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Effect of Some Organic Acids on Seed Vigor and Health of Some Rice Cultivars

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ABSTRACT
The present study was conducted at Laboratory of Seed Technology Research Unit, Mansoura, Seed Technology Research Department, ARC, Egypt during 2013. Organic acids are known for their antibacterial and antifungal properties. The purpose of the research was to study, the effect of salicylic acid and benzoic acid and propionic acid treatment on rice seed germination, vigor and infestation with fungi. Seed samples cultivars Giza 178, Giza 177 and Sakha 106 were soaked with organic acid solutions at concentrations 10, 15, 20 mM and control were soaked in distilled water for 24 h to each treatments. Giza 178 cv. gave the highest results of seed and seedling vigor characters followed by Giza 177 cv. and Sakha 106, respectively. Seed treatment with salicylic acid 15 mM was the best treatments followed by benzoic acid 10 mM and propionic acid 15 mM recorded the highest values of seed germination, seed and seedling vigor as concentrations. SA reduced seed infestation with fungi with increasing concentrations followed by propionic acid and benzoic acid, respectively. However, increased to propionic acid concentration were negatively affected seed germination and vigor with all cultivars.

Key words: Organic acids, seed vigor, seed-borne diseases, rice

INTRODUCTION

Rice (*Oryza sativa*) is principal cereal crop of Egypt. Seed-borne diseases cause enormous losses to our crop. The infected seeds may fail to germinate, transmit diseases from seed to seedling and from seedling to growing plants (Fakir *et al*., 2002). Microorganisms play an important role in affecting the quality of seed of which fungi are the largest group. These pathogens are disastrous as they reduce seed vigor and weaken the plant at its initial growth stages. Organic acid are known for years for their antibacterial and antifungal properties which have been widely used food stuff industry and agriculture (De Muynck *et al*., 2004; Laitila *et al*., 2002; Lavermicocca *et al*., 2003; Pao *et al*., 2008; Sathe *et al*., 2007). Salicylic acid was the best priming agent to increase germination and subsequent seedling growth in fine rice. However, the seed and seedling vigor depended on the concentration of SA and soaking time (Anwar *et al*., 2013). SA an endogenous growth regulator of phenolic nature influences many physiological processes such as, seed germination (Cutt and Klessig, 1992), photosynthesis and plant growth rate (Khan and Abdullah, 2003; Mahmood *et al*., 2010; Rafique *et al*., 2011) and endogenous hormone levels (Iqbal *et al*., 2006) in crop plants. SA also prevents the damaging action of various stress factors in many plan species (Sakhabutdinova *et al*., 2003; Afzal *et al*., 2005; Iqbal and Ashraf, 2010). Salicylic acid was applied as a foliar spray and led to reduction in the incidence of chocolate spot of faba been caused by *Botrytis fabae* (Abbas *et al*., 2006). For damping-off control, benzoic acid inhibited *Rhizoctonia solani in vitro* and efficiently controlled both pre-emergence damping-off and post-emergence mortality of *Phaseolus vulgaris*. Benzoic acid and salicylic acid and sodium benzoate and hydroquinone were effective against the seed-borne fungi of peanut i.e., *Cephalosporium* sp., *Fusarium* spp., *R. solani*, *Sclerotium bataticola* and *Verticillium* sp. (Elwakil and El-Metwally, 2000). Ibrahim and Kishk (2014) found that SA played an important role in enhancing seed viability and increased seedling length, less number of total fungi and reduced of disease incidence in seedlings when applied as seed soaking of onion seeds. Benzoic acid, salicylic acid and ascorbic acid significantly reduced the linear growth of three fungi and spore germination of *Fusarium* spp., they increased shoot, root length, dry weight and reduced damping-off of

three tomato cultivars (Shahda, 2000). Galal *et al*. (2000) found that the mycelia dry weight of both *Alternaria radicina* and *A. tenuissima in vitro* were completely inhibited at 10 mM benzoic acid and salicylic acid. Daw *et al*. (2008) demonstrated that exogenous SA treatment of rice could increase production of the enzymes implicated in resistance to oxidative stresses and antifungal compounds which appear to play a significant role in rice resistance to the invasion of blast fungal pathogens. Biocontrol agents *Trichoderma* and *Pseudomonas fluorescens* when applied along with SA showed appraisable increase in the biometric parameters in rice against *Rhizoctonia solani* and decrease the percentage of rate of infection compared with control and other treatments (Anitha and Das, 2011). Salontai *et al.* (1987) found that seed treatment of maize and barley seeds intended for storage with propionic acid+acetic acid, each at 0.5% gave good protection to, bacteria, yeasts, *Fusarium*, *Penicillium*, *Aspergillus*, *Alternaria*, *Mucor*, *Trichoderma* and *Geotrichum* spp. Mazzani *et al*. (1998), tested a mixture propionic acid, sorbic acid and benzoic acid for the control of toxigenic fungi in maize grains. The most effective control of *Aspergillu sflavus*, *Fusarium moniliforme* and *Penicillium* spp. was achieved with acid mixture at 2 kg t^{-1} grain. This study was aimed to study the effect of salicylic acid, benzoic acid and propionic acid on seed germination, vigour and health of rice seeds.

MATERIALS AND METHODS

Seed samples of three rice cultivars (Giza 178, Giza 177, Sakha 106) obtained from Seed Certification Testing Station, Dakhlia Governorate, Egypt season 2013 for two times at the laboratory of Seed Technology Research Unit, Mansoura, Egypt. Seed were soaked with Salicylic acid, benzoic acid and propionic acid for 24 h in concentration 10, 15, 20 mM and other seed soaked for 24 h in distilled water (control) from each cultivars. Seeds treated were air dried in lab condition on filter paper sheets for 10-12 h. Eight replicates of 25 seeds from each treatment and cultivar (200 seeds) were placed in Petri dishes (12 cm) containing 3 layers of moistened blotters and incubated in the growth chamber at 25±2°C and evaluated the following characters:

Seed vigor test:

- **Germination percentage (G%):** It was calculated by counting only normal seedling 14 day after planting according to ISTA (1999)
- **Speed Germination Index (SGI):** It was calculated in the Association of Official Seed Analysis (AOSA., 1983) by following equation:

$$SGI = \frac{\text{No. of germinated seed}}{\text{Days of first count}} + \frac{\text{No. of germinated seed}}{\text{Days of final count}}$$

The seeds were considered germinated when the radicle was at least 2 mm long

- **Germination Energy (GE):** It was recorded on the 4th day after planting. It is the percentage of germinated seeds 4 days after planting relative to the total number of seeds tested (Ruan *et al*., 2002)
- **Germination Rate (GR):** It was defined according to the following equation of Bartlett (1937):

$$GR = \frac{a + (a+b) + (a+b+c) \ldots\ldots (a+b+c+m)}{n(a+b+c+m)}$$

where, a, b and c are No. of seedlings in the first, second and third count, m is No. of seedlings in final count, n is the number of counts.

Seedling vigor test: At the final count, ten normal seedlings from each replicate were randomly taken to measure seedling characters:

- **Seedling length:** It was measured of ten normal seedlings 14 days after planting
- **Seedling dry weight:** Ten normal seedlings were dried in hot-air oven at 85°C for 12 h to obtain the seeding dry weight (g) according to Krishnasamy and Seshu (1990)
- **Seedling vigor index:** It was calculated according to on the following equation of Abdul-Baki (1980):

$$\text{Seedling vigor index} = \text{Dry weight(g)} \times \text{Germination percentage}$$

Mycological analysis: The seed health analysis was performed on 200 seeds from each treatment, other two hundred of untreated seed of cultivar under study as control (ISTA., 1999). The seeds were plated in Petri dishes (9 cm) with eight replicates (25 seeds/Petri dishes) and incubated at 22±2°C for 7 days, under 12 h alternating cycles of NUV light and darkness. After incubation the fungi were identified on the basis of their growth and sporulation using a stereomicroscope and if necessary, compound microscope (Machado *et al*., 2002; Mathur and Kongdal, 2003). The total number of infected seeds by fungus in each dish was recorded and the calculated to the following equation:

$$\text{Fungi (\%)} = \frac{N1 - N2}{N1} \times 100$$

Where:
N1 = Number of treated seeds
N2 = Number of seeds with fungal growth

Statistical analysis: All obtained data of characters were subjected to the statistical analysis according to the technique of analysis of variance (ANOVA) of completely randomized design, as described by Gomez and Gomez (1984).

RESULTS

Results of germination percentage, seed seedling vigor traits of the studied rice cultivars as affected by the treatment under study are presented in Table 1. Rice cultivars were significantly varied among them on seed and seedling vigor traits. Giza 178 cv. gave the highest values of germination percentage (92.1%), speed germination index (41.1), germination energy (88.8) and germination rate (0.78). Also, the highest values of seedling vigor traits were obtained by Giza 178 cv. (28.6%), followed by Giza 177 cv. (87.8%) as germination percentage, (40.2) speed germination, (85.0) germination energy and (24.6) seedling vigor index while Sakha 106 cv. were less values of germination percentage, speed germination, germination energy, germination rate and seedling vigor index. The effect of organic acid on vigor characters were highly significant, salicylic acid, benzoic acid gave the highest germination percentage (89%), speed germination (42.1), germination energy (87.9) with benzoic acid and (87.4) with SA, also SA gave highest values of seedling vigor index was (26.8) while propionic acid gave the lowest values of germination %, speed germination, Germination Energy (GE), Germination Rate (GR) seedling vigor index. Regarding the effect of concentration of organic acid, results in Table 1 demonstrated that seed treatment with 15 mM gave the highest values of seed and seedling vigor. On the other hand, the lows values of seed germination, seed and seedling vigor were obtained of by seed treatment with 20 mM with high significant.

Interactions effect between organic acid and concentrations on GP, SGI and seedling dry weight (g) and seedling vigor index are shown in Fig. 1, SA (15 mM) was the

Table 1: Effect of rice cultivars, organic acid and concentrations on rice seed germination, seed and seedling vigor characters

Characters Treatments	Germination (%)	Speed germination index	Germination energy	Germination rate	Shoot length (cm)	Root length (cm)	Seedling dry weight	Seedling vigor index
Cultivars								
Giza 178	92.10	41.10	88.80	0.78	10.20	9.00	0.310	28.60
Giza 177	87.80	40.20	85.00	0.65	10.10	7.40	0.280	24.60
Sakha 106	83.00	39.60	81.00	0.67	7.50	7.30	0.240	19.90
f-test	**	**	**	**	**	**	**	**
LSD at 5%	2.71	0.42	2.00	0.04	0.68	1.13	0.070	6.34
Seed treatment								
Propionic acid	84.50	36.50	79.40	0.66	7.60	6.80	0.230	19.60
Benzoic acid	89.20	42.10	87.90	0.73	10.40	8.30	0.300	26.90
Salicylic acid	89.10	42.10	87.40	0.73	10.00	8.70	0.310	26.80
f-test	**	**	**	**	**	**	**	**
LSD at 5%	0.94	0.33	2.17	0.02	0.46	0.54	0.005	0.57
Concentration								
Distilled water	85.70	40.00	85.00	0.66	8.50	7.90	0.260	22.80
10 mM	89.80	42.30	88.00	0.71	9.70	8.30	0.290	25.60
15 mM	90.40	42.60	88.50	0.74	10.30	8.30	0.300	27.20
20 mM	84.40	36.10	78.10	0.68	8.70	7.20	0.250	21.80
f-test	**	**	**	**	**	**	**	**
LSD at 5%	1.08	0.38	2.50	0.03	0.53	0.62	0.006	0.65

**Significant at 5%

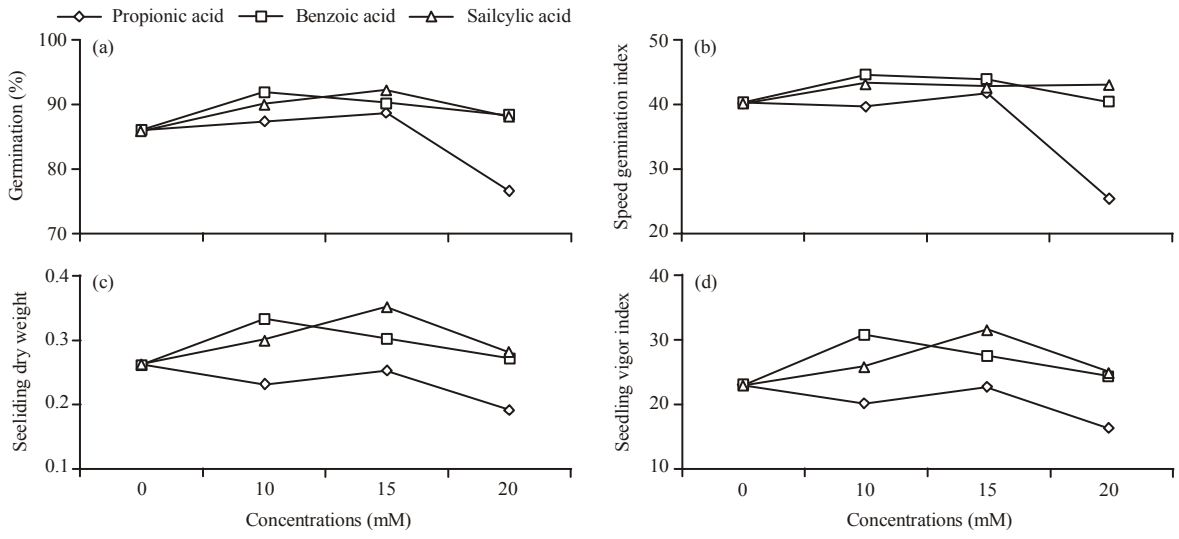

Fig. 1(a-d): Effect of interaction between organic acids and concentrations on (a) Germination percentages, (b) Speed germination index, (c) Seedling dry weight and (d) Seedling vigor index of treated rice seeds

Table 2: Effect of organic acid on percentage of infected seeds with fungi of c.v. Giza 178, Giza 177 and Sakha 106 rice

	Propionic acid			Benzoic acid			Salicylic acid			
Fungi	10	15	20	10	15	20	10	15	20	Control
Giza 178										
Alternaria longissima	0	0	0	0	0	0	0	0	0	0
Alternaria padwickii	32	24	12	26	40	24	28	16	4	64
Alternaria tenuis	16	12	0	0	0	0	12	16	8	28
Bipolaris oryzae	28	18	0	16	16	10	12	11	3	40
Cladosporium oryzae	0	0	0	0	0	0	0	0	0	0
Curvularia oryzae	0	0	0	0	0	0	0	0	0	0
Fusarium graminearum	0	0	0	0	16	12	11	0	4	13
Fusarium moniliforme	12	11	0	12	0	8	0	4	0	8
Fusarium semitectum	12	0	0	0	12	4	4	12	4	12
Helminthosporium sp.	70	0	0	0	0	0	8	2	0	8
Rhizoctonia solani	0	0	0	2	0	1	0	0	0	11
Sarocladium oryzae	0	0	0	0	0	0	0	0	0	12
Stempyhlium botryosum	o	0	0	11	0	0	0	4	0	0
Trichothecium roseum	0	0	0	0	0	0	0	0	0	8
Giza 177										
Alternaria longissima	0	0	0	0	0	0	16	12	0	24
Alternaria padwickii	42	20	12	20	12	12	12	8	2	32
Alternaria tenuis	0	0	0	0	8	0	28	12	4	16
Bipolaris oryzae	12	8	4	12	12	8	12	8	4	28
Cladosporium oryzae	0	0	0	0	0	0	4	0	0	8
Curvularia oryzae	0	0	0	4	0	0	0	0	0	8
Fusarium graminearum	8	4	0	8	0	0	0	8	2	12
Fusarium moniliforme	8	2	0	12	4	0	4	0	0	4
Fusarium semitectum	0	0	0	8	0	4	0	0	4	12
Helminthosporium sp.	12	4	2	0	0	0	0	0	0	18
Rhizoctonia solani	0	0	0	12	0	0	0	4	0	8
Sarocladium oryzae	4	2	0	0	0	2	0	4	2	2
Stempyhlium botryosum	0	0	0	0	0	0	0	0	0	0
Trichothecium roseum	0	0	0	4	0	0	8	0	0	8
Sakha 106										
Alternaria longissima	0	0	0	8	4	4	12	8	4	12
Alternaria padwickii	28	21	2	12	8	0	12	16	4	40
Alternaria tenuis	0	0	0	0	8	2	4	4	2	16
Bipolaris oryzae	12	12	2	12	16	4	16	0	0	21
Cladosporium oryzae	0	0	0	0	0	0	0	0	0	0
Curvularia oryzae	0	0	0	0	0	0	0	0	0	8
Fusarium graminearum	12	8	4	8	2	0	4	0	0	4
Fusarium moniliforme	12	4	2	4	4	0	12	2	0	16
Fusarium semitectum	4	0	0	12	4	4	8	8	0	12
Helminthosporium sp.	8	2	0	8	4	0	0	0	0	12
Rhizoctonia solani	8	2	0	0	0	0	0	0	0	8
Sarocladium oryzae	12	4	4	12	0	0	0	0	0	12
Stempyhlium botryosum	8	8	0	12	12	0	0	0	0	12
Trichothecium roseum	0	0	0	0	0	0	0	0	0	0

Test was carried out using blotter technique. Two hundred seeds were tested. Incubation was carried at 22±2 for 7 days (ISTA., 1999)

most effect on germination character (92.3%), SGI (42.6) and seedling vigor index (31.7), followed by benzoic acid (10 mM) (92%) as GP, SGI (44.4) seedling dry weight (0.33 g) and seedling vigor index (30.8). While propionic acid was the lowest values of all characters especially 20 mM where gave (76.7%) as GP, SGI (25.1), seedling dry weight (0.19 g) and seedling vigor index (16.1).

Effect of organic acids treatments on percentage of infection by seed-borne fungi of seed cultivars (Giza 178, Giza 177 and Sakha 106) are presented in Table 2. The results gave evidence to the presence of fourteen fungal species among the tested cultivars. The fungus *Alternaria padwickii* was the highest percentage on Giza 178 cv. seeds with (64%) followed by Sakha 106 cv. with (40%) and Giza 177cv. with (32%). *Bipolaris oryzae* recorded (40%), (28%) and (21%) on cvs Giza 178, Giza 177 and Sakha 106, respectively. Genus *Fusarium* recorded high percentages on seeds of their cultivars especially *Fusarium graminearum* and *F. moniliforme*.

Sarocladium oryzae presented on seeds of Giza 178 cv. and Sakha 106 cv. with (12%) and (2%) on Giza 177 as control. On other hand, effect of concentration of organic acid on percentages of fungi on seed treatments of cv. Giza 178 Table 2, *A. padwickii* decreased to 24, 12 and 4% about 20 mM concentration of both benzoic acid, propionic acid and salicylic acid, respectively. *Bipolaris oryzae* recorded 10, 3 and 0% with 20 mM of both benzoic acid, salicylic acid and propionic acid, respectively as compared with check. Genus

Table 3: Organic acids concentrations and their effect on the total fungi, germination percentage and seedling vigor index of three rice cultivars

	Giza 178			Giza 177			Sakha 106		
Concentration of organic acid	Total fungi	G (%)	SVI	Total fungi	G (%)	SVI	Total fungi	G (%)	SVI
Propionic acid (mM)									
Distilled water	204	91	26.4	180	86	24.1	173	81	17.8
10	107	92	22.1	86	88	20.2	104	82	18.0
15	65	93	23.3	40	89	23.1	61	84	21.0
20	12	78	15.6	18	77	15.4	14	75	12.8
Benzoic acid (mM)									
Distilled water	204	91	26.4	180	86	24.1	173	81	17.8
10	67	97	35.9	80	92	32.5	88	87	24.4
15	84	95	31.4	36	90	28.8	62	86	22.4
20	51	95	28.5	26	88	26.4	14	82	18.0
Salicylic acid (mM)									
Distilled water	204	91	26.4	180	86	24.1	173	81	17.8
10	75	94	36.1	84	90	23.4	68	85	21.3
15	75	96	39.4	56	93	34.4	38	88	24.6
20	23	92	31.3	18	89	22.3	10	84	20.2

LSD G% = 3.24, LSD SVI = 1.96

Table 4: Effect of organic acids on percentage pathogenic infected seeds of rice

	Propionic acid (mM)			Benzoic acid (mM)			Salicylic acid (mM)			
Fungi	10	15	20	10	15	20	10	15	20	Control
A. padwickii	34	22	9	20	20	12	17	13	3	45
B. oryzae	17	13	2	13	15	7	13	6	2	30
F. graminearum	7	4	1	5	6	4	5	3	2	10
F. moniliforme	11	6	1	9	3	3	5	2	0	9
R. solani	3	1	0	5	0	0	0	1	0	9
S. oryzae	6	2	1	4	0	0	0	1	1	9

Fusarium effected with concentrations of organic acids where less percentages of this genus with increased concentrations. *Rhizoctonia solani* and *Sarocladium oryzae* recorded less value of percentages with each concentrations of organic acid as compared with check. Seed treatment of cv. Giza 177 lead to low of number fungi such as *A. padwickii* with 20 mM salicylic acid to (2%), *Bipolaris oryzae* to (4%), *F. graminearum* to (2%) while propionic acid lead to decreased this fungi to 12, 4 and 0%, respectively, also benzoic acid gave (12%), (8%), (0%), (0%) and (0%) with 20 m M concentration of *A. padwickii*, *B. oryzae*, *F. graminearum*, *F. moniliforme* and *R.* solani, respectively. Also in Table 2, cv. Sakha 106 showed that, *A. padwickii*, *B. oryzae*, *Fusarium* spp., *R. solani* and *S. oryzae* recorded notes low with increase concentrations of organic acid arrive to (0%) compared with control.

Organic acids concentrations and their effect on total fungi, germination percentage and seedling vigor index of three rice cultivars under study.

Table 3 found that increase of total fungi lead to decreased in germination percentage and seedling vigor index, benzoic acid (10 mM) gave (67) of Total fungi, (98%) GP and (35.9) SVI as higher rate of cv. Giza 178 while (80) Total fungi gave (92%) GP and (32.5) SVI of cv. Giza 177. Also, Total fungi (88%), (87%) GP and (24.4) SVI were cv. Sakha 106.

Propionic acid (15 mM) concentration recorded the highest number of GP (93%), (23.3) SVI and Total fungi was (65%) of cv. Giza 178 while cv. Giza 177 recorded (40) Total fungi, (89%)GP and (23.1)SVI, also cv. Sakha 106 was (61) Total fungi, (84%) GP and (21) SVI as compared with distilled water treatment. Concentration (15 mM) of SA gave (75) Total fungi, (96%) GP and (39.4) SVI of cv. Giza 178. Giza 178 cv. recorded after treatment with same concentration the less of Total fungi (56) with highest of GP (93%) and (34.4) SVI, Sakha 106 cv. with SA was (38) of Total fungi, (88%) GP and (24.6) SVI, where with increased significantly of GP and SVI.

Table 4 illustrated that all fungi affected with treatments seeds of organic acid as compared with control, SA was the most effective followed by propionic acid and benzoic acid on percentage of pathogenic fungi, the percentage of seeds with fungi decreased with increasing concentrations of organic acid.

DISCUSSION

Differences in germination percentage among cultivar were observed, Giza 178 cultivar showed superiority over cv. Giza 177 and cv. Sakha 106 of seed and seedlings characters. These results reported that Giza 178 was the best of shoot and root length as compared with Giza 177 and Sakha 106 were similar with Sedeek (2001). Salicylic acid gave the highest values of seed and seedling vigor characters followed by benzoic acid and propionic acid, these results were confirmed with (Anwar *et al*., 2013).

Ikegawa *et al*. (1996) found increased in shoot and root length especially treated with SA may be related to the action of cellulose and pectinases on host cell walls which would decrease the level of lignin cell wall-bound phenolic

compounds. Priming with SA for 20 h proved much effective in increasing germination rate of rice (Basra *et al*., 2006). Also, our results were harmony with (Anwar *et al*., 2013), they found that SA was the best priming agent to increase germination and subsequent seedling growth in fine rice. However, the seed and seedling vigor depended on the concentration of SA and the soaking time. Also El-Kallal (2007) reported that enhanced shoot and root growth of *Fusarium* infected plants of tomato when treated plants with jasmonic acid and salicylic acid together. Benzoic acid and salicylic acid reduced the liner growth of three fungi and spore germination of *Fusarium* spp. at 20 mM; also they increased shoot, root length, dry weight and control of damping off of tomato (Shahda, 2000).

Organic acid decreased the number of seeds infected with fungi with increased concentration where SA the more effective on fungi followed by propionic acid and benzoic acid similar of earlier study by Elwakil and El-Metwally (2000), Ibrahim and Kishk (2014) and Szopinska (2013) reported that some organic acids reduced seed infestation, however negatively affected seed germination and vigor of Zinnia. Also, our results were similar with (Shabana *et al*., 2008) they noticed that benzoic acid or salicylic acid at 9 mM completely inhibited the growth of *Bipolaris oryzae*. *In vitro* and under field conditions, spraying of benzoic acid at 20 mM led to a significant reduction in disease severity and incidence on the plant leaves, in addition to a significant increase in the grain yield its components of rice Cowan (1999) explained the mechanisms thought to be responsible for the phenolics toxicity to microorganisms on the basis of enzyme inhibition by the oxidized compounds, possibly thought reaction with sulfhydryl groups or thought more nonspecific interaction with the proteins. The site(s) and number of hydroxy groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity. SA is phytohormone and has a significant impact on plant growth and development and is recognized as an endogenous where signal mediating in plant defense, against pathogens. Where in the pathogen-induced pathway, systemic acquired resistance is mediated by an endogenous signal that is produced in the infected leaf and translocated in the phloem to other plant parts, where it activates resistance mechanisms (Hayat and Ahmad, 2007). Effect propionic acid as seed treatment, results show there enhancing of germination percentage and vigor characters with 15mM while 20 mM gave decrease in the same characters and number of seed infected fungi, harmony with earlier studies, Salontai *et al.* (1987) and Mazzani *et al.* (1998).

CONCLUSION

Using Giza 178 cultivar was the best as compared with other cultivars followed by cv. Giza 177 then cv. Sakha 106, were gave the highest value of seed germination, seed and seedling vigor characters under experiment conditions.

The effect on vigor characters: Salicylic acid with 15 mM for 24 h gave the highest value followed by benzoic acid rate 10 mM and propionic acid 15 mM.

Effect of organic acids on number of seeds infected with fungi: SA reduced seed infestation with fungi followed by propionic acid and benzoic acid, however increased to propionic acid concentration were negatively affected seed germination and vigor.

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