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Control of Phytopathogenic Fungi by Generally Recognized as Safe (GRAS) Acids

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The efficacy of Generally Recognized as Safe (GRAS) acids (citric, formic, lactic, malic, phosphoric and propionic acids) was evaluated as possible alternatives to synthetic fungicides for the control of the economically important phytopathogenic fungi including *Fusarium culmorum*, *F. nivale*, *F. solani*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Uromyces appendiculatus*. The concentration of acids that caused a 50% reduction (ED_{50}), the minimum inhibition concentration (MIC), and the minimum fungicidal concentration (MFC) values for mycelial growth, spore germination and germ tube elongation indicated that formic acid, propionic acid and phosphoric acid were generally more toxic to the tested fungi than the other acids. Therefore, formic and propionic acid were selected for further testing in soil. Formic acid and propionic acid completely inhibited the mycelial growth of *S. sclerotiorum* at 0.2%. These acids also completely inhibited the mycelial growth of both *F. culmorum* and *R. solani* at 0.6% in soil tests. Selected concentrations of acids were tested for efficacy against *U. appendiculatus* on bean plants in pots under controlled conditions. In these tests, control efficacy against *U. appendiculatus* of all acids ranged from 0 to 85.7%, with citric acid being the most effective treatment. The results of this study showed that the acids tested could become natural alternatives to synthetic fungicides for control of phytopathogenic fungi.

Key words: Soil-borne fungi, Bean rust, Antifungal activity, Alternative control, Natural substances, Soil tests.

Fungal plant diseases are one of the major causes of agricultural losses. For many years, a variety of different synthetic chemicals such as benzimidazoles, aromatic hydrocarbons and sterol biosynthesis inhibitors have been used as antifungal agents to inhibit the growth of plant pathogenic fungi[1,2]. The widespread use of pesticides has significant drawbacks including increased cost, concern about pesticide residues on food, and potential threat to human health and the environment[3]. In addition, the effective use of these chemicals is not possible in areas where the fungi have developed resistance[4]. In order to overcome the resistance problem, higher concentrations of fungicides were used. However, overdosing may increase the risk of toxic residues in the food products.

In recent years there has been considerable pressure by consumers to reduce or eliminate chemical fungicides in foods[5,6]. Worldwide 'organically grown' fruit, which has not been treated with fungicides, is becoming popular

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among consumers. Under these circumstances, there is an urgent need for alternative methods of controlling plant diseases without the use of synthetic fungicides. Organic acids may provide a reliable alternative to currently used synthetic fungicides to control phytopathogenic fungi.

Except for phosphoric acid, the acids used in this study are organic acids. Organic acids such as lactic, malic, and citric acid are natural substances found in various fruits and fermented products that exhibit antimicrobial activity against foodborne pathogens[7]. All acids used in this study have approved as "generally recognized as safe" (GRAS) for use in human food by the U.S. Food and Drug Administration.

Organic acids have been widely used as preservatives in foods and buffer agents in medical solutions[8,9]. Several studies have reported the inhibitory effect of acids such as saturated fatty acids, formic and propionic acids, lactic acid and medium-chain fatty acids against different microorganisms[10-12]. In addition to their suppressing effect on the growth of food spoilage microorganisms, organic acids were shown to possess antibacterial activities against various humanpathogens[13,14]. In ecological farming, natural antimicrobial compounds like organic acids can be used for seed disinfection as an alternative, or in combination with physical treatment[15].

The objective of the present work was to evaluate the efficacy of Generally Recognized as Safe (GRAS) acids (citric, formic, lactic, malic, phosphoric and propionic acids) for the control of phytopathogenic fungi including *Fusarium culmorum, F. nivale, F. solani, Macrophomina phaseolina, Rhizoctonia solani, Sclerotinia sclerotiorum* and *Uromyces appendiculatus.*

MATERIALS AND METHODS

Acids tested

All acids (citric, formic, lactic, malic, phosphoric and propionic acids) used in this study were purchased from Merck (Darmstadt, Germany).

Fungi

The phytopathogenic fungi used in this study were: *Fusarium culmorum*, *F. nivale*, *F. solani*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Uromyces appendiculatus*. Except for *U. appendiculatus*, the fungi were routinely maintained on potato dextrose agar (PDA, Difco, Le Pont de Claix, France) and were stored in PDA slants at 5ºC for further use. *U. appendiculatus* was maintained on the bean as a host plant. The cultures were obtained from the fungal collection of Uludag University, Faculty of Agriculture, Department of Plant Protection. These selected pathogens cause yield losses innumerous economically important crops.

Efficacy of acids on mycelial growth

The desired quantities of acids were added to autoclaved and cooled PDA medium at 50ºC to obtain concentrations of 0.01, 0.02, 0.03, 0.04, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0% (v/v or w/v). The acid-amended medium was dispensed (10 ml per plate) aseptically into 6-cm diameter Petri plates. A mycelial disc (5-mm-diameter) taken from 7-day-old culture of the respective fungus was placed in the center of each acid-amended PDA. The plates were then sealed with Parafilm and incubated at 25ºC in the dark for 2-5 days. Mycelial growth was measured daily at two perpendicular colony diameters until the growth in the control plates reached the edge of the plates. The plates without the acid were used as control. Percentage inhibition reported is the ratio of mycelial growth compared with that of the control. The concentrations of acids that caused a 50% reduction (ED_{50}) of mycelial growth were calculated by probit analysis (SAS Institute, Cary, NC, USA). The minimum inhibition concentration (MIC) that completely inhibited the mycelial growth was also determined by using the probit analysis. The minimum fungicidal concentration (MFC) was also determined by parallel experiments. The nature of toxicity (fungistatic/fungicidal) of the acids was determined by following the method of Thompson[16] and Tripathi *et al*.[17]. The inhibited fungal discs with no growth were taken from acid-treated Petri plates, and then re-inoculated separately onto the fresh medium, and revival of their growth was observed for the next 9 days at 25°C. The concentration that completely inhibits the fungi and irreversibly when transferred to fresh medium was stated as MFC.

Four replicates were used for each concentration of acid and each replicate comprised one Petri dish. Analysis of variance was performed, and mean values were separated by LSD test ($P \leq 0.05$). Experiments were conducted twice.

Efficacy of acids on spore germination and germ tube elongation

The efficacy of acids on spore germination and germ tube elongation of *F. culmorum*, *F. nivale*, *F. solani* and *U. appendiculatus* and was determined. Different concentrations (0.01, 0.02, 0.03, 0.04, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0%, v/v or w/v) of acids were added aseptically to autoclaved and cooled (to 50ºC) PDA medium, followed by the addition of 100 µg/ml streptomycin sulfate (Fluka, BioChemika, China). The medium was dispensed (10 ml per dish) aseptically into 6-cm-diameter Petri dishes. Spores were harvested from 1-week-old PDA cultures of *Fusarium* spp. grown at 25ºC. Ten milliliters of sterile water, containing 0.01% Tween-20, was added to Petri dish cultures of *Fusarium* spp., the spores were gently dislodged from the surface with a bacteriological loop, and suspensions were filtered through three layers of cheesecloth to remove mycelial fragments. Fresh urediniospores of *U. appendiculatus* were obtained from pustules on infected leaves of potted bean plants in a climate-controlled room (see pot experiments for details). The spore concentrations of *Fusarium* spp. and *U. appendiculatus* were determined with a hemocytometer.

The efficacy of acids on spore germination and germ tube elongation of *Fusarium* spp. and *U. appendiculatus* was tested by placing 100 µl aliquots of spore suspension (10^4 spores/ml) of each pathogen in the Petri dishes containing PDA medium with appropriate acid concentrations. Control treatments consisted of PDA medium containing 100 µg/ml streptomycin sulfate. The Petri dishes were incubated at 20-25ºC for 6-12 h in darkness and then spore germination percentages were determined in ten microscopic fields. A total of 100 spores per replicate were observed. Spores were considered germinated when germ tube length was equal to or greater than spore length. The percentage inhibition of spore germination was calculated as compared to the control. Four replicates were used for each concentration of acids and each replicate comprised one Petri dish. Analysis of variance was performed, and mean values were separated by LSD test ($P \leq 0.05$). Experiments were conducted twice.

Soil tests with soil-borne fungi

Cornmeal-sand medium was used in soil tests to evaluate the efficacy of acids. The medium was prepared as described by Ocamb *et al.*[18] with a slight modification to favour the growth of fungi[19]. The ratio of cornmeal to sand was 1:8 and 45 g of medium was placed in 7-cm-diameter glass Petri plates. The Petri plates including medium were sterilized in a laboratory oven at 130°C for 5 h. Three mycelial discs (5-mm-diameter) taken from 7-day-old culture of the respective fungus in PDA medium were placed in 0.5 cm depth of cornmeal-sand medium. The discs were placed in the center of the plates as forming a triangle and the distance between fungal discs was 1 cm. The desired concentrations of formic and propionic acid (0.01, 0.02, 0.03, 0.04, 0.05, 0.1, 0.2, 0.4 and 0.6%, v/v) were prepared as described above and 12 ml from each solution was added to cornmeal-sand medium homogeneously. Control plates received 12 ml of sterile distilled water. The plates were sealed with Parafilm and incubated at 25°C in the dark for 4-5 days. Mycelial growth area of fungi was measured after removal of the lids of Petri plates by placing a transparent acetate paper that has squares on it with an area of 1 cm^2 and 1 mm^2 each. Mycelial growth was measured daily at two perpendicular colony diameters until the growth in the control plates reached the edge of the plates. Percentage inhibition reported is the ratio of mycelial growth compared with that of the control. Five replicates were used for each concentration of acids and each replicate comprised one Petri dish. Analysis of variance was performed, and mean values were separated by LSD test ($P \leq 0.05$). Experiments were conducted twice.

Pot experiments

The efficacy of acids was tested under controlled conditions in a climate-controlled room as described below. Two bean plants (cv. Gina) were grown in pots that were 9.5-cm-diameter. Gina was highly susceptible to *U. appendiculatus*[20,21]. Different concentrations (0.01, 0.02, 0.05, 0.1 and 0.2%, v/v or w/v) of acids were prepared in distilled water containing 0.01% Tween-20. Tested concentrations were applied to the primary leaves of 12-day-old bean plants with a hand sprayer. Control treatments consisted of distilled water containing 0.01% Tween-20. Synthetic fungicide, Mancozeb (Dithane M-45 Special, 80 WP, Dow Agro Sciences, Turkey, label rate, 0.2 %) was used as reference.

Table 1. ED_{50}[a], MIC[b] and MFC[c] values (%, v/v or w/v) of acids in inhibiting mycelial growth of phytopathogenic fungi

Acids	*Fusarium culmorum*			*Fusarium nivale*			*Fusarium solani*			*Macrophomina phaseolina*			*Rhizoctonia solani*			*Sclerotinia sclerotiorum*		
	ED_{50}	MIC	MFC	ED_{50}	MIC	MFC	ED_{50}	MIC	MFC	ED_{50}	MIC	MFC	ED_{50}	MIC	MFC	ED_{50}	MIC	MFC
Citric acid	0.303	0.6	>2	0.574	1.2	>2	0.128	1.2	>2	0.086	1	>2	0.192	0.4	>2	>2	>2	>2
Formic acid	0.036	0.05	0.1	0.064	0.1	0.2	0.061	0.1	0.2	0.042	0.05	0.1	0.022	0.04	0.1	0.022	0.03	0.04
Lactic acid	0.172	0.6	1.2	0.177	0.8	1.2	0.140	0.8	>2	0.075	0.4	>2	0.212	0.4	>2	0.6	1.4	>2
Malic acid	0.342	0.6	>2	0.371	0.8	>2	0.084	1	>2	0.080	0.6	>2	0.156	0.4	>2	1.050	>2	>2
Phosphoric acid	0.038	0.1	1.6	0.071	0.2	>2	0.072	0.2	>2	0.065	0.2	>2	0.050	0.1	>2	0.278	1.8	>2
Propionic acid	0.028	0.05	0.2	0.057	0.1	0.6	0.054	0.1	0.4	0.033	0.1	0.2	0.012	0.04	0.1	0.010	0.02	0.04

[a]The concentration that caused a 50% reduction, [b]Minimum inhibition concentration, [c]Minimum fungicidal concentration

Table 2. ED_{50}[a] and MIC[b] values (%, v/v or w/v) of acids in inhibiting germination and germ tube elongation of the spores of phytopathogenic fungi

Acids	*Fusarium culmorum*				*Fusarium nivale*				*Fusarium solani*				*Uromyces appendiculatus*			
	Spore germination		Germ tube elongation		Spore germination		Germ tube elongation		Spore germination		Germ tube elongation		Spore germination		Germ tube elongation	
	ED_{50}	MIC	ED_{50}	MIC	ED_{50}	MIC	ED_{50}	MIC	ED_{50}	MIC	ED_{50}	MIC	ED_{50}	MIC	ED_{50}	MIC
Citric acid	0.867	1	0.055	1	0.509	0.8	0.091	0.8	0.533	1	0.165	1	0.018	0.1	<0.01	0.1
Formic acid	0.042	0.05	0.018	0.05	0.071	0.1	0.041	0.1	0.065	0.1	0.017	0.1	<0.01	0.02	<0.01	0.02
Lactic acid	0.651	0.8	0.222	0.8	0.470	0.8	0.162	0.8	0.506	0.8	0.217	0.8	0.011	0.1	<0.01	0.1
Malic acid	>1	>1	0.287	>1	0.506	0.8	0.157	0.8	0.531	1	0.264	1	0.019	0.1	<0.01	0.1
Phosphoric acid	0.15	0.2	0.049	0.2	0.132	0.2	0.063	0.2	0.118	0.2	0.060	0.2	<0.01	0.02	<0.01	0.02
Propionic acid	0.066	0.1	0.016	0.1	0.070	0.1	0.019	0.1	0.067	0.1	0.011	0.1	<0.01	0.02	<0.01	0.02

[a]The concentration that caused a 50% reduction, [b]Minimum inhibition concentration

The plants were left to air-dry for 2 h, and then inoculated with $3x10^4$ urediniospores/ml suspensions of *U. appendiculatus*. The urediniospore suspension was also applied with a hand sprayer. Inoculated plants were covered with polyethylene bags to maximize the relative humidity so as to facilitate infection. Covered plants were kept at 19 ± 1°C for 24 h in darkness, and then kept at 22 ± 1°C for 10 days without the polyethylene bags. The light intensity inside the climate room was 10.000 lux with a 12 h supplemental photoperiod. The efficacy of acids was assessed 10 days after the inoculation. Disease development was evaluated by counting the number of pustules within three randomly selected 1 cm^2 areas per leaf. Data were converted to the control percentage as compared to controls. Three replicates were used for each concentration of acids and each replicate comprised three pots. Analysis of variance was performed, and mean values were separated by LSD test (P≤0.05). Experiments were conducted twice.

RESULTS AND DISCUSSION

ED_{50}, MIC and MFC values of acids in inhibiting mycelial growth of phytopathogenic fungi are presented in Table 1. The lowest ED_{50} values against all pathogens were recorded for propionic acid, formic acid and phosphoric acid. The ED_{50} values ranged from 0.010 to 0.057%, 0.022 to 0.064% and 0.038 to 0.278% for propionic acid, formic acid and phosphoric acid, respectively. ED_{50} value was 0.010 of propionic acid against *S. sclerotiorum*.

Generally, similar results were found in MIC values. The lowest MFC values were recorded for formic acid and propionic acid. The MFC values ranged from 0.04 to 0.2% and 0.04 to 0.6% for formic acid and propionic acid, respectively. Gowda *et al*.[22] reported that the antifungal properties of chemicals at different levels were tested on potato dextrose agar. Among the chemical compounds, propionic acid at 0.1-0.5%, ammonia at 0.5%, copper sulfate at 0.08-0.5% and benzoic acid at 0.1-0.5% completely inhibited *Aspergillus parasiticus* growth. Urea, citric acid and sodium propionate had moderate antifungal properties (36-64% reduction). Citric acid below 0.2% had poor antifungal effect. Propionic acid at 0.05-0.5%, sodium propionate at 0.1-0.5%, benzoic acid at 0.2% and ammonia at 0.5% completely inhibited aflatoxin production. Among the chemical compounds tested

Table 3. The efficacy of formic acid and propionic acid on mycelial growth of soil-borne fungi in soil tests

Acids	Concentration (%, v/v)	Inhibition over control (%)			
		Fusarium culmorum	*Macrophomina phaseolina*	*Rhizoctonia solani*	*Sclerotinia sclerotiorum*
Formic acid	0.01	0.0 f*	0.0 f	0.0 h	0.0 g
	0.02	0.0 f	0.0 f	0.0 h	0.0 g
	0.03	0.0 f	0.0 f	0.0 h	0.0 g
	0.04	0.0 f	0.0 f	7.8 g	0.0 g
	0.05	0.0 f	0.0 f	22.3 f	23.7 de
	0.1	6.7 e	48.7 cd	23.5 ef	34.0 bc
	0.2	10.0 e	54.0 bc	31.6 d	100 a
	0.4	28.2 c	59.3 b	62.0 c	100 a
	0.6	100 a	70.0 a	100 a	100 a
Propionic acid	0.01	0.0 f	0.0 f	0.0 h	0.0 g
	0.02	0.0 f	0.0 f	0.0 h	15.6 f
	0.03	0.0 f	0.0 f	0.0 h	16.8 ef
	0.04	0.0 f	0.0 f	0.0 h	22.2 def
	0.05	0.0 f	0.0 f	0.0 h	27.7 cd
	0.1	16.8 d	29.5 e	0.0 h	35.2 b
	0.2	21.3 d	33.6 e	28.5 de	100 a
	0.4	43.8 b	44.3 d	69.9 b	100 a
	0.6	100 a	51.2 cd	100 a	100 a

*Means within columns by unlike letters differ significantly according to LSD test (P≤0.05)

in feeds, propionic acid, sodium propionate, benzoic acid and ammonia were the best antifungal compounds, followed by urea and citric acid. Rusul *et al*.[23] reported similar trends in the reduction in fungal growth and aflatoxin production by *A. parasiticus* with increasing concentrations of propionic acid (0.25-1%). Ghosh *et al*.[24] reported complete inhibition of mould growth and aflatoxin biosynthesis by *A. flavus* at 0.5% propionic acid. Propionic acid is a highly effective fungal inhibitor often used in the food industry[25].

ED_{50} and MIC values of acids in inhibiting germination and germ tube elongation of the spores of phytopathogenic fungi are presented in Table 2. The lowest ED_{50} values for spore germination and germ tube elongation against all pathogens were recorded in propionic acid, formic acid and phosphoric acid. The ED_{50} and MIC values for spore germination and germ tube elongation showed that all acids used in this study were more toxic to *U. appendiculatus* than to *Fusarium* spp. (Table 2).

Among the acids screened in *in vitro* experiments, propionic acid and formic acid showed the best overall performance against all tested fungi and therefore they were selected for further testing in soil experiments.

The efficacy of formic acid and propionic acid on mycelial growth of soil-borne fungi in soil tests is presented in Table 3. In soil tests, the percentages of mycelial growth inhibition of formic acid and propionic acid ranged from 6.7 to 100% and 15.6 to 100%, respectively. Among the fungi tested, the most susceptible fungus was *S. sclerotiorum* against both acids. Both formic acid and propionic acid completely inhibited *S. sclerotiorum* at 0.2%, *F. culmorum* and *R. solani* at 0.6% in soil tests (Table 3). The results of soil tests demonstrated that the application of formic and propionic acid significantly limited the growth of soil-borne fungi.

Experimental soil test results on the potential of acids to control fungal infections of crops under controlled conditions contribute to the assessment of the potential application of the acid under field conditions in sustainable agriculture.

In vivo efficacy of acids on the pustules caused by *U. appendiculatus* is presented in Table 4. In pot experiments, control efficacy of all tested acids ranged from 0 to 85.7%. Among acids tested, citric acid was the most effective followed by malic acid. Control efficacies of citric acid treatments were 85.7, 68.6, 22.5 and 16.0% at 0.2, 0.1, 0.05 and 0.02%, respectively. Control efficacy of malic acid was 67.8% at 0.2%. Both formic acid and phosphoric acid caused only slight phytotoxicity on bean leaves at 0.1%. The application of higher acid concentrations in *in vivo* experiments could not have been possible due to phytotoxicity. None of the acids tested was as effective as the fungicide Mancozeb. It completely inhibited rust development at 0.2% (label rate).

In vivo antifungal efficacy of acids was much lower than that of *in vitro.* In this study, the difference between the *in vivo* and *in vitro* efficacy

Table 4. *In vivo* efficacy of acids on the pustules caused by *Uromyces appendiculatus* in pot experiments

Acids	Concentration (%, v/v or w/v)	Control efficacy (%)
Citric acid	0.01	0.0 k*
	0.02	16.0 hi
	0.05	22.5 gh
	0.1	68.6 c
	0.2	85.7 b
Formic acid	0.01	0.0 k
	0.02	0.0 k
	0.05	34.9 f
	0.1	50.1 d**
Lactic acid	0.01	13.0 ij
	0.02	20.6 gh
	0.05	24.0 g
	0.1	42.2 e
	0.2	43.4 de
Malic acid	0.01	0.0 k
	0.02	0.0 k
	0.05	0.0 k
	0.1	37.4 ef
	0.2	67.8 c
Phosphoric acid	0.01	0.0 k
	0.02	0.0 k
	0.05	7.9 j
	0.1	39.5 ef**
Propionic acid	0.01	0.0 k
	0.02	20.5 gh
	0.05	21.0 gh
	0.1	33.3 f
	0.2	37.1 ef
Mancozeb	0.2	100 a

*Means within columns by unlike letters differ significantly according to LSD test (P≤0.05).
**Slight phytotoxicity

shows that volatile acids such as formic, and propionic acid may evaporate from the surface of bean leaves. Specific acid-host tissue interactions may involve biochemical reactions, such as a host defense mechanism contributing to the control of *U. appendiculatus*. In addition, the interaction between acid and PDA medium may play an important role.

To the best of our knowledge, this is the first report where the efficacy of generally recognized as safe (GRAS) acids against seven economically important phytopathogenic fungi including *Fusarium culmorum, F. nivale, F. solani, Macrophomina phaseolina, Rhizoctoniasolani, Sclerotinia sclerotiorum* and *Uromyces appendiculatus* was evaluated.

CONCLUSIONS

Our results indicated that the use of acids to control infections by phytopathogenic fungi may be a valid alternative to synthetic fungicides. The findings in this study will provide a non-toxic and environmentally safe option for alternative control of phytopathogenic fungi. These acids can be used alone in organic growing or in rotation with synthetic fungicides in an IPM program in the conventional agriculture. However, this was a preliminary study regarding the efficacy of acids on phytopathogenic fungi. The efficacy of acids should be investigated in natural growing soil and conditions on different host-pathogen interactions before their use is recommended. It is necessary to conduct further research on the mechanism by which the acids take action on controlling the phytopathogenic fungi. Because acids are natural and environmentally-friendly products, they may offer new strategies to control phytopathogenic fungi in future sustainable agriculture. The findings of the present investigation could be an important step towards using natural acids as fungicides in the plant disease control.

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REFERENCES

1. Bajpai, V.K., Lee, T.J., Kang, S.C. Chemical composition and *in vitro* control of agricultural plant pathogens by the essential oil and various extracts of *Nandina domestica* Thunb. *J. Sci. Food Agric*., 2009; **89**: 109-116.
2. Al-Reza, S.M., Rahman, A., Ahmed, Y., Kang S.C. Inhibition of plant pathogens *in vitro* and *in vivo* with essential oil and organic extracts of *Cestrum nocturnum* L. *Pestic. Biochem. Phys*., 2010; **96**: 86-92.
3. Paster, N., Bullerman, L.B. Mould spoilage and mycotoxin formation in grains as controlled by physical means. *Int. J. Food Microbiol*., 1988; **7**: 257-265.
4. Schillberg, S., Zimmermann, S., Zhang, M.Y., Fischer, R. Antibody-based resistance to plant pathogens. *Trans. Res*., 2001; **10**: 1-12.
5. Feng, W., Zheng, X. Control of *Alternaria alternata* by cassia oil in combination with potassium chloride or sodium chloride. *J. App. Microbiol.*, 2006; **101**: 1317-1322.
6. Dubey, N.K., Srivastava, B., Kumar, A. Current status of plant products as botanical pesticides in storage pest management. *J. Pesticides*., 2008; **1**: 182-186.
7. Beuchat, L.R., Golden, D.A. Antimicrobials occurring naturally in foods. *Food Technol.*, 1989; **43**: 134-142.
8. Mansour, M., Linder, M., Milliere, J.B., Lefebvre, G. Combined effects of nisin, lactic acid and potassium sorbate on *Bacillus licheniformis* spores in milk. *Lait*, 1998; **78**: 117-128.
9. Phillips, C.A. The effect of citric acid, lactic acid, sodium citrate and sodium lactate, alone and in combination with nisin, on the growth of *Arcobacter butzleri*. *Lett. Appl. Microbiol*., 1999; **29**: 424-428.
10. Cherrington, C.A., Hinton, M., Chopra, I. Effect of short-chain organic acids on macromolecular synthesis in *Escherichia coli*. *J. Appl. Bacteriol.*, 1990; **68**: 69-74.
11. Dibner, J.J., Buttin, P. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. *J. Appl. Poul. Res*., 2002; **11**: 453-463.
12. Marounek, M., Skrivanova, E., Rada, V. Susceptibility of *Escherichia coli* to C-2-C-18 fatty acids. *Folia Microbiol*., 2003; **48**: 731-735.
13. Lee, Y.L., Cesario, T., Owens, J., Shanbrom, E., Thrupp, L.D. Antibacterial activity of citrate and acetate. *Nutr*., 2002; **18**: 665-666.

14. McWilliam Leitch, E.C.M., Stewart, C.S. Susceptibility of *Escherichia coli* O157 and non-O157 isolates to lactate. *Let. Appl. Microbiol.*, 2002; **35**: 176-180.
15. Dorota Szopinska, D. The effects of organic acids treatment on germination, vigour and health of zinnia (*Zinnia elegans* Jacq.) seeds. *Acta Sci. Pol. Hortorum Cultus.,* 2013; **12**: 17-29.
16. Thompson, D.P. Fungitoxic activity of essential oil components on food storage fungi. *Mycologia*, 1989; **81**: 151-153.
17. Tripathi, P., Dubey, N.K., Banerji, R., Chansouria, J.P.N. Evaluation of some essential oils as botanical fungitoxicants in management of post-harvest rotting of citrus fruits. *World J. Microbiol. Biotechnol*., 2004; **20**: 317-321.
18. Ocamb, C.M., Juzwik, J., Martin, F.B. *Fusarium* spp. and *Pinus strobus* seedlings: root disease pathogens and taxa associated with seed. *New For*., 2002; **24**: 67-79.
19. Arslan, U., Ilhan, K., Karabulut, O.A., Vardar, C. Evaluation of antifungal activity of food additives against soilborne phytopathogenic fungi. *World J. Microb. Biot.,* 2009; **25**: 537-543.
20. Arslan, U., Ilhan, K., Karabulut, O.A. Evaluation of food additives and low-toxicity compounds for the control of bean rust and wheat leaf rust. *J. Phytopathol*., 2006; **154**: 534-541.
21. Arslan, U., Ilhan, K., Karabulut, O.A. Antifungal activity of aqueous extracts of spices against bean rust (*Uromyces appendiculatus*). *Allelopathy J.,* 2009; **24**: 207-213.
22. Gowda, N.K.S., Malathi, V., Suganthi, R.U. Effect of some chemical and herbal compounds on growth of *Aspergillus parasitucus* and aflotoxin production. *Anim. Feed Sci. Technol*., 2004. **116**: 281-291.
23. Rusul, G., Elgazzar, F.E., Marth, E.H. Growth and aflatoxin production by *Aspergillus parasiticus* NRRL 2999 in the presence of acetic or propionic acid and at different initial pH values. *J. Food Prot*., 1987; **50**: 909-914.
24. Ghosh, M.K, Chhabra, A., Atreja, P.P., Chopra, R.C. Effect of treating with propionic acid, sodium bisulfite and sodium hydroxide on the biosynthesis of aflatoxin on groundnut cake. *Anim. Feed Sci. Technol.,* 1996; **60**: 43-49.
25. Skrinjar, M., Danev, M., Dimic, G. Interactive effects of propionic acid and temperature on growth and ochratoxin a production by *Penicillium aurantiogriseum*. *Folia Microbiol*., 1995; **40**: 253-256.