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Original Research Article

The Effect of Acetic, Formic and Propionic Acids on Plant Pathogenic Fungi

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ABSTRACT

The efficiency of low or non-toxic chemicals is alternatives to fungicide usage. Especially, GRAS (Generally Recognized as Safe) compounds are quite suitable to prevent plant disease development. Propionic, formic and acetic acid were selected to state antifungal activities on some soilborne plant pathogens that are in the GRAS chemicals list. GRAS compounds were tested on, *Macrophomina phaseolina*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Fusarium oxysporum* and *Rhizoctonia solani* to understand the efficiencies of organic acids on the plant pathogen development. The mycelial growth inhibition of propionic, formic and acetic acids was determined. Minimum inhibition concentration (MIC) and minimum fungicidal concentrations (MFC) of the organic compounds were stated also. Propionic was significantly better than formic and acetic acid. Propionic acid at 0.7%, formic acid at 0.9% and acetic acid at 1.8% concentration was totally inhibited mycelial growth of all fungi, respectively. Organic compounds efficiency was variable and shown a different impact on fungi based on their resistance. *B. cinerea*, *S. sclerotiorum* and *F. oxysporum* resistance was higher than *R. solani* and *M. phaseolina*.

Keywords: GRAS, Plant pathogen, Mycelial growth, Minimum inhibition concentration (MIC), Minimum fungicidal concentration (MFC)

INTRODUCTION

In simple terms, GRAS (Generally Recognized as Safe) chemicals are environmental friendly, low or non-toxic compounds that are allowed to contact food and food additives (FDA 2016). Most of GRAS chemicals are currently used in the food industries as a food additives and consume by humans. GRAS compounds are not only used in the food industry, but also used in conventional farming. In agricultural pest management, some of the GRAS ingredients are allowed to use in the organic farming. GRAS compounds are one of the alternative methods to synthetic pesticide to prevent plant pests (Corral *et al.* 1988). Organic farming has strict rules and limitation about pesticide usage related to pesticide residues. Besides, in most of the countries, using synthetic pesticides in organic farming is not allowed and forbidden. Thus, an alternative method or compounds are needed instead of pesticide usage to prevent plant diseases. Furthermore, pesticides threaten human, animal and environmental health and cause development of resistance in plant pathogens. Organic acids could be a solution of the resistance problems and health risks caused by pesticides. And also, organic acids chemical bond and structures are easily degradable in soil or on plant foliar. There will be no harmful residue after degradation. That's why they are environmentally friendly chemicals or compounds. Organic acids can be used to prevent plant disease development and to reduce mentioned risks. In agricultural farming, soilborne plant pathogens are one of the major problems (Koike *et al.* 2003). Soilborne pathogens cause economical and yield losses at field and after harvest and even postharvest and storage period. Soilborne pathogens cause root decay, damping off, root softening and death of the plant (Dreistadt S. H. 2001). Some of the important soilborne pathogens are *Fusarium, Rhizoctonia, Macrophomina* and *Sclerotinia* (Agrios 1988). The objective of the study is to determine inactivating capacity of propionic, formic, and acetic acids on plant pathogen mycelial growth. Mentioned organic acids are widely used to prevent plant disease development, reduce germ tube elongation and inactivation of spore germination. Organic acids show respectable results on soilborne pathogen mycelial inactivation (Goepfert and Hicks 1969, Kunte *et al.* 1998, Sholberg 1998).

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MATERIALS AND METHODS

Fungi Isolate

The fungi cultures were isolated from infected plants in Bursa region. After the identification of the fungi, they were storaged at +4^{o}C in potato dextrose agar (PDA, Difco, Detroit, USA) media until use. The fungi isolate that used in this study were; *Macrophomina phaseolina* (Goid.), *Botrytis cinerea* (Pers.), *Sclerotinia sclerotiorum* (de Bary), *Fusarium oxysporum* (Schlecht. emend. Snyder and Hansen), and *Rhizoctonia solani* (J. G. Kühn).

Gras Chemicals

In this study some of the organic acids that in the GRAS chemicals list were used. The used acids were propionic acid (Merck, Germany), formic acid (Merck, Germany), and acetic acid (Merck, Germany). Propionic acid ($C_3H_6O_2$) is used as a food additive and as a preservative compounds. In addition, propionic acid inhibits fungi and bacteria development and also is used in pesticide making. Formic acid (CH_2O_2) is occurring naturally and notably from ants. Formic acid has a preservative effect and has antibacterial properties. Moreover, it is used as miticide. Acetic acid ($C_2H_4O_2$) is one of the most widely using organic acid in all. It is generally used as food an additive as a regulator and as a condiment. As a food additive, it is approved for use in many countries (Davidson and Juneja 1990).

Soilborne Cultures

Soilborne plant pathogens were cultured in PDA media for 7 days at 25 °C. After an incubation period, all pathogens mycelial and if available conidia was looked under the light microscope and identified as a pure culture. Afterwards, they were used in the experiment.

In vitro Toxicity of Gras Chemicals

In the toxicity experiments, application concentrations were stated for all organic acids separately. Propionic and formic acid application doses were from 0.1% to 1% with a 0.1% addition. On the other hand, acetic acid application doses were from 0.1% to 2% and the addition was 0.1% until 1% concentration, when reach %1 concentration of acetic acid, addition was 0.2% afterwards. Organic acid concentration was added to PDA agar media that was cooled 50^{o}C before the addition of the organic acids. Afterwards, PDA that has organic acids were poured into 6 cm petri dishes. From each media culture 5 mm disc (plate) was obtained via cork borer and placed the center of the petri dishes. The inhibition effect of the organic acids against soilborne pathogens was tested by placing pathogens culture disc to the PDA media that containing suitable organic acids concentration. The PDA media was not included organic acids used as a control. Every petri dish was placed in an incubator for the incubation period for 3-6 days at 25^{o}C. On a daily basis, all petri dishes were observed. After the incubation period, all soilborne pathogens radials and mycelial growth were measured. The minimum inhibition concentration (MIC) and MFC (minimum fungitoxic concentration) of the organic acids were detected.

Statistical Analysis

Statistical analysis was performed in one way "ANOVA" to incidence data. Mean values of the data were separated by using LSD test ($P \leq 0.05$). At tables, values in columns followed by the same letter are not significantly different according to LSD test ($P \leq 0. 05$). Experiments were conducted three times consecutively.

RESULTS

Organic Acid Activity

All organic acids have shown inhibition on soilborne pathogens mycelial growth. Yet, all of them inactivation activity was variable. This variation has come from fungi resistancy. All fungi have unique resistant mechanisms against organic acids. Some of the fungi that used in this study such as *B. cinerea* and *F. oxysporum* were more resistant than others.

Propionic Acid Activity

Propionic acid was one of the most successful application when compared with others. Propionic acid has shown variable inhibition effect on soilborne fungi. The variation of the efficiency was came from soilborne pathogens resistance capacity. In all soilborne pathogens, *B. cinerea* was one of the most resistant (Figure 1.). Propionic has shown inhibition at even lowest concentration (Table 1.). Also, the inhibition rate of the propionic acid was more superior with increasing concentrations. The lowest concentration of the propionic acid was delayed approximately the half of the all soilborne mycelial growth. *R. solani* was inhibited at 0.4% concentration. At 0.5% concentration addition the *R. solani*, *M. phaseolina* and *S. sclerotiorum* were inhibited. Similarly, at 0.6% concentration *F. oxysporum* and at 0.7% concentration *B. cinerea* were inhibited (Table 1.). Propionic acid has shown a great effect at 0.7% concentration and inhibited all pathogens mycelial growth totally (Figure 2.).

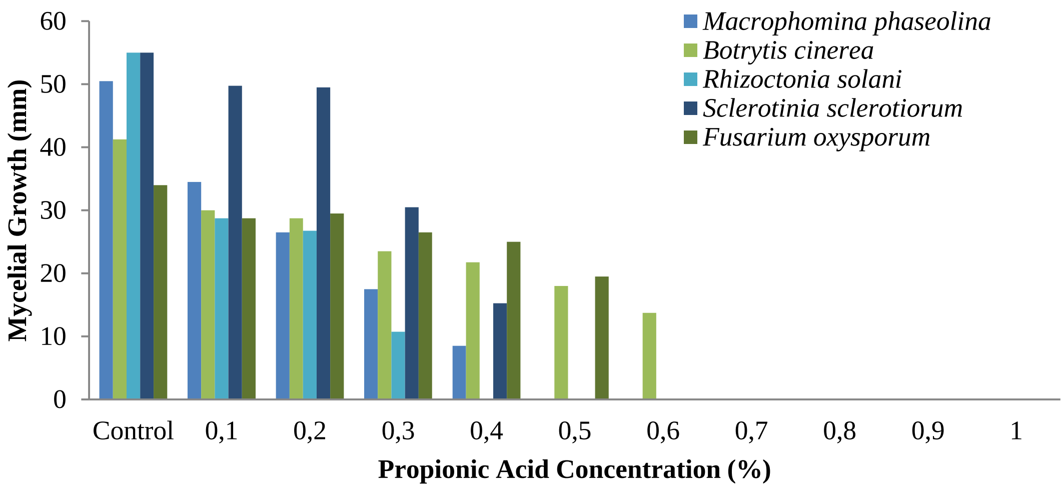

Figure 1. Mycelial growth inhibition of propionic acid on soilborne pathogens.

Table 1. The effect of the organic acids concentration on fungi mycelial growth.

	Mycelial Growth (mm)				
	Soilborne Plant Pathogens				
Application (% Concentration)	***M. phaseolina***	***B. cinerea***	***R. solani***	***S. sclerotiorum***	***F. oxysporum***
Control	50,5 ab	41,25 c	55 a	55 a	34 ab
Propionic acid					
0.1	34,5 ef	30 e	28,75 fg	49,75 b	28,75 f
0.2	26,5 g	28,75 e	26,75 gh	49,5 b	29,5 ef
0.3	17,5 h	23,5 f	10,75 j	30,5 e	26,5 g
0.4	8,5 ı	21,75 fg	0 l	15,25 g	25 gh
0.5	0 j	18 gh	0 l	0 h	19,5 ı
0.6	0 j	13,75 hı	0 l	0 h	0 m
0.7	0 j	0 j	0 l	0 h	0 m
0.8	0 j	0 j	0 l	0 h	0 m
0.9	0 j	0 j	0 l	0 h	0 m
1	0 j	0 j	0 l	0 h	0 m
Formic acid					
0.1	47,75 c	55 a	47,25 b	55 a	34,75 a
0.2	48,75 bc	55 a	38,5 d	45,25 c	31,75 cd
0.3	24 g	55 a	24,75 h	32,5 e	29,25 f
0.4	18,75 h	55 a	4,25 k	22,75 f	25 gh
0.5	0 j	55 a	0 l	0 h	18,75 ı
0.6	0 j	53,75 a	0 l	0 h	11,75 l
0.7	0 j	35,25 d	0 l	0 h	0 m
0.8	0 j	24 f	0 l	0 h	0 m
0.9	0 j	0 j	0 l	0 h	0 m
1	0 j	0 j	0 l	0 h	0 m
Acetic acid					
0.1	51,5 a	47,5 b	40,75 c	55 a	31,75 cd
0.2	49,75 abc	41,5 c	37 d	39,5 d	32,5 bcd
0.3	42,5 d	47,75 b	34,25 e	33,25 e	31 de
0.4	37 e	45,75 bc	29 f	22 f	33,25 abc
0.5	33,75 f	46,25 b	18,75 ı	16,5 g	32,25 cd
0.6	25 g	36 d	11,25 j	0 h	23,75 h
0.7	17,5 h	29,75 e	0 l	0 h	17 j
0.8	0 j	22,75 f	0 l	0 h	14,5 k
0.9	0 j	22,5 f	0 l	0 h	12,5 l
1	0 j	13,5 hı	0 l	0 h	12,25 l
1.2	0 j	12,5 ı	0 l	0 h	0 m
1.4	0 j	10,75 ı	0 l	0 h	0 m
1.6	0 j	4 j	0 l	0 h	0 m
1.8	0 j	0 j	0 l	0 h	0 m
2	0 j	0 j	0 l	0 h	0 m

Values in columns followed by the same letter are not significantly different according to LSD test ($P \leq 0.05$).

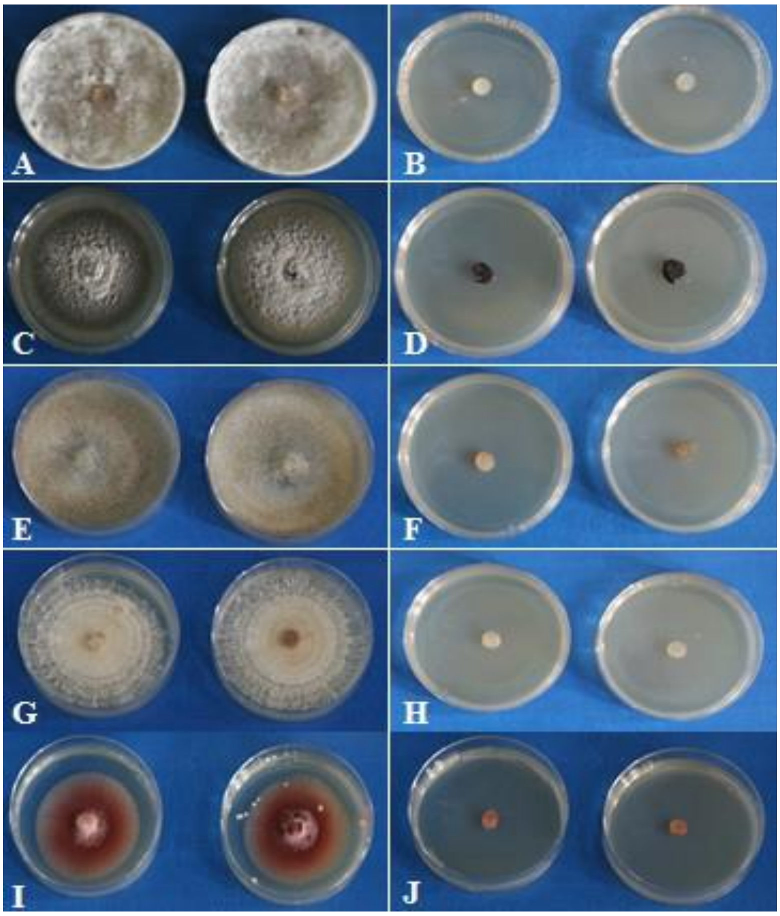

Figure 2. Efficiency of propionic acid application on soilborne plant pathogens at 0.7% concentration. **A:** *S. sclerotiorum* control, **B:** *S. sclerotiorum* propionic acid application, **C:** *M. phaseolina* control, **D:** *M. phaseolina* propionic acid application, **E:** *B. cinerea* control, **F:** *B. cinerea* propionic acid application, **G:** *R. solani* control, **H:** *R. solani* propionic acid application, **I:** *F. oxysporum* control, **J:** F. *oxysporum* propionic acid application.

Formic Acid Activity

Formic acid was the second most effective organic acid and was shown similar results with propionic acid. Correlatively, *B. cinerea* was most resistant fungi against formic acid as expected. And also, soilborne pathogens resistance capacities were much higher than propionic acid or in the other words formic acid application was not effective as propionic acid. Formic acid inhibition efficiency was unsatisfying at lower concentration. To reach the satisfactory results, was needed higher concentration of the formic acid (Table 1.). At least 0.3% concentration of the formic acid was needed to inhibit half of the fungi mycelial growth except *B. cinerea* (Figure 3.). Formic acid application at 0.4% concentration was delayed only *R. solani* and *M. phaseolina* mycelial growth. Formic acid inhibition activity was improved with increasing concentration. *S. sclerotiorum* was inhibited at 0.5% concentration. Formic acid was shown inhibition on *F. oxysporum* at least 0.7% concentration. Increasing concentration of the formic acid affected *B. cinerea* mycelial growth. Mycelial growth of the soilborne pathogens was totally was inhibited at 0.9% concentration of the formic acid (Figure 3.).

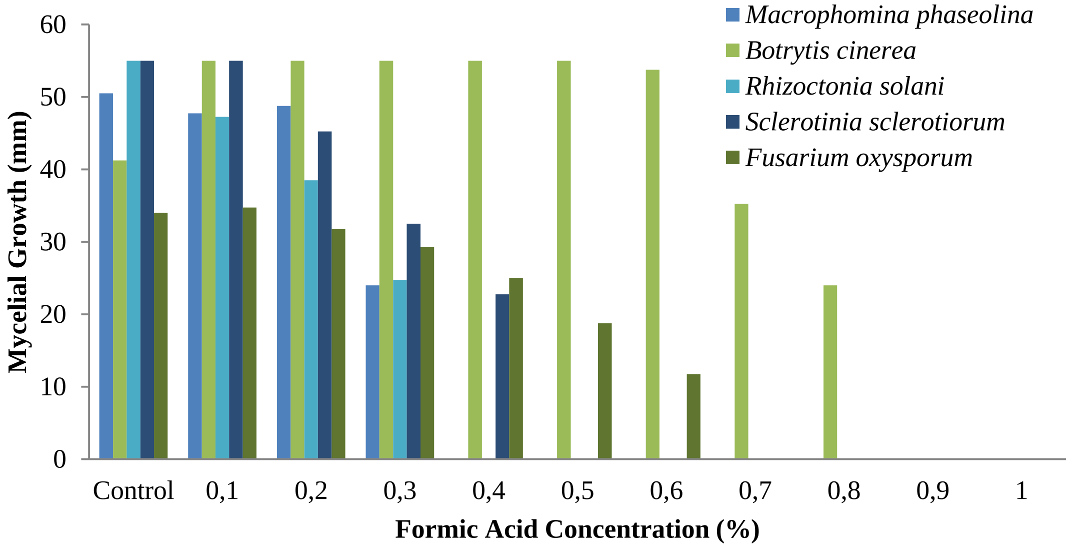

Figure 3. Mycelial growth inhibition of formic acid on soilborne pathogens.

Acetic Acid Activity

Acetic acid was ineffective one when compared with propionic and formic acid. Acetic acid low concentrations was not effective as others (Table 1.). To inhibit half of the mycelial growth of the soilborne was needed approximately 0.4% concentration or higher (Figure 4.). Again, *B. cinerea* was most resistant fungi against acetic acid. To inhibit total mycelial growth of the *B. cinerea* was needed higher than 1.6% concentration or higher. Accordingly, *F. oxysporum* was second most resistant fungi and inhibition of the *F. oxysporum* was occurred at 1.2% concentration. It was found that *S. sclerotiorum*, R. *solani*, *M. phaseolina*, *F. oxysporum*, and *B. cinerea* mycelial growth was inhibited at 0.5%, 0.7%, 0.8%, 1.2%, and 1.8%, respectively.

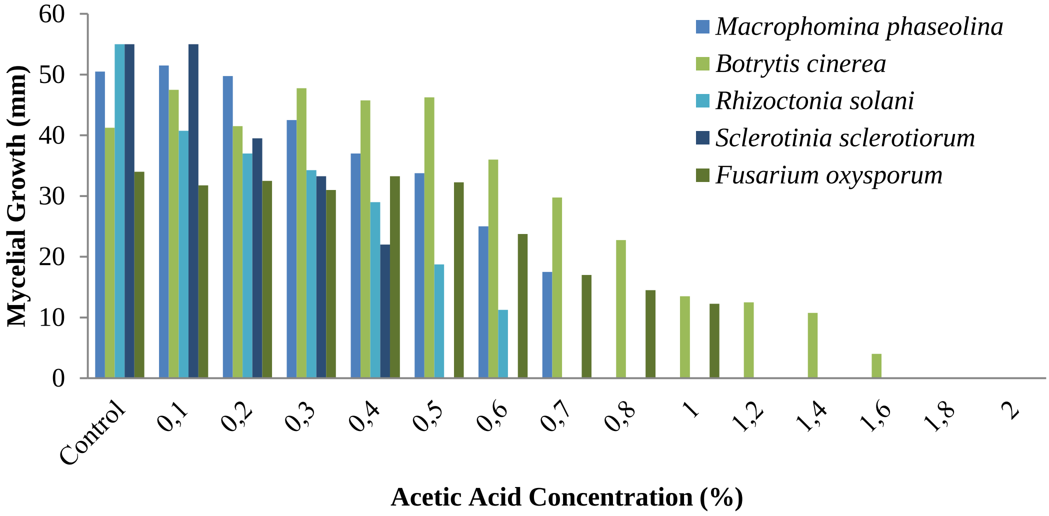

Figure 4. Mycelial growth inhibition of acetic acid on soilborne pathogens.

MIC and MFC Values Comparison of the Organic Acids

Organic acids efficiency was tested within 2 sections. One of them was mycelial growth inhibition and the second one was determining MIC and MFC values of the organic acids. MIC means inhibition activity is reversible, but MFC means inhibition activity is irreversible. MIC activity is removed from the area when the organic acids and the mycelia interaction has no longer. On the other hand, by the MFC activity, development of the fungi mycelia permanently inhibit, even if fungi mycelia has placed another media.

MIC values Comparison

Propionic acid inhibition activity was higher than formic and acetic and so, propionic acids MICs was much lesser than formic and acetic (Table 2.). Propionic was most effective organic acid that tested in the study. Approximately, 0.4% or higher concentration enough for the MIC effect against all soilborne. Lowest MIC values were recorded at 0.4 % concentration of the propionic acid against *R. solani*. At 0.5% concentration of the propionic acid was enough to stop the mycelial growth of the *M. phaseolina* and *S. sclerotiorum* (Table 2.). To inhibit *F. oxysporum* mycelial growth temporary was needed at least 0.6% concentration. MIC values of *B. cinerea* was needed 0.7% or higher concentration. Formic acid has shown similar effects as propionic acid on soilborne pathogens. Specially, *R. solani* and *S. sclerotiorum* MIC value was exactly the same with propionic acid. *B. cinerea* and *F. oxysporum* were resistant fungi. Formic has shown a lesser effect than propionic acid against *B. cinerea* and *F. oxysporum*. MIC value of the formic acid concentration against *B. cinerea* and *F. oxysporum* were 0.9% and 0.7%, respectively. Acetic acid MIC values were much higher than propionic and formic acid as expected, excluding *S. sclerotiorum*. Furthermore, acetic acid MIC values were approximately two times more than propionic and formic. But, there was an exceptional situation about *S. sclerotiorum*. Inhibition of the *S. sclerotiorum* was observed at 0.5% concentration of the acetic acid (Table 2.). Acetic acid was effective as propionic and formic against *S. sclerotiorum* and MIC values of the organic acids were the same.

Table 2. MIC and MFC values of the organic acid on soilborne plant pathogens.

	Soilborne Plant Pathogens									
	M. phaseolina		***B. cinerea***		***R. solani***		***S. sclerotiorum***		***F. oxysporum***	
Application (% Concentration)	*MIC	**MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Propionic Acid	0,5	0,6	0,7	0,9	0,4	0,5	0,5	0,6	0,6	0,7
Formic Acid	0,4	0,5	0,9	>1	0,4	0,6	0,5	0,6	0,7	0,9
Acetic Acid	0,8	>1,2	1,8	>2	0,7	1	0,5	0,7	1,2	>1,4

*MIC; Minimum inhibition concentrations
**MFC; Minimum fungicidal concentrations

MFC Values Comparison

MFC refers, permanent inhibition of the mycelial growth. And even if the interaction of the fungi and organic acid removed, fungi will not develop. For this reason, resistant fungi have higher MFC value than sensitive ones. *B. cinerea* was most resistant fungi against all organic acid, so its MFC values for propionic, formic and acetic were 0.9%, over 1% and over 2% concentrations, respectively. *B. cinerea* was followed by *F. oxysporum* and its MFC values, based on propionic, formic and acetic were 0.7%, 0.9% and over 1.4%, respectively. *M. phaseolina*, *R. solani* and *S. sclerotiorum* has relatively similar MFC results in themselves (Table 2.). MFC values of the *M. phaseolina* were 0.6%, 0.5% and over 1.2%, according to propionic, formic and acetic, respectively. On the other hand, propionic, formic and acetic acid MFC values against *R. solani* were 0.5%, 0.6% and 1%, respectively. And finally 0.6%, 0.6% and 0.7% concentrations of the propionic, formic and acetic acid for *S. sclerotiorum*, respectively (Table 2.).

DISCUSSION

Recent years, organic farming has become more and more important. And also, attention and awareness to the organic farming is rising due to the health issues, environment contamination and pesticide residue problems caused by conventional farming and pesticide usage. However, synthetic pesticide usage also increased to prevent disease development to get more quantities and quality products. These interactions occur a vicious cycle. Using synthetics to get more products cause more problem. Thereof, organic acids could be a solution about this problem and also an alternative to the synthetic pesticide to prevent plant diseases. For these purpose; some of the organic acids, which were propionic, formic and acetic, were used to get effective results against soilborne plant pathogen. Propionic, formic and acetic acid has shown remarkable results on soilborne pathogen mycelial growth inhibition. Inhibition of the mycelial growth of the fungi by organic acid was occurred with 2 different effect mechanism. One of the mechanism was contact and other one was volatile. The mechanism was worked in two steps; step one was contact of the organic acid with fungi. Step one was showing direct interaction on mycelia and fungi was inhibited. The second step was; volatile effect. Organic acid was vaporized in PDA media and volatile compounds of the organic acid was shown inhibition on fungi. These organic acids have a volatile effect on plant pathogen (Sholberg and Gaunce 1995; 1996). For these reason efficiency was raised with contact and volatile combination. Most of the study has done about their volatile effect on many other fungi and get significant results. Propionic, formic and acetic acid contact activity was effective as well as their volatile effect against soilborne plant pathogen development. Development of mycelial growth of the fungi was inhibited (Sholberg, 1998; Ushiwata *et al.* 2009). Organic acids show respectable results on soilborne pathogen inactivation (Goepfert and Hicks 1969, Kunte *et al.* 1998, Sholberg 1998). Furthermore, MIC and MFC values was observed. MFC was critical for these kind of study and organic acids has shown great activity on soilborne pathogen. The main objective about plant diseases, prevent was inhibited development. But, sometimes inhibition was occurred temporary by antimicrobial agent and antimicrobial agent removes from the contact or volatile area, effect of the inhibition disappears. Thus, development of the plant diseases starts again.

To get over this problem was needed permanent solution. And to get permanent solution was needed MFC value. Cause, MFC values of the organic acid inhibit fungi development totally and these effects aren't reversed. In this study organic acid has shown great inactivation on soilborne pathogen and MFC was observed. In further studies will be about their combination and volatile effect together to achieve more reliable results against plant pathogens. Similarly, find out new possible usage of the organic acids in organic farming.

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