathogen in the soil, it infects many plant families, causing seed rot and damping off before and after the germination such as tomato crop (Parmeter and Whitney, 1970; Carisse *et al.,* 2001; Harikrishnan and Yang, 2002, Thornton *et al.,* 2004). *R. solani* is soil borne pathogen that kills the host by producing many enzymes and toxins that have an effective role in its pathogenicity ability (Dillard, 1987). The fungus attacks the plant at all stages of its growth and is more dangerous in the early stages of plant (Jabr *et al.,* 2008). Agricultural chemical applications by using chemical pesticides have become less useful and more dangerous in the remote perspective due to these applications are not consistent with modern trends that work to protect the environment from pollution as well as an emergence of the resistance characteristic in those pathogens against the pesticides (Calhelha *et al.,* 2006; Kim and Hwang, 2007; Kaewchai *et al.,* 2009). Numerous attempts have been conducted to find alternatives to these pesticides. Biological control agents considered as alternatives to the synthetic pesticides due to its more safety and less impact on the environment (Cotxarrera *et al.,* 2002; Brimner *et al.,* 2003). The yeast, *Saccharomyces cerevisiae* has high efficiency in control many pathogens due to their prevalence in the root area as well as the simplicity of its nutritional requirements and its ability to form colonies on dry surfaces for a long period of time and its production of many lethal toxins for microorganisms (Slavikova and Vadkerti, 2003; El-Tarabily, 2006; Young, 2012; Augusto and Pascholati, 2010; Franca *et al.,* 2015; Chen *et al.,* 2015; Fakruddin *et al.,* 2017). Also the plant extracts are promising alternatives to chemical pesticides due to they contain various plant parts of phenolic compounds and volatile oils. The leaves of *Myrtus communis* contain many of secondary metabolite compounds that are effective against bacteria, fungi and viruses, such as Tannins, Flavonoids, Glycosides and Phenols (Al-Tkirity, 1997; Martin *et al.,* 1999; Hayder *et al.,* 2004). The leaves of *Populus euphratica* contain the effective chemical compounds such as Glycosides, Flavonoids, Polyphenols, Alkaloids, Saponins and Tannins against fungi and bacteria (Prior *et al.,* 2001; Patricia *et al.,* 2010; Resen *et al.,* 2016). Aim of the current study is to assess the effectiveness of *Saccharomyces cerevisiae*, *Populus euphratica* and *Myrtus communis* in the inhibition of *Rhizoctonia solani* growth in vitro.

Materials and Methods

A laboratory experiment was conducted at a college of agriculture, university of Diyala during 2018. leaves of *Myrtus communis* and *Populus euphratica* were collected from orchards located in Baqubah city, while *Saccharomyces cerevisiae* was obtained from the market.

Isolation and diagnosis of *Rhizoctonia solani*

Rhizoctonia solani was isolated from infected tomato seedlings that collected from an agricultural field in Diyala province, the infected roots were cut into small pieces at length (0.5 cm) and sterilized superficially with 1% sodium hypochlorite solution for 1 minutes and washed twice with sterile distilled water to remove chlorine, the pieces were dried on sterile filter paper and transferred to the medium of potato dextrose agar (PDA) with added 100 mg/L-1 of the antibiotic (ampicillin) in petri dishes (9 cm) and incubated at 25±2 °C for four days, pieces of mycelium were transferred by a sterile needle to PDA medium in petri dishes to purpose of fungus purification, then incubated at 25±2 °C for four days, the fungus was diagnosed to the species level according to the characteristics that mentioned by (Parmeter and Whitney, 1970), where the fungal growth appeared white on the PDA medium that turned to dark brown color, the mycelium of fungus appeared with divided barriers and branched to right angles with a clear narrowing in the branching areas under the microscope , then preserved at 4 °C until use.

Preparation of alcoholic extracts of *M. communis* and *P. euphratica*

250 g powder from the leaves of *M. communis* and *P. euphratica* separately were soaked in 1 liter of ethyl alcohol 96% in glass bottles 2 liter and placed on a shaker for 24 hours for continuous stirring, the extracts were filtered by double layered muslin cloth, then centrifuged at 3000 RPM for 10 minutes. Supernatant was dried by oven at 40°C to evaporate the solvent and each extract was preserved until use.

Effectiveness of *S. cerevisiae* against *R. solani* on PDA medium

Yeast of *S. cerevisiae* was grown in medium of Nutrient Yeast Dextrose Broth (NYDB) that consist of 8 g nutrient broth, 6 g yeast extract, 10 g dextrose and 1000 ml distilled water after sterilized by autoclave at 121 °C and a pressure of 1.5 kg/cm² for 20 minutes, then incubated at 25 ± 2 °C for 48 hours. The number of yeast cells of *S. cerevisiae* was calculated by a hemocytometer slide which reached $3x10^8$ cells/ml. The test was carried out with two methods, the first included pouring 1 ml of yeast suspension to petri dishes 9 cm, then pouring 20 ml of PDA medium (Potato Dextrose Agar) with three replicates, while the control treatment included pouring 1 ml of sterilized distilled water instead of yeasts, an agar disk 3 mm from the tip of *R. solani* colony at age of 3 days was transferred in the center of each the dish and incubated at 25 ± 2 °C, then after filling the control dishes with the fungus growth, the percentage of inhibition was calculated according to the equation:

% Inhibition = growth rate in control - growth rate in treatment / growth rate in control x 100

While the second method included pouring 1 ml of yeast suspension to the edge of the dish containing the PDA medium, then making the dish slant from side to side so that the yeast suspension covers the edge of the dish completely, whereas the control treatment without adding yeast suspension, an agar disk 3 mm from the tip of the *R. solani* colony at age of 3 days was transferred in the center of each the dish and incubated at 25 ± 2 °C, then after filling the control dishes with the fungus growth, the percentage of inhibition was calculated according to the previous equation.

Toxicity test of alcoholic extracts of *M. communis* and *P. euphratica* in the growth of *R. solani* and yeast *S. cerevisiae* on PDA

The concentrations 2500, 5000, 7500 and 10000 ppm were tested for each extract separately against *R. solani*, the concentrations of the extracts were added to flasks contains sterile PDA medium before solidification at a temperature approximately 45 °C, then the PDA medium of each extract separately was poured in petri dishes 9 cm with three replicates for each concentration, while the control treatment included only PDA medium**,** then an agar disk 3 mm from the tip of the *R. solani* colony at age of 3 days was transferred in the center of each the dish. The dilution process was conducted for yeast *S. cerevisiae*, 1 ml of the sixth dilution was added to the poisoned medium with the previous concentrations separately in the dish before solidification. The results were taken after the control dishes were filled with the fungus growth through calculating the average of two orthogonal diameters for each colony, then the percentage of inhibition was calculated according to the previous equation. Effective concentration value (EC50) that mean a concentration which leading to inhibition of 50 % of fungus growth was adopted to compare the toxicity of the extracts by drawing the toxicity line which is one of the important statistical analyzes to study the results of pesticides tests and other used chemicals in pests control (Shaaban and Al-Mallah, 1993). By drawing this line, it is possible to determine the inhibition percentages that are recorded at concentrations not used in the study, including effective concentration (EC50). During the drawing process, the used concentrations in the study are converted to the logarithm of the concentration (x), the response degree of the tested organisms to the extracts is directly proportional with the extract concentration logarithm, the inhibition percentages are converted to their corresponding of probability units (Probit), then the slope (b) is calculated by the concentration logarithm values and the experimental Probit units (Ye) by using the straight line equation, then calculate value of (a).

Straight line equation Yc = a + bx

Yc = Calculated Probit values, x = concentration logarithm

$$\bar{\gamma}e = \frac{\sum Ye}{n}$$

Ye = experimental Probit values

$$\bar{x} = \frac{\sum x}{n}$$

$$\sum x\ Ye = \frac{(\sum x)(\sum Ye)}{n}$$

$$\sum x^2 = \frac{(\sum x)^2}{n}$$

$a = \bar{\gamma} - b\bar{x}$ (Shaaban and Al-Mallah, 1993).

Through the value of b and a and the concentration logarithm, the calculated Probit values (Yc) were calculated. To ensure the drawing accuracy, the concentration logarithm values represent the x-axis while the calculated Probit values represent the y-axis.

Synergistic Effectiveness of *S. cerevisiae* with alcoholic extracts of *M. communis* and *P. euphratica* against *R. solani* on PDA

This test was carried out by adding 1 mL of yeast suspension to petri dishes 9 cm and pouring 20 ml of PDA medium (Potato Dextrose Agar) with three replicates, while synergistic treatment included adding 1 mL of yeast suspension to petri dishes 9 cm ,then pouring the poisoned medium with a concentration of 10000 ppm for each extract, while the control treatment included adding 1 ml of sterile distilled water instead of yeast suspension, an agar disk 3 mm from the tip of the *R. solani* colony at age of 3 days was transferred in the center of each the dish and incubated at 25 ± 2 °C, then after filling the control dishes with the fungus growth, the percentage of inhibition was calculated according to the previous equation.

Results and Discussion

Effectiveness of *S. cerevisiae* against *R. solani* on PDA medium

The results in Fig (1) revealed that rate of *R. solani* growth was reduced significantly by *S. cerevisiae* in first and

second methods which reached 2.2 cm and 4.8 cm respectively as compared with control 9 cm, whereas inhibition percentage of *R. solani* was increased significantly by *S. cerevisiae* in first and second methods which reached 75.55 % and 46.66 % respectively as compared with control 0 % (Fig 2,3). The results of this test indicate to the ability of *S. cerevisiae* to produce of bio compounds that inhibit the growth of many microorganisms and fungi (Slavikova and Vadkertiova, 2003; El-Tarabily, 2006; Augusto and Pascholati, 2010; Young, 2012; Franca *et al.,* 2015; Chen *et al.,* 2015; Fakruddin *et al.,* 2017).

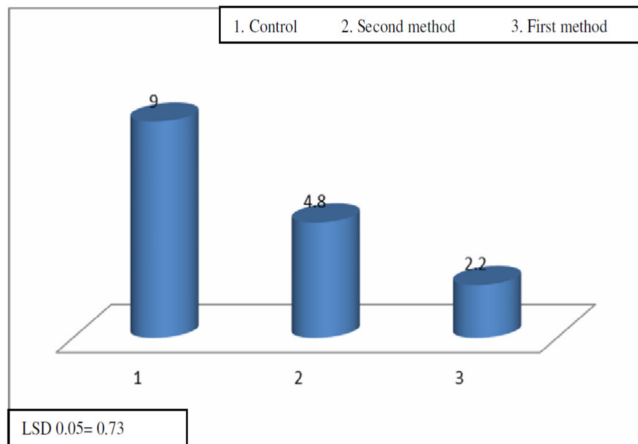


Fig. 1 : Effect of *S. cerevisiae* in vitro on rate of *R. solani* growth (cm)

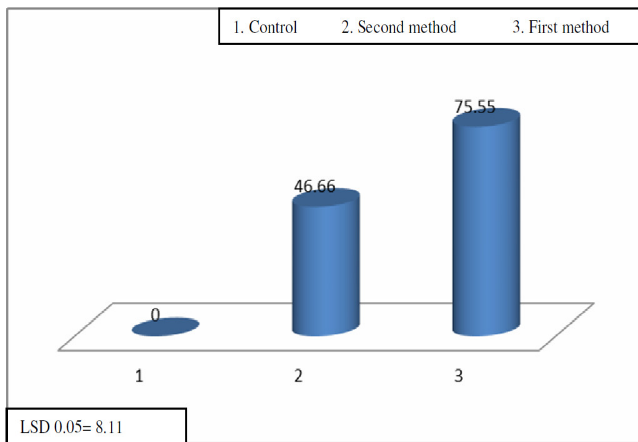


Fig. 2 : Effect of *S. cerevisiae* in vitro on inhibition percentage of *R. solani* growth (cm)

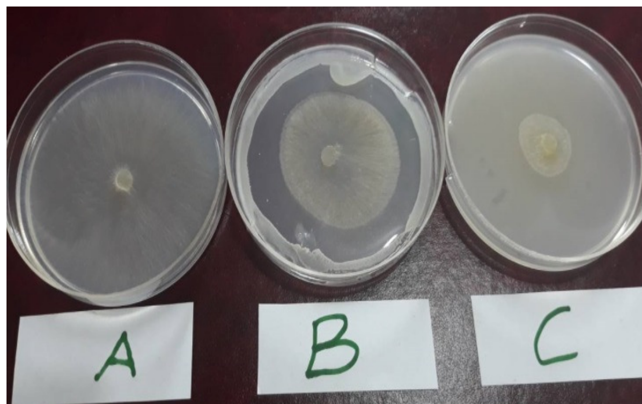
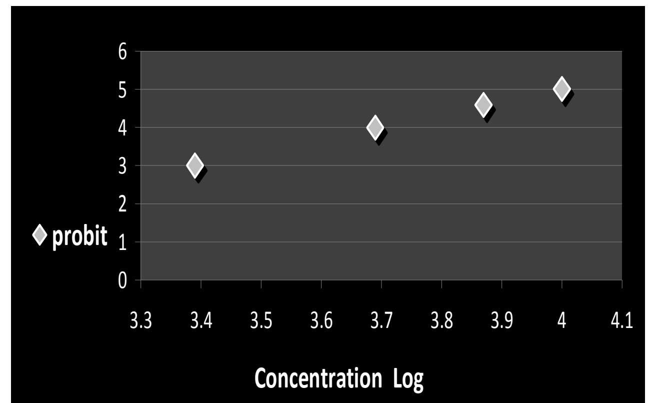


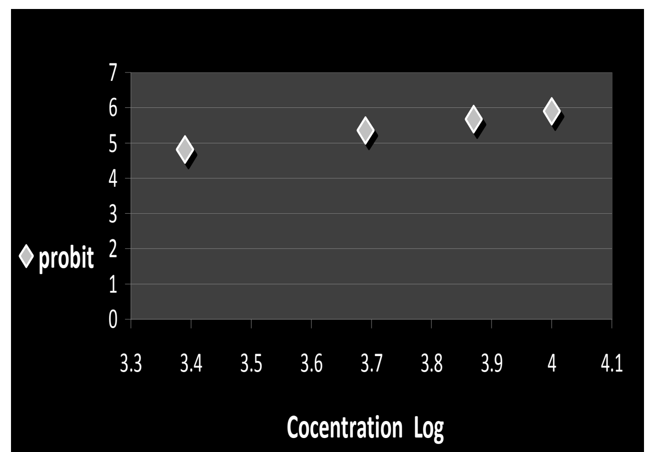
Fig. 3 : Effect of *S. cerevisiae* on *R. solani* growth on PDA medium, where A- Control, B- Second method, C- First method

Toxicity test of alcoholic extracts of *M. communis* and *P. euphratica* in the growth of *R. solani* and yeasts *S. cerevisiae* on PDA

The toxicity line was drawn for extracts of *M. communis* and *P. euphratica* separately through the values of concentration logarithm and the calculated probit values, then the effective concentration (EC50) for each extract in the yeast of *S. cerevisiae* and the fungus of *R. solani* was obtained (Fig 4 and 5).The yeast showed the high tolerant ability to the extracts toxicity compared to the tolerant ability of *R. solani* to their toxicity. The effective concentration (EC50) of *M. communis* extract in the yeast *S. cerevisiae* reached 9120 ppm, while the effective concentration (EC50) of *M. communis* extract in *R. solani* reached 3311 ppm, whereas the effective concentration (EC50) of *P. euphratica* extract in the yeast *S. cerevisiae* reached 8709 ppm, while the effective concentration (EC50) of *P. euphratica* extract in *R. solani* reached 3019 ppm. The inhibitory effect of both extracts in the growth of pathogenic fungus can be attributed to their contain numerous of chemical compounds that inhibit the growth of pathogenic fungus (Al-Tkirity, 1997; Martin *et al.,* 1999 Prior *et al.,* 2001; Hayder *et al.,* 2004 Patricia *et al.,* 2010; Resen *et al.,* 2016). Also, the high ability of the yeast to tolerate the extracts toxicity may be due to the ability of its cellular system to crashing the toxic biological compounds in these extracts or the concentrations of these toxic substances in both extracts may be within yeast tolerance range.

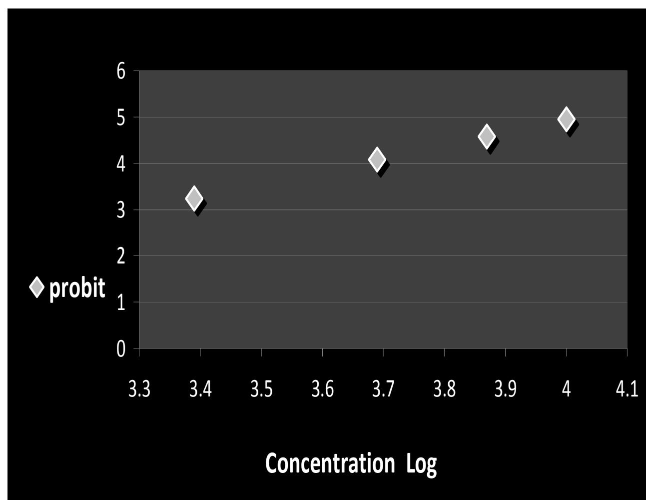


A. *M. communis* + *S. cerevisiae*

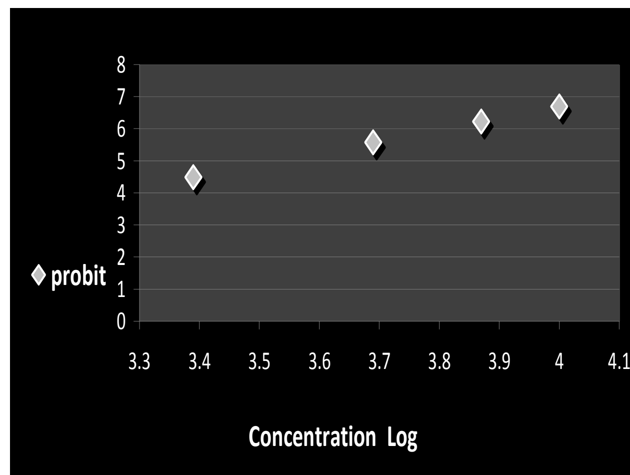


B. *M. communis* + *R. solani*

Fig. 4 : Determination of the effective concentration (EC50) to *M. communis* extract, where A= *S. cerevisiae*, B= *R. solani*



A. *P. euphratica* + *S. cerevisiae*



B. *P. euphratica* + *R. solani*

Fig. 5 : Determination of the effective concentration (EC50) to *P. euphratica* extract, where A= *S. cerevisiae*, B= *R. solani*

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The Fig. (6,7) showed that effect of *M. communis* and *P. euphratica* concentrations on growth of *R. solani*, where the fungus growth reduced gradually with the increase in levels of extracts concentrations from 2500 ppm to 10000 ppm as compared with control, the concentration of 10000 ppm was superior in reduction of fungus growth, so it selected for subsequent synergistic test

D C B A Co.

Fig. 6 : Effect of *M. communis* concentrations on growth of *R. solani*, where Co.= Control, A=2500 ppm, B=5000 ppm, C=7500 ppm and D= 10000 ppm

D C B A Co.

Fig. 7 : Effect of *P. euphratica* concentrations on growth of *R. solani*, where Co.= Control, A=2500 ppm, B=5000 ppm, C=7500 ppm and D= 10000 ppm

Synergistic effectiveness of *S. cerevisiae* and alcoholic extracts of *M. communis* and *P. euphratica* against *R. solani* on PDA

The results in Table (1) showed that efficiency of both alcoholic extracts and yeast and synergies among them in inhibiting the fungal growth with significant differences from control. This effect was very clear for *S. cerevisiae* + *M. communis* followed by *S. cerevisiae* + *P. euphratica*, which reached 82.22% and 78.88% respectively, then *P. euphratica*, *S. cerevisiae* and *M. communis*, which reached 75.55%, 74.44% and 71.11% respectively compared to control 0%. The synergistic action of *S. cerevisiae* with the alcoholic extracts of *M. communis* and *P. euphratica* showed significant superiority compared to using them separately against *R. solani*, this may be due to the ability of yeast to tolerate the toxicity of the alcoholic extracts . The inhibitory effect of both alcoholic extracts in the fungus growth can be attributed to their contains many of chemical compounds such as Flavonoids, Alkaloids, Polyphenols, Tannins, Glycosides, and Saponions that inhibit the growth of fungus

growth (Al-Tkirity, 1997; Martin *et al.,* 1999 Prior *et al.,* 2001; Hayder *et al.,* 2004 Patricia *et al.,* 2010; Resen *et al.,* 2016). The effectiveness of yeasts in the inhibition of pathogenic fungus can be attributed to their ability to compete for the place and food and have ability to parasitize on the pathogenic fungus as well as the production of many antibiotics with the inhibitory effect against many microorganisms (Slavikova and Vadkertiova, 2003; El-Tarabily, 2006; Young, 2012; Augusto and Pascholati, 2010; Franca *et al.,* 2015; Chen *et al.,* 2015; Fakruddin *et al.,* 2017).

Conclusion

The findings of this study showed that the tested yeast of *S. cerevisiae* and alcoholic extracts of *M. communis* and *P. euphratica* for each or synergies among them showed inhibiting effects against the tested pathogen *R. solani*, so it can be exploited the yeast and these plant extracts as natural fungicides to inhibit the growth of *R. solani.*

Table 1 : Effect of *S. cerevisiae*, *M. communis* and *P. euphratica* on inhibition percentage of *R. solani* growth

Treatments	Rate of fungus growth / cm	% Inhibition
Control	9	0
M. communis	2.6	71.11
P. euphratica	2.2	75.55
S. cerevisiae	2.3	74.44
S. cerevisiae +*M. communis*	1.6	82.22
S. cerevisiae +*P. euphratica*	1.9	78.88
LSD 0.05	0.32	**3.6**

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