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Evaluation Of The Effect Of Benzoic Acid On Some Plant Pathogenic Fungi

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Abstract
The objective of the study was to evaluate in vitro growth inhibition activity of benzoic acid against some pathogenic fungi. The pathogens used in the test were seven species of Fusarium (*F.moniliforme*, *F.oxysporium*, *F.solani*, *F.graminearum*, *F.sambucinum*, *F.equisetum* and *F. semitectum*), *Rhizoctenia solani* and *Verticillium dahliae*. The rates used were 0.4mg, 0.8mg and 1.2mg/Petri dish of benzoic acid mixed with 1 ml DMSO. PDA+1ml of DMSO were used as a control. All the concentrations of benzoic acid used in the study have shown great inhibition activity in all the fungi. The minimum effect was shown in the lowest concentration, which was 0.4mg/Petri dish. However, the inhibition percentage increased with the increase of the acid concentration. The lowest inhibition percentage (37.88%) was shown in the application of 0.4mg/Petri dish with *R.solani,* followed by that of with *F. semitectum* (56.63%) and *Verticillium dahliae* (60.81%). Most susceptible fungus was *F.sambucinum,* in this pathogen 73.94% growth inhibition was observed in 0.4mg/Petri dish, 100% growth inhibition in 0.8mg/Petri dish and 100% inhibition in 1.2mg/Petri dish, whereas the most resistant fungus was *F.moniliforme,* the only pathogen that revealed growth in all the tested acid concentrations. This finding indicated that benzoic acid has the potential to inhibit the growth of some plant pathogenic fungi.
Keywords: Benzoic acid, Pathogenic fungi, inhibition percentage.

INTRODUCTION

Fungi are the most important microorganisms that cause diseases in plants. Plant pathogenic fungi and oomycetes are the major causative agents of infectious crop diseases [1]. The association between fungi and plants is ancient. Management of plant diseases are a big concern for plant pathologists and their allied agro-experts. In this regard, scientists became very busy of getting environmental friendly way of solution to minimize the effect of plant diseases caused by fungi.

Organic acids usually used to control food-related microbes and they are not commonly used as a control agent for other microbes such as plant pathogenic fungi. They play a role for biological soil disinfestations as they suppress the growth of soil pathogens like *Fusarium oxysporum* and *Ralstonia solanacearum* [2]. Because of their antimicrobial activities, organic acids are used for seed disinfection. Seed treatment results showed that Banzoic acid yielded best after salicylic acid when Elmahdy, A.A et al., 2015 [3] studied it to see their effect on rice seed germination, vigor and infestation with fungi. Bracey et al., 1998 [4] reported that; Acidification of the cell and membrane disruption are among the inhibitory mode of organic acids. Some other researches revealed that, organic acids stop or interrupt metabolic activities [5].Among organic acid Benzoic acid its derivatives known to have high antifungal activity and that is why it is used largely for food preservations.

Due to their chemical structures, effects of the acids on the growth of fungi might be different. Formic acid, acetic acid and propionic acid had a similar strong inhibitory effect on *Aspergillus flavor* as [6] stated. N.V. Narendranath et al. 2001, [7] reported that the inhibitory effect of acetic and lactic acids in a same medium revealed synergism. Researches concern organic acids and their effect on plant pathogenic fungi are very limited, so the aim of this study was to investigate the effect of Benzoic acids on the mycelial growth of some important plant pathogens.

MATERIALS AND METHODS

Benzoic acid

Benzoic acid was used to test its antifungal activities against seven *Fusarium* species, *Rhizoctonia solani* and *Verticillium dahliae.* Acid amounts were dissolved in 1ml DSMO to mix it well. The doses used during the study were 0.4 mg, 0.8 mg and 1.2 mg/Petri dish (90 x 15mm Petri dishes).

Fungal strains

Fusarium species (*F. oxysporum, F. sambucinum, F. semitectum, F. moniliforme, F. solani, F. equisetum and F. graminearum*), *Rhizoctenia solani* and *Verticillium dahlia* were obtained from the department of Plant pathology, mycology laboratory in Ataturk university, Erzurum Turkey. Then, these fungal isolates were cultured in Potato Dextrose Agar (PDA) and kept at 4 ^{0}C. The isolates were sub cultured in a new slant of PDA and left in the incubator at 25 ^{0}C for 7 days until used.

Culture Preparation

For checking the antifungal activities of the used acid, Potato Dextrose Agar was the basal medium used. 250 ml sterile water allocated into 500 ml Erlenmeyer Flasks, added 10 g of PDA and sterilized in the autoclave at 121C^o in 15min. When the media was removed from the Autoclave, it was cooled a short while and then mixed it with the dissolved acid solutions gently. Flasks added only 1ml of DMSO used as control. After that, the treated media were poured into Petri dishes under aseptic conditions and waited until solidified. 5 mm fungal discs taken from 1-week-old cultures and inoculated at the center of the Petri dishes. All the tests were in a same procedure.

Growth inhibition

After the application of fungi with the acid, the growth of fungal mycelia measured in 7 days and then their average was taken to use in the statistical analysis. Using the following formula, the inhibition percentage caused by the acid concentrations were calculated,

Inhibition percentage (%) =

$$\frac{\text{Diameter of the control—diameter of the treatment}}{\text{Diameter of the control}} \times 100$$

STATISTICAL ANALAYSIS

To compare the means of the mycelial growth, the data was subjected one-way analysis of variance (ANOVA) in SPSS software and the treatment differences were tested using Duncan's multiple range test. P<0.05 level of significance was considered for significance.

RESULTS AND DISCUSSION

Three different concentrations of benzoic acid were applied to the growth media of the fungi to see the effect that it can have the mycelial growth of the fungi. Inhibition percentages caused by this three concentrations 0.4mg, 0.8mg and 1.2mg/ Petri dish compared to the control with their mean differences adapted from ANOVA results presented as

Table1: Effect of Benzoic acid concentrations on the growth of the fungi and its percent of inhibition.

Fungi	Doses						
	1.2mg/Petri dish		**0.8mg/Petri dish**		**0.4mg/Petri dish**		**Control**
	Mean of mycelial growth(cm)	Inhibition percentage %	Mean of mycelial growth(cm)	Inhibition percentage %	Mean of mycelial growth(cm)	Inhibition percentage %	Mean of mycelial growth(cm)
R.solani	0.0± 0.0c*	**100**	1.02± 0.11e	**79.56**	3.10 ±0.20e	**37.88**	4.99±0.33
V.dahliae	0.0 ±0.0c	**100**	0.67±0.0c	**84**	1.67±0.019c	**60.81**	4.26±0.04
F.oxysporum	0.0 ±0.0c	**100**	0.0 ±0.0c	**100**	1.53± 0.10c	**62**	4.04±0.16
F.solani	0.0 ±0.0d	**100**	0.0 ±0.0d	**100**	1.47±0.02d	**66**	4.33±0.12
F.semitectum	0.0 ±0.0d	**100**	0.0 ±0.0d	**100**	1.73±0.31d	**56**	4.0±0.03
F.sambucinum	0.0 ±0.0b	**100**	0.0 ±0.0b	**100**	0.91±0.02b	**74**	3.5±0.34
F.graminerum	0.0 ±0.0c	**100**	0.0 ±0.0c	**100**	1.50±0.**19c**	**61.6**	3.91±0.41
F. moniliforme	0.54±0.01a	**85.9**	0.82±0.10a	**78.6**	1.35±0.05a	**64.8**	3.84±0.10
F. equisetum	0.0 ±0.0c	**100**	0.92±0.12c	**78**	1.42±0.01c	**66.5**	4.25±0.17

*Mean + standard deviation.

As indicated in the table above, the effect was concentration dependent manner. The highest dose of 1.2mg/Petri had the greatest effect on the growth, where no growth seen except one fungus (*F.moniliforme),* which we can say the most resistant fungus among the species used in this test. In this pathogen the percentages of inhibitions caused by benzoic acid concentrations (0.4, 0.8, 1.2mg/Petri) were 64.8, 78.6 and 85.9%, respectively. *Rhizotonia solani*, *Verticillium dahliae* and *F.equisetum* revealed small growth at 0.8mg/ Petri dish and no growth seen at 1.2mg/Petri dish, at 0.8mg dose of benzoic acid growth inhibitions of these three fungi were 79.56, 84 and 78%, respectively.

For the smallest dose (0.4mg/Petri dish), all the fungi used demonstrated growth though it was various. At this dose, *R.solani* was the most resistant compared to others, the percentage of inhibition caused by benzoic acid was only 37.88%, while for the others it was between 56-74%.

Mechanisms that allow acids to inhibit or interrupt the growth of fungi are their ability to affect fungal cell functioning. They alter many parts of the cell and they have an effect on the respiration of the cell. Benzoic acid is in the first line of the acids used for food preservations because of its inhibitory effects on yeast and fungi [8]. Most of the works and researches, which relate antimicrobial activities of organic acids such as benzoic acid and its derivatives, show great effect on the growth of microorganisms. B.E. Amborabé et al. 2002, [9] tested the antifungal activity of benzoic acid and its derivatives against *Eutypa lata* and found growth inhibition both in solid and liquid medium at low ph. The degree of inhibition may differ from one fungus to another due to fungal structure and susceptibility. RK Pundir et al. 2010, [10], who found 75 to 100% of mycelial growth inhibition against food associated fungi, got near result to our finding.

Furthermore, it is known that, the action of organic acids as antimicrobial agents is generally improved by anions, which interfere with the dissociation of the acid molecule; certain specific cations may also significantly increase the effectiveness of organic acids by increasing the solubility of the acid in the microbial cell membrane [11]. We can conclude that benzoic acid have antifungal activity against tested plant pathogens.

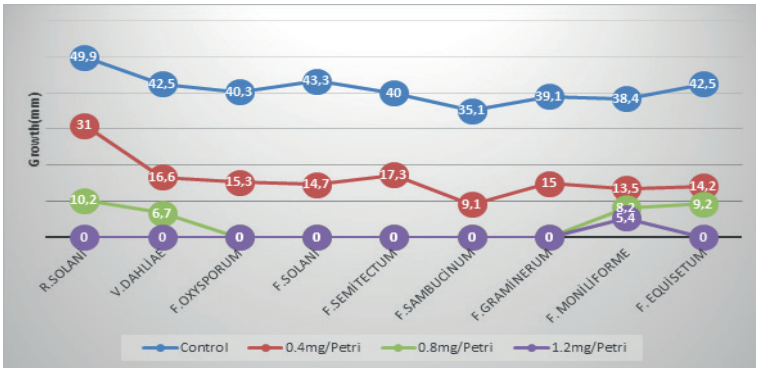

Figure 1 effect of benzoic acid concentrations on mycelial growth of the fungi compared with the control

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