EFFECT OF SOME ORGANIC ACIDS ON SOME FUNGAL GROWTH AND THEIR TOXINS PRODUCTION

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ABSTRACT

The effect of eight organic acids (propionic, acetic, formic, lactic, tartaric, citric, oxalic and malic acids) as antifungal agents on the growth of four fungi (Aspergillus flavus, Penicillium purpurogenum, Rhizopus nigricans and Fusarium oxysporum) were studied. The high acidity appeared for oxalic acid being 0.14 at the high concentration (10%), while the lowest acidity recorded for propionic acid and acetic acid being 2.71 and 2.56 at the low concentration (5%). It was observed that, there was no relationship between the efficacy of organic acid and its final pH. Acetic acid (10%) has the highest inhibitory effect on A. flavus being 45.21%, but tartaric acid (5%) and citric acid (5%) gave the same lowest inhibition effect (0.42%). The lowest value of mycelium dry weight (MDW) of P. purpurogenum was 5.92 g/l when acetic acid was used (10%), but the highest value was 9.38 g/l when tartaric acid (5%) was used. Formic acid (10%) had a strong effect on the inhibition growth of R. nigricans being 28.65%, similar to propionic acid (10%), acetic acid (10%), lactic acid (10%), tartaric acid (10%) and citric acid (10%) being 26.57%, 26.38%, 26.19%, 23.53% and 24.48%, respectively. But malic acid (5%) and oxalic acid (5%) were having a week effect on R. nigricans being 5.31% and 6.45%, respectively. Lactic acid (10%) has the highest inhibitory effect on F. oxysporum being 34.45% and the lowest value was in the case of tartaric acid (5%) being 1.68%. Four treatments were used to determine aflatoxin B_1 production. The highest inhibition (50%) was observed by R. nigricans in the presence of formic acid (10%). Acetic acid in 10% level inhibited the toxic secretion of A. flavus and P. purpurogenum to become 25% and 40%, respectively. Lactic acid (10%) gave 35% inhibition of toxin production in the presence of F. oxysporum.

KEYWORDS

Antifungal, Organic Acids, Aspergillus flavus, Penicillium purpurogenum, Fusarium oxysporum, Rhizopus nigricans & Aflatoxin B_1

1. INTRODUCTION

Large amounts of food and feed are lost every year due to spoilage by yeasts and fungi [1]. So that, preservative agents commonly used include weak organic acids such as acetic, lactic, benzoic and citric acids, which inhibit the microbial growth in various foods. The effect of organic acids on the fungal growth, which contaminate food and feed, has been investigated by several authors [2; 3 and 4].

PH affects on the permeability of the cell membrane and on the enzymes that are active in degrading the substrate [5, 6 and 7]. Organic acids generally used as safe agents to preserve foods, these organic acids reduce cytoplasmic pH and stop metabolic activities. On the other hand, organic acids caused the death by the susceptible organisms act on the plasmic membrane

by neutralizing its electrochemical potential and increasing its permeability [8]. Organic acids, which used in food preservation is considered; simple, fast, acid, cheap and efficient. Moreover, most of them are not limited in the acceptable daily intake for humans. These characteristics favor their use in food preservation. Many authors have been testing the effect of organic acids on the microbial growth, and they have taken a consideration in the sensory changes such as color and flavor. Organic acids are considered weak acids (do not fully dissociate in water) but do so in a pH dependent manner. The pK_a is defined as the acid dissociation constant [4]. The decreasing of pH resulting a greater concentration of protons and increasing the diffusion of acid across the plasmic membrane and the cytoplasm. However, the substitution of the don able proton with a monovalent (Na^+, K^+) or multivalent (Ca^{+2}) cation significantly increases the solubility of organic acid in aqueous systems. Thus, a balance must be occur between the need to maintain acid solubility with the need to achieve maximal activity by pH reduction. The inhibitory effect of organic acids is based in the "weak acid preservative theory". Lactic and acetic acid have been used as fungal inhibitors, they are present in the fermented foods and easy to obtain, their maximum concentration is depending on the sensor parameters. Resistance towards weak acids dependent on the plasma membrane H^+-ATPase. This can be explained by the low permeability of the plasma membrane to undissociated acid. Because of an energy spent during protons pumping to membrane outside [9 and 10].

On the other hand, the higher acid resistance may be explained by the ability of some microbes to consume, acetate while growing on fermentable sugar. The observation of higher acid resistance was explained by two theories, the fungal deacidafication and the formation of alcohols. *P. camembert* metabolizes the lactate to CO_2 and H_2O, which results in deacidafication [4, 11 and 12].

High amount of aflatoxin B_1 was produced even initial spore inoculum levels were low [13]. *A. flavus* and *A. parasiticus* producing aflatoxins were isolated from different Egyptian commodities [14]. However, high fungi counts (i.e. 10^6 colony forming unite (cfu) or higher) are generally producing mycotoxins [15]. Fungi can produce their mycotoxins under laboratory conditions or naturally in various agricultural products [16]. Fungi cause a significant yield reduction and economic losses because its commonly contaminate crops and foods. In addition, they changes the appearance, taste, texture and odor of food, and also unsafe for human consumption because of there mycotoxins. The consumption of foods which contaminated with mycotoxin has been associated with several cases of human poisoning, sometimes resulting in death [17]. Nowadays, mycotoxins have been receiving worldwide attention and several groups of mycotoxins are known such as ergot, aflatoxins, ochratoxins, citrinin, patulin and fumonisines [18]. *Fusarium*, *Aspergillus* and *Penicillium* were noted to be the major fungal populations in feed and foods. *F. moniliforme* present in the feed and food for about a year. The predominant naturally occurring fungi belonging mainly to *Penicillium purpurogenum*, *Aspergillus glaucus* and *A. candidus* [8]. Aflatoxin (AF) B_1 and mixtures of AFB_1, AFG_1 and AFM_1 are proven as a human carcinogens and are classified in the Group 1 carcinogen status [10].

So that, the aim of our research was a trial to inhibit the fungal growth and aflatoxin B_1 production in the presence of some organic acids.

2. MATERIALS AND METHODS

2.1. Fungal isolates and maintenance

Four local fungal isolates, namely *A. flavus*, *P. purpurogenum*, *F. oxysporum* and *R. nigricans* were obtained from Agric. Microbiology Dept., Fac. of Agric., Damietta University, Damietta, Egypt. The fungal isolates were maintained on potato dextrose agar (PDA) medium slants at 5°C

till use [19]. Fungal isolates were subcultured on a new slant of PDA and incubated in a digital incubator (Switc, MPM Instruments s.r.l., Bernareggio/Made in Italy) at 25°C for 10 days before use.

2.2. Fungal spore suspensions and inoculum size

Spores suspension was prepared as described by [20]. Fungi were grown on a PDA slant at 25°C for 10 days. 10 ml of sterilized saline solution (0.09%) was added to the slants and the spores were loosened by gently brushing with a sterile inoculating loop. A vortex mixer (VM-300 power: 220 VAC, 50Hz, 0.16A/Made in Taiwan-Associated with Cannicinc U.S.A.) was used for one minute to remove all spores from slant [21]. Spores count was performed in a Hematocytometer slide (model Buerker MOM BUDA pest) and the following equation was used for fungal spore count: Spore count equation (spores/ml) = mean of spore count in 10 squares x slid factor (2.5×10^6) x dilution rate. Spores suspension corresponding to give approximately $1x10^6$ spores per ml using a micropipette (Micro Volume Pipettor - Accumax A made in China), thus the count of spores was fixed in the following experiment [2]. The spores suspension stocks were stored at 4°C.

2.3. Organic acids

Propionic acid (99%, BDH) acetic acid glacial (99.8%, Almasria for Chemicals) formic acid (85%, BDH) lactic acid (Chemicals Ltd, Poole, England), tartaric acid (99%, ADWIC) citric acid anhydrous (99%, ADWIC), oxalic acid (99.5%, ADWIC), and malic acid (99%, LOBA Chemie, India) were used as antifungal agents. The addition of these acids was at the time of inoculation to reduce the fungal growth and their mycotoxins production. The pH value of each eight organic acids in water was determined.

2.4. Fungal inhibition

The basal medium was potato dextrose broth (PDB), this medium was used to evaluate the spores germination and the growth of our fungi by surface culture technique [3]. Fifty ml of PDB was put into a 250 ml Erlenmeyer flask and autoclaved at 121℃ for 20 min. After cooling, the flasks were then treated with three levels (0%, 5% and 10%) of organic acids as an antifungal agents using a Millipore filter (0.2 µm, Flow Pore D made by Sartorius, W. Germany). All flasks were inoculated with $1xl0^6$ spores from each spores suspension stocks (*A. flavus*, *P. purpurogenum*, *F. oxysporum* and *R. nigricans*). After 8 days of incubation in the digital incubator at 25°C, the inhibition percentage was calculated by the difference between the growth of mycelium dry weight in the absence or in the presence of the antifungal agent [2]. All experiments were conducted in three replicates.

2.5. Determination of mycelium dry weight (MDW)

Through a double-layered Whatman filter paper No. 1 the mycelial mat resulting from surface culture technique was filtered and washed twice with distilled water, dried in an oven (Nemmert, W. Germany) at 80°C to a constant weight (g/l) [22]. Mycelial mat was determined using an electronic balance (type BL-32OH Shimadzu Corporation, Japan). The supernatant was collected and used for mycotoxins determination. The final pH was determined in the culture filtrate also using a pH meter, (model Hanna pH 211 microprocessor pH meter).

2.6. Determination of aflatoxin B_1

Culture filtrates were extracted three times with 100 ml volume of chloroform. The residue was dissolved in 5 ml methanol (70%). ELISA technique was used to determine aflatoxin B_1 [23]. Aflatoxin B_1 (standard) was purchased from Sigma Chemical Co. (USA).

3. RESULTS AND DISCUSSION

3.1. The effect of organic acids as antifungal agents

PH values of the eight organic acids when dissolved in water at levels of 5 and 10% were presented in Table 1. It was clear showed that, when the acid concentration increased the pH degree decreased. There is an opposite relation between pH degree and the acidity. The high acidity appeared for oxalic acid being 0.14 at the high concentration (10%), while the lowest acidity recorded for propionic acid and acetic acid being 2.71 and 2.56 at the low concentration (5%).

Table 1. PH values of organic acids in water

Organic acids	State	The pH values	
		5%	10%
Propionic acid (v/v.)	Liquid	2.71	2.45
Acetic acid (v/v)		2.56	2.28
Formic acid (v/v)		1.75	1.53
Lactic acid (v/v)		2.09	1.83
Tartaric acid (w/v)	Solid	1.48	1.29
Citric acid (w/v)		1.18	1.12
Oxalic acid (w/v)		0.43	0.14
Malic acid (w/v)		1.91	1.72

The mechanism of inhibition fungi growth by organic acids is generally not considered a pH phenomenon. It is known that, growth and morphology of fungi are influenced by the pH of media [24]. Some mechanisms have been suggested to explain the inhibitory mode of organic acids. Organic acids resulting a decreasing in pH value, this may influence the growth by acidifying the cell, which will consume a great amount of energy to maintain the intracellular pH homeostasis [3]. Other explanations have also been proposed including the membrane disruption, the interruption of metabolic reactions, and the accumulation of toxic anions. Three of the fungi (*P. roqueforti*, *P. commune* and *F. sporotrichoides*) and one yeast species (*Kluyveromyces marxianus*) did not grow at pH 3 [1]. The inhibition of microbial growth increases by lowering pH of the media, and most microorganisms are susceptible to antimicrobial effects in the presence of organic acids. This phenomenon is due to the hydrophobic feature of most organic acids, which allows free diffusion of the protonized form through cell membrane. This diffusion process takes place spontaneously due to pH and osmolarity gradients that exist between the inner and outer sides of the cell. The intracellular pH is higher than the extracellular, and the acid undergoes dissociation as soon as it enters the cytoplasm and then decreases the intracellular pH by releasing the proton. In order to counter the decrease of cytoplasmic pH, resulting from the ionization of the entered acid, the cell allocates the main part of its energy content to eliminate these newly formed protons which results in slower growth kinetics [10].

Results recorded in Table 2. showed that, the effect of organic acid levels (0, 5 and 10%) on *A. flavus* growing in PDB medium. The inhibition percentage was calculated by the difference

between dried mycelium weight in the absence or presence of the antifungal agent (organic acids) after 8 days of incubation at 25°C. The final pH and MDW of the control were 3.87 and 9.60 g/l, respectively. Acetic acid (10%) has the highest inhibitory effect on *A. flavus* being 45.21% and the final pH was 3.25, but tartaric acid (5%) and citric acid (5%) gave the same lowest inhibition effect (0.42%) and final pH was 3.12 and 3.24, respectively. Other authors, [25] studied the inhibition growth of some species of fungi (*A. flavus*, *A. niger*, *A. fumigatus*, *P. glabrum F. moniliforme* and *Cladosporium sphaerospermum*) using organic acids (lactic, acetic, formic, oxalic, and propionic) in concentrations 3, 5, 10, 20 and 50 ml/l and they found that, lactic and oxalic acids did not prove any activity on the chosen concentrations. Propionic acid in concentration 20 ml/l inhibited the growth of five fungi (*A. fumigatus*, *A. niger*, *P. glabrum*, *F. moniliforme*, and *C. sphaerospermum*). Propionic acid exhibited a fungicidal action; however, calcium propionate had essentially no effect on *A. flavus* growth [2]. Our results within the line of [8] who reported that, acetic acid was more effective than lactic acid and had the best inhibitor of fungi growth. Different acetic and lactic acid concentrations were studied by [10] for antifungal activity against different strains of *A. flavus*: They determined that the increase of acid in the medium decreases the growth rate and extends the lag phase.

Table 2. Effect of organic acids levels on growth of *Aspergilus flavus*

Organic acids	**5%**			**10%**		
	Final pH	**MDW (g/l)**	**Inhibition %**	**Final pH**	**MDW (g/l)**	**Inhibition %**
Propionic acid	3.96	7.06	26.46%	3.49	5.72	40.42%
Acetic acid	3.76	6.92	27.92%	3.25	5.26	45.21%
Formic acid	3.15	7.50	21.88%	2.56	6.26	34.79%
Lactic acid	3.43	9.16	4.58%	2.62	8.58	10.63%
Tartaric acid	3.12	9.56	0.42%	2.40	8.00	16.67%
Citric acid	3.24	9.56	0.42%	2.60	7.90	17.71%
Oxalic acid	1.96	8.86	7.71%	1.58	8.78	8.54%
Malic acid	2.31	9.46	1.46%	2.62	9.06	5.63%

Table 3. showed the effect of organic acids levels on the growth of *P. purpurogenum*. The final pH and MDW of the control experiment were 4.24 and 10.02 g/l, respectively. The lowest value of MDW was 5.92 g/l when acetic acid (10%) was used, but the inhibition effect and final pH were 40.92% and 3.31, respectively. The highest value of MDW was 9.38 g/l when tartaric acid (5%) was used. Propionic acid (20 ml/L) inhibited the growth of *P. glabrum* [25]. The growth of two isolates of *Penicillium* sp. were completely inhibited by the presence of ascorbic acid or propionic acid. Calcium-propionate and Na-benzoate did not exhibit any inhibitory effects on these two cultures [2].

Table 3. Effect of organic acids levels on the growth of *Penicillium purpurogenum*

Organic acids	**5%**			**10%**		
	Final pH	**MDW (g/l)**	**Inhibition %**	**Final pH**	**MDW (g/l)**	**Inhibition %**
Propionic acid	4.25	8.10	19.16%	3.49	6.84	31.74%
Acetic acid	4.03	7.66	23.55%	3.31	5.92	40.92%
Formic acid	3.00	9.08	9.38%	2.39	8.32	16.97%
Lactic acid	3.27	9.36	6.59%	2.68	8.22	17.96%
Tartaric acid	3.63	9.38	6.39%	2.44	8.28	17.37%
Citric acid	2.89	9.16	8.58%	2.56	8.00	20.16%
Oxalic acid	2.18	8.50	15.17%	1.75	6.74	32.74%
Malic acid	3.12	8.50	15.17%	2.68	7.94	20.76%

Similar results were obtained from [24] who published that, the values of final pH of cultivation broth for lactic acid and acetic acid were 3.35 and 3.81, respectively, these tow acids displayed little efficacy in controlling fungi growth. Our results were agreeing with that obtained by [1] who found that, *P. roqueforti* was the most sensitive to organic acids. Also, they evaluated the antifungal activity (propionic acid, lactic acid and acetic acid) against *A. fumigatus*, *P. roqueforti*, *P. commune*, *A. nidulans*, *F. sporotrichoides*. The minimal inhibitory concentration values of propionic, acetic and lactic acid were established for all fungi at pH 3, 5 and 7. Propionic acid, followed by acetic acid, was the most potent antifungal acid.

The final pH and MDW values in the control flask of *R. nigricans* were 3.89 and 10.54 g/l, respectively. Formic acid (10%) had a strong effect on the inhibition growth of *R. nigricans* being 28.65% (Table 4.), similar to propionic acid (10%), acetic acid (10%), lactic acid (10%), tartaric acid (10%) and citric acid (10%) being 26.57%, 26.38%, 26.19%, 23.53% and 24.48%, respectively. But malic acid (5%) and oxalic acid (5%) were a weak effect being 5.31% and 6.45%, respectively. The final pH values were varied between 1.58 and 3.96 in the case of oxalic acid (10%) and propionic acid (10%), respectively. On the other hand, the values of MDW were varied between 7.52 and 9.98 g/l when formic acid (10%) and malic acid (5%) was used, respectively. The mixtures of acetate, lactate and propionate have a synergistic inhibitory effect on the indicator strains [26].

Table 4. Effect of organic acids levels on the growth of *Rhizopus nigricans*

Organic acids	**5%**			**10%**		
	Final pH	**MDW (g/l)**	**Inhibition %**	**Final pH**	**MDW (g/l)**	**Inhibition %**
Propionic acid	3.65	8.66	17.84%	3.96	7.74	26.57%
Acetic acid	3.47	8.72	17.27%	3.17	7.76	26.38%
Formic acid	2.91	8.54	18.98%	2.59	7.52	28.65%
Lactic acid	2.79	8.94	15.18%	2.40	7.78	26.19%
Tartaric acid	2.80	8.84	16.13%	2.41	8.06	23.53%
Citric acid	2.64	8.64	18.03%	2.58	7.96	24.48%
Oxalic acid	2.03	9.86	6.45%	1.58	9.46	10.25%
Malic acid	2.95	9.98	5.31%	2.81	9.32	11.58%

The effect of organic acid levels on the growth of *F. oxysporum* was presented in Table 5. The final pH and MDW of the control experiment were 3.98 and 10.74 g/l, respectively. Lactic acid (10%) has the highest inhibitory effect on *F. oxysporum* being 34.45% and the lowest value was in the case of tartaric acid (5%) being 1.68%.

Table 5. The effect of organic acids levels on the growth of *Fusarium oxysporum*

Organic acids	**5%**			**10%**		
	Final pH	**MDW (g/l)**	**Inhibition %**	**Final pH**	**MDW (g/l)**	**Inhibition %**
Propionic acid	3.85	8.86	17.51%	3.17	8.16	24.02%
Acetic acid	3.16	8.20	23.65%	3.23	9.78	8.94%
Formic acid	3.08	9.76	9.13%	2.45	8.70	18.99%
Lactic acid	3.73	9.14	14.90%	2.49	7.04	34.45%
Tartaric acid	3.89	10.56	1.68%	2.27	10.52	2.05%
Citric acid	3.64	10.16	5.40%	2.71	8.50	20.86%
Oxalic acid	3.45	10.02	6.70%	1.44	9.98	7.08%
Malic acid	3.85	10.22	4.84%	2.56	9.32	13.22%

Our results were in reserve direction with those [25] who reported that, lactic and oxalic acids did not prove any activity on the growth of *F. moniliforme,* but the best organic acid on the fungal growth inhibition was propionic acid. The growth of *Fusarium* sp. was completely inhibited by the presence of propionic acid, sorbic acid or Na-benzoate [2]. 10 inorganic and 12 organic salts for their inhibitory activity against *F. sambucinum* were evaluated [27] They found that, several salts inhibited completely mycelial growth and spore germination of *F. sambucinum*. Among these salts, sodium benzoate, sodium metabisulphite, potassium sorbate, trisodium phosphate and aluminium salts were fungi toxic. Sodium propionate, sodium carbonate and sodium citrate inhibited the growth of *F. sambucinum* but to a lesser extent.

3.2. Effect of organic acids on aflatoxin B_1 production:

Four tests were chosen to determent aflatoxin B_1 (AB_1) production based on the highest inhibitory effect on fungal growth being: *A. flavus*, *P. purpurogenum*, *R. nigricans* and *F. oxysporum* treated with acetic acid (10%), acetic acid (10%), formic acid (10%) and lactic acid (10%), respectively. All organic acids under study reduced aflatoxin B_1 secretion. The highest one (50%) was observed for *R. nigricans* in the presence of formic acid (10%) (Table 6). Acetic acid in 10% level inhibited the toxic secretion of *A. flavus* and *P. purpurogenum* to become 25% and 40%, respectively. Lactic acid (10%) gave 35% inhibition of toxin production in the presence of *F. oxysporum*. Propionic acid and butyric acid were added as sub-lethal doses (1–20%) to a growth medium of *A. flavus* for supporting growth and subsequent aflatoxin production [28]. In the same manner [8] reported that lactic acid bacteria inhibited the fungal growth and biosynthesis of mycotoxin.

Table 6. The effect of organic acids on aflatoxin B_1 production.

Tested fungi	**Aflatoxin B1 production (ppb)**		**Inhibition (%)**
	Treated	**Control**	
Aspergillus flavus	8	12	25%
Penicillium purpurogenum	6	10	40%
Rhizopus nigricans	4	8	50%
Fusarium oxysporum	6.5	10	35%

3.3. Relationship between chemical structure of organic acid and the fungal growth inhibition:

From Figs. 1, 2 and 3, it was clear that, formic acid (H-CO-OH), acetic acid (CH_3-CO-OH) and propionic acid (CH_3-CH_2-COOH) was the highest inhibitory effect on *A. flavus* growth. This effect may be due to the similarity in their chemical structure and also may be due to their pK_a, which are almost the same being 3.77, 4.79 and 4.87, respectively. The effect of propionic acid concentration (129, 258 and 516 ppm) on the growth of *A. parasiticus* were studied by [17], the increasing of propionic acid concentration showed that decreasing in the growth rate. Our results were similar to those obtained by [3] who measured the residual concentration of dissolved oxygen in the culture medium after 4 days. The findings of those may explain why acetic acid showed the strongest inhibition of the fungal growth, where the growth inhibition by this organic acid was closely related to the inhibition of respiration.

The results in Figs. 4 and 5 appeared that, the lactic acid (CH3-CHOH-COOH) and tartaric acid (COOH-CHOH-CHOH-COOH) almost had the same effect on the *P. purpurogenum* and *R. nigricans* growth, that may be due to the isomerism in their chemical structure. The minimal inhibitory concentration (MIC) values for lactic acid concentration are nearly tenfold higher than MIC values for acetic acid on the *A. flavus*. Lactic and acetic acid mixtures showed a synergistic

effect, reducing the concentration necessary of every acid in the mixture for fungal inhibition compared with the individual MIC values [10].

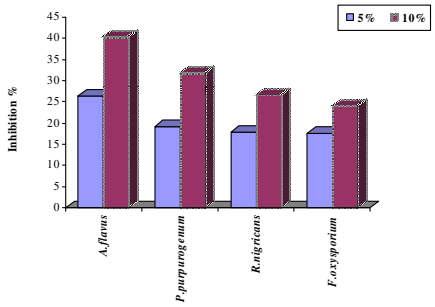

Figure 1. The effect of propionic acid concentrations on the fungal growth

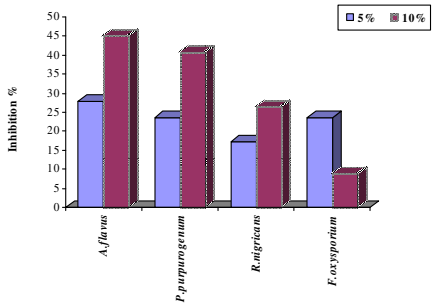

Figure 2. The effect of acetic acid concentrations on the fungal growth

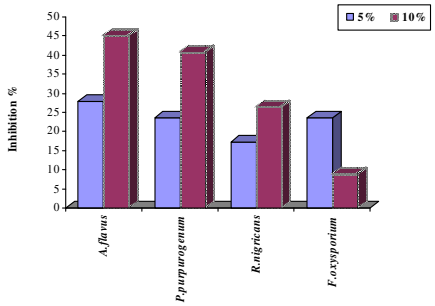

Figure 3. The effect of formic acid concentrations on the fungal growth

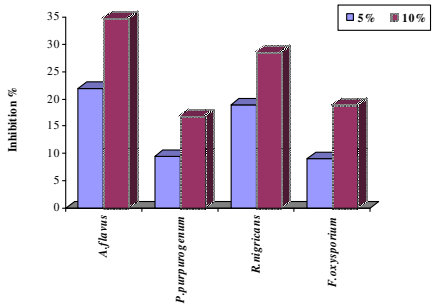

Figure 4. The effect of lactic acid concentrations on the fungal growth

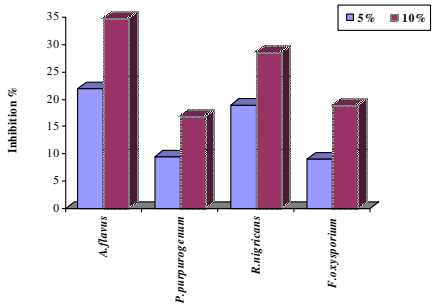

Figure 5. The effect of tartaric acid concentrations on the fungal growth

Citric acid ($C_3H_5O(COOH)_3$), oxalic acid (COOH-COOH) and malic acid (COOH-CHOH-CH_2-COOH) gave highest effect on *P. purpurogenum* growth (Figs. 6, 7 and 8) that may be due to the similarity in their chemical structure and containing more than one carboxylic group.

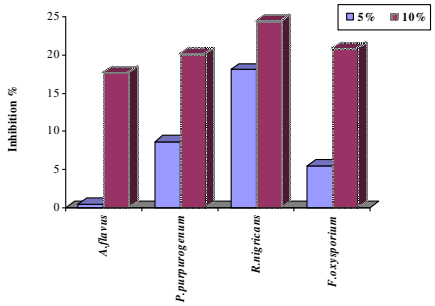

Figure 6. The effect of citric acid concentrations on the fungal growth

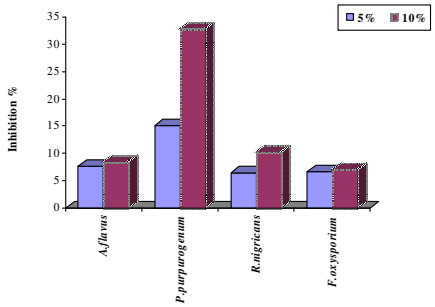

Figure 7. The effect of oxalic acid concentrations on the fungal growth

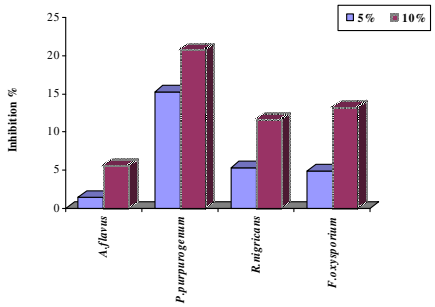

Figure 8. The effect of malic acid concentrations on the fungal growth

This observation is in agreement with the results of those [29] who reported that, the inhibitory effect of organic acids on microbial growth has been studied. Organic acids are not members of a homologous series, but vary in the numbers of carboxy groups, hydroxy groups and carbon–carbon double bonds in the molecule. Properties correspond to polar groups, the number of double bonds, molecular size, and solubility in non- polar solvents.

4. CONCLUSIONS

All tested organic acids, which used as antifungal were variations in the effect of fungal growth. There was a little correlation between the final pH of the organic acids and its efficacy on the tested fungi. Acetic acid (10%) has the highest inhibitory effect on *A. flavus* followed by *P. purpurogenum* being 45.21% and 40.92, respectively. On the other hand tartaric acid (5%) and citric acid (5%) gave the same lowest inhibition effect (0.42%) on *A. flavus*, but tartaric acid (5%) affected in the growth of *F. oxysporum* being 1.68%. All organic acids, which used in this study reduced aflatoxin B_1 production. The treatment of *R. nigricans* in the presence of formic acid (10%) was the highest inhibitory effect being 50%.

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